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Tissue Expression of Protease Activated Receptor 2 in Patients with Pruritus in Chronic Kidney Disease

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Abstract

***Background*: Uremic pruritus has been defined as itching that is directly related to kidney disease, without any other comorbid condition that causes itching. The exact pathogenesis of uremic pruritus remains unclear but, protease-activated receptor 2 (PAR2) is claimed to have a pivotal role in the pathogenesis of uremic pruritus. *Aim*: Evaluation of PAR2 activity in the epidermis of patients with chronic kidney disease (CKD) complaining of pruritus. *Subjects and Methods:* Case-control study on 48 individuals (24 CKD patients complaining from pruritus and 24 matched healthy controls). Skin biopsy specimens from all participants were taken from a similar area of the back of forearms then immunohistochemical analysis for PAR2 activity in the epidermis was performed. *Results:* Data revealed that epidermal PAR-2 expression was significantly higher in CKD patients compared to healthy controls. PAR2 staining intensity in skin biopsies was moderate in 9 (37.5%) patients and strong in 15 (62.5%) patients in the cases group. On the other hand, PAR2 staining was moderate in all 24 (100%) cases in the control group which was statistically significant. *Conclusion:* Epidermal PAR-2 expression is increased in CKD patients with pruritus.**

Keywords: PAR2, Chronic kidney disease, Pruritus.

Introduction

Pruritus is an unpleasant perception that provokes the desire to itch, often resulting from the activation of free nerve endings by pruritogenic or itch-inducing stimuli in the skin. The propagation of itch can result from both inflammatory as in atopic dermatitis or noninflammatory diseases as in uremic pruritus and cholestatic pruritus(1). Chronic kidney disease (CKD) associated pruritus has been defined as itching that is directly related to kidney disease without another comorbid condition(2). The severity of pruritus may vary over time from barely noticeable to severe itching that causes constant restlessness(3). The pathophysiology of uremic pruritus remains not fully understood. Several theories such as systemic inflammatory process, parathyroid hormone elevation, phosphorus and calcium disequilibrium, and/or a neuropathic process. Neurotrophins are a group of neurological mediators that have a role in pruritic skin diseases(4). Histamine has long been the gold standard itch mediator,

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when it is applied to human skin it causes local vasodilatation and gives rise to a characteristic redness, flare, and swelling response accompanied by itch sensation[5]. In addition to histamine and serotonin, mast cells also release other proteases that can cause strong non-histaminergic itch, originally, proteases cause itch via protease-activated receptor 2 (PAR2). The PARs are a family of G protein-coupled receptors with N-terminal domains; Proteases are regarded as hormone-like multifunctional signaling molecules under both physiological and pathophysiological conditions[6]. PAR2 has been primarily localized to the stratum granulosum layer of epidermal keratinocytes. Functional PAR2 is also expressed by several immune cells such as neutrophils, eosinophils, monocytes, macrophages, dendritic cells, mast cells, and T cells[7]. The connection between PAR2 and non-histaminergic itch has been well documented, in atopic dermatitis increased PAR2 expression was found in epidermal nerve fibers and keratinocytes. PAR2 has also been linked to pruritus of post-burn wounds, hepatic dysfunction, and end-stage renal disease[8]. In this study, cutaneous PAR2 expression has been investigated in CKD patients having pruritus; this not only provides new insight into the pathogenesis of itching in CKD patients but also allows the development of clinical applications and potential therapeutic targets against PAR2.

Subjects and Methods

This case-control study included 48 participants (24 CKD patients complaining of pruritus and 24 matched healthy controls). Patients were recruited from the nephrology department and outpatient clinic, at Suez Canal university hospital (SCU), Ismailia, Egypt; during the period between January to September 2020. Patients of CKD having pruritus with a glomerular filtration rate less than 60, aged 30 to 70 years old were recruited. Patients with HCV seropositive; any skin disease causing itching; hyperphosphatemia exceeding 6 mg/dl and hyperparathyroidism exceeding 9 folds above baseline were excluded. All participants were subjected to detailed history and total body skin examination. Assessment of pruritus severity was done through a 4 items itch questionnaire score as follows: Pruritus without the need to scratch (score 1), pruritus with the need to scratch but without excoriation (score 2), pruritus unrelieved by scratching without excoriation (score 3), pruritus accompanied by excoriation (score 4), totally restless (score 5)[9]. Biochemical parameters such as serum calcium, phosphorous, urea, and intact-parathyroid hormone, before and after dialysis, were assessed in this study to rule out other possible causes of itching. Skin biopsy specimens from all participants were taken from a similar area of the back of the forearms under sterile conditions. Each skin biopsy specimen is 4 mm and was fixed in neutral buffered formalin and processed for paraffin sections (5 mm). Immunohistochemical analysis was performed at the pathology department, Faculty of Medicine, SCU. Each biopsy was subjected to Hematoxylin & Eosin (H&E) to detect general skin structure and Immunohistochemical stain for demonstration of PAR2 with rabbit anti-clonal antibody according to the manufacturer's instructions (ABclonal antibody. Catalog#A1583).

Statistical Analysis

Data were analyzed using SPSS software package version 20.0. (Armonk, NY: IBM Corp). The data were expressed as mean and standard deviation or number and frequencies according to the type of variables. Chi-square and t-test were used to

compare qualitative and quantitative variables respectively. In cases where the descriptive data were highly skewed, Mann-Whitney test and Fisher's Exact test were employed. The Spearman and Pearson correlation coefficients were used to analyze the degree of association. The difference was statistically significant when p-< 0.05.

Results

A total of 24 patients including 16 males and 8 females participated in the study; the mean age of the patients was 49.21 ± 11.41 ranging from 32 to 66 years. The control group included 16 males and 8 females their mean age was 40.46 ± 9.51 ranging from 31 to 65 years. The onset of CKD ranged from 2 to 12 years with a mean of 6.17 ± 2.81 and median (IQR) of 5.50 (4.0-8.0), most patients (21) had CKD for more than 3 years, while only 3 patients had less than 3 yrs. CKD duration. Regarding the site of itching, 7 of the patients had itching in the abdomen, 3 in the back, 9 in the upper limbs, 9 in the lower limb, and 8 had itching in the body. The duration of itching ranged from 1 to 6 years, 9 patients had itching for less than 3 years and 15 patients had itching for more than 3 years.

Table 1: Relation between PAR2 staining with skin parameters in cases group

	PAR2 staining				Test of sig.	p
	Moderate (n = 9)		Strong (n = 15)			
	No.	%	No.	%		
Site of Itching						
Abdomen	2	22.2	5	33.3	(^{2}=0.336	^{FE}p=0.669
Back	2	22.2	1	6.7	(^{2}=1.244	^{FE}p=0.533
Lower limb	2	22.2	7	46.7	(^{2}=1.434	^{FE}p=0.389
Upper limb	6	66.7	3	20.0	(^{2}=5.227*	^{FE}p=0.036*
All body	0	0.0	8	53.3	(^{2}=7.200*	^{FE}p=0.009*
Duration of itching (Yrs.)						
Range	1.0 – 2.0		3.0 – 6.0		U=0.0*	<0.001*
Mean ± SD.	1.56 ± 0.53		4.13 ± 1.19			
Severity of itching						
1	2	22.2	0	0.0	(^{2}=16.650	^{MC}p <0.001*
2	4	44.4	0	0.0		
3	3	33.3	4	26.7		
4	0	0.0	11	73.3		
Range	1.0 – 3.0		3.0 – 4.0		U=6.0*	<0.001*
Mean ± SD.	2.11 ± 0.78		3.73 ± 0.46			
Median	2.0		4.0			
Skin lesions excoriations						
Absence	9	100.0	4	26.7	(^{2}=12.185*	^{FE}p=0.001*
Presence	0	0.0	11	73.3		

*(^{2}: Chi-square test MC: Monte Carlo FE: Fisher Exact U: Mann Whitney test. p: p value for association between different categories. *: Statistically significant at p ≤ 0.05*

The mean duration of itching was 3.17±1.61 and the median (IQR) was 3.0 (2.0-4.0). Regarding 4 items itch questionnaire for evaluation of the severity of itching, 2 cases (8.3%) had score 1, 4 cases (16.7%) scored 2, 7 cases (29.2%) had score 3 and 11 cases (45.8%) had a score 4, the mean severity of itching was 3.13 ± 0.99 ranged from score 1 to score 4 while the median was 3.0 (2.5-4.0). PAR2 staining intensity in

skin biopsies was found to be moderate in 9 (37.5%) patients and strong in 15 (62.5%) patients. On the other hand, PAR2 staining was found to be moderate in all 24 (100%) cases of the control group which was statistically significant.

Table 2: Relation between PAR2 staining with CKD parameters in cases group.

	PAR2 staining				Test of sig.	P
	Moderate (n = 9)		Strong (n = 15)			
	No.	%	No.	%		
Onset of CKD (years)						
Range	2.0 – 4.0		5.0 – 12.0		U=0.0*	<0.001*
Mean ± SD.	3.56 ± 0.73		7.73 ± 2.37			
Median	4.0		7.0			
Duration of dialysis session (hrs.)						
Range	4.0 – 4.0		4.0 – 4.0		U=0.0	1.000
Mean ± SD.	4.0 ± 0.0		4.0 ± 0.0			
Median	4.0		4.0			
Onset of dialysis (yrs)						
Range	1.0 – 3.0		3.0 – 8.0		U=1.000*	<0.001*
Mean ± SD.	2.0 ± 0.71		5.53 ± 1.73			
Median	2.0		5.0			
Type of dialyzer						
High flux dialyzer	9	100.0	15	100.0	–	–

χ^2: Chi square test MC: Monte Carlo FE: Fisher Exact U: Mann Whitney test.
*p: p value for association between different categories. *: Statistically significant at p ≤ 0.05*

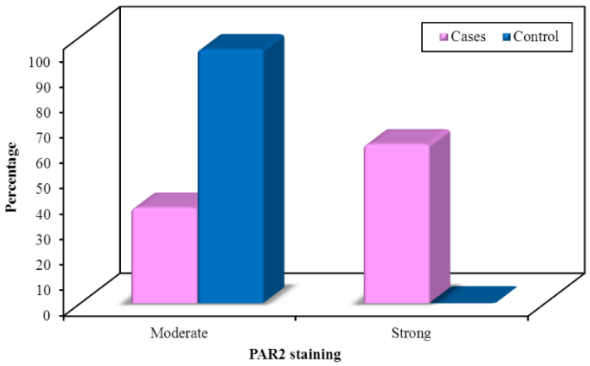

Figure 1: Comparison between the two studied groups according to PAR2 staining intensity in skin biopsies.

The mean duration of itching in cases with moderate PAR2 staining was 1.56 ± 0.53 ranging from 1 to 2 years, while in cases with strong PAR2 staining was 4.13 ± 1.19 ranging from 3 to 6 years. According to 4 items itch questionnaire, the mean severity of itching in cases with moderate PAR2 staining was 2.11 ±0.78 ranging from score 1 to 3, and the mean severity of itching in cases with strong PAR2 staining was 3.73 ± 0.46 ranging from score 3 to 4. Regarding skin lesions, there is no skin lesions in all

cases that had moderate PAR2 staining but 11 (73.3 %) of the cases that had strong PAR2 staining had skin lesions (excoriations) and 4 cases had no skin lesions, Table (1).

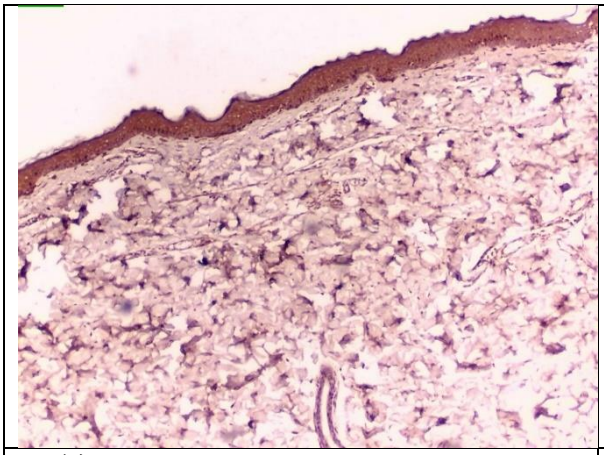

Fig (2): Skin of a patient with ESRD and pruritus showing strong expression of PAR-2 in the epidermis (Anti PAR-2, Hx. and DAP, 40X).

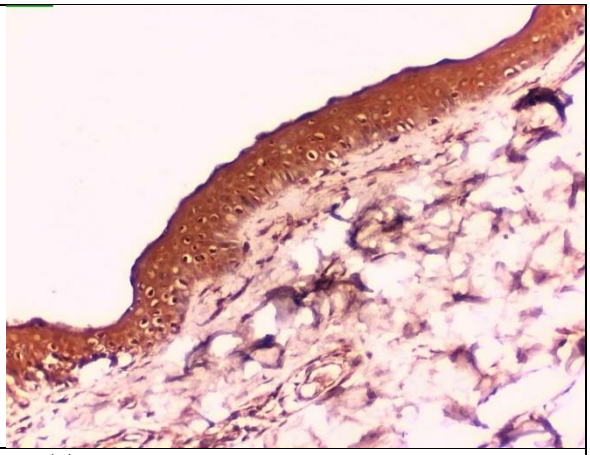

Fig (3): Skin of a patient with ESRD and pruritus showing strong expression of PAR-2 in the epidermis (Anti PAR-2, Hx. and DAP, 100X).

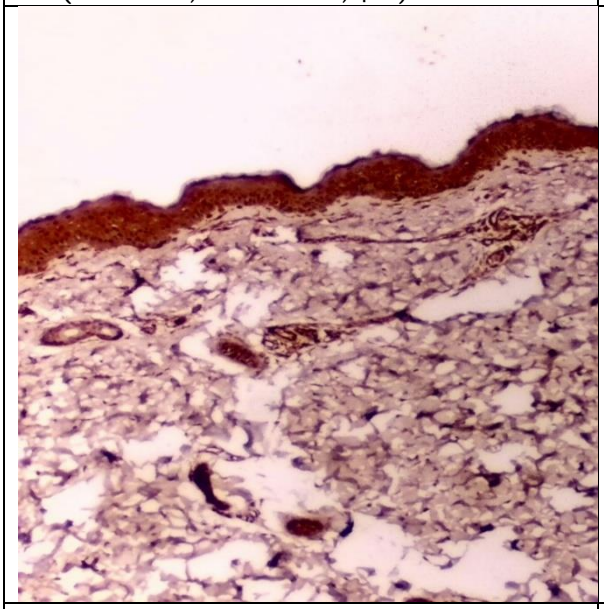

Fig (4): Skin of a patient with ESRD and pruritus showing moderate expression of PAR-2 in the epidermis (Anti PAR-2, Hx. and DAP, 40X).

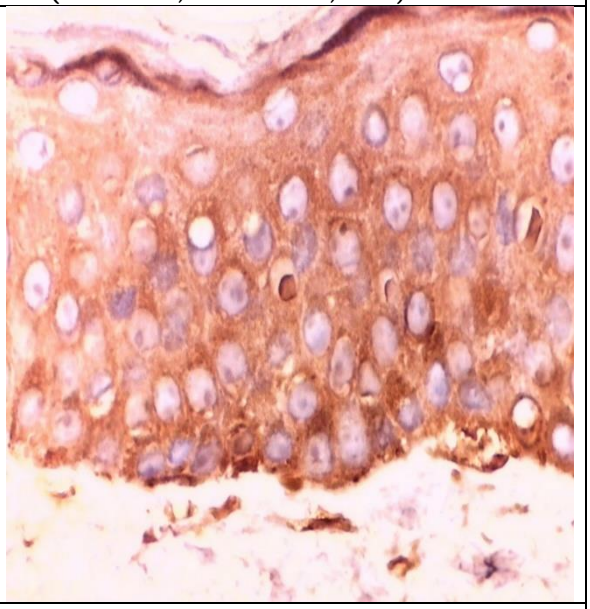

Fig (5): Skin of a patient with ESRD and pruritus showing strong expression of PAR-2 in the epidermis (Anti PAR-2, Hx. and DAP, 400X).

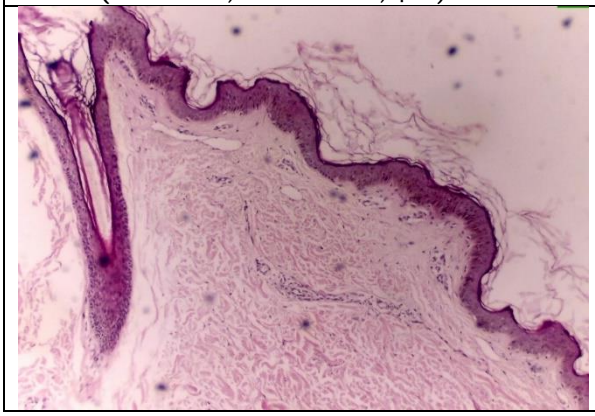

Fig. (6): Skin of a patient with ESRD and pruritus showing mild hyperkeratosis (Hx&E, 40X).

In moderate PAR2 staining 8 cases had no family history of CKD while 1 had a positive family history of CKD. In strong PAR2 staining 10 cases had a positive family history of CKD while 5 cases had no family history of CKD. The mean onset of CKD in moderate PAR2 staining was 3.56 ±0.73 ranging from 2 to 4 years and the mean onset of CKD in strong PAR2 staining was 7.73 ±2.37 ranging from 5 to 12 years. The mean duration of dialysis sessions was 4.0 ±0.0. In moderate PAR2 staining the mean onset of dialysis was 2.0 ± 0.71 ranging from 1 to 3 years while in strong PAR2 staining the mean onset of dialysis was 5.53 ± 1.73 ranging from 3 to 8 years. The type of dialyzer was a high flux dialyzer as shown in table (2).

Discussion

Histamine is a common mediator of various cutaneous disorders associated with itching sensation, also plays a pathogenic role in some patients with uremic pruritus. However, because many of these patients are refractory to treatment with antihistamines, there must be additional histamine-independent mediators that need to be identified. Uremic pruritus is a common complication in ESRD patients. The exact pathogenesis remains unclear. Several studies report some risk factors including elevated calcium and phosphate levels, hyperparathyroidism, elevated serum magnesium, and aluminum concentrations, and inadequate dialysis[10]. A uremic condition in CKD patients leads to upregulation of proteolytic activity and protein energy wasting that may result in increased PAR-2 expression in the skin of these patients[11]. Proteases are regarded as hormone-like multifunctional signaling molecules. In the skin, trypsin-like serine proteases activate PAR-2, that is abundantly expressed in the epidermis of patients with pruritic skin diseases, as atopic dermatitis and uremic pruritus[12]. In atopic dermatitis, PAR-2 in the skin is markedly expressed on primary afferent nerve fibers and markedly related to pruritus. Provocation of an itching sensation by intracutaneous injection of endogenous PAR-2 agonists suggests that this receptor is one of the histamine-independent pruritic mediators, so PAR-2 might be a new therapeutic target for the treatment of itching in atopic dermatitis[13]. The present study found that uremic pruritus might be related to PAR-2 expression as epidermal PAR-2 expression was significantly increased in ESRD patients compared to healthy controls. This came in agreement with Kim and his colleagues who revealed that skin samples from CKD patients exhibited marked increases in PAR-2 expression in the cytoplasm of epidermal cells compared to healthy control[11]. Also, Sung and his colleagues have investigated the association between the severity of pruritus in ESRD patients and levels of cutaneous serine protease activity, as well as PAR-2 expression, they hypothesized that uremic pruritus might be related to PAR-2 expression as epidermal PAR-2 expression was significantly increased in ESRD patients compared to healthy controls.[13] In the same manner Inayat and her colleagues reported that the elevated levels of serine protease and PAR-2 could also play vital roles in the pathogenesis of CKD pruritus[14]. On studying the demographic criteria of case and control groups, mean ages were 49.21 and 40.46 years old respectively. Male gender represented the largest percentage of both groups (66.7%) with no statistically significant differences. Sung and his colleagues reported the median age of ESRD patients with pruritus was 66 years (ranges from 41 to 79) and the male gender was 75%[13]. In this study, the mean severity of itching according to 4 items itch questionnaire in

cases that had moderate PAR2 staining was 2.11 ±0.78 ranging from a score of 1-3, while the mean severity of itching in cases that had strong PAR2 staining was 3.73 ± 0.46 ranged from score 3-4. Also Sung and his colleagues found a significant positive correlation between PAR-2 expression and the visual analogue scale for pruritus scores in ESRD patients[13]. On comparing PAR2 staining in patients and the control group, this study showed that PAR2 staining intensity in skin biopsies was found to be moderate in 9 patients and strong in 15 patients in the cases group. On the other hand, it was moderate in all 24 cases in the control group which is considered statistically significant. This came in agreement with Leigh and his colleagues who reported that tryptase and its receptor PAR2 were found to be elevated in itchy AD and psoriatic skin. Tryptase$^+$ mast cells were significantly increased in itchy AD (40.5.25 ± 6) and itchy psoriatic (42.75 ± 4.5) skin when compared with healthy (6.75 ± 2.25) and non-itchy AD (28.25 ± 9.5) and psoriatic (32.35 ± 4.5) skin[15]. In the current study, on assessing the relationship between PAR2 staining and duration of itching, severity of itching, presence of skin excoriations, family history of CKD, the onset of CKD, and onset of dialysis there was a significant relationship, while, there was no significant relationship between PAR2 staining in relation to the site of itching and duration of dialysis. Another study done by Harlim showed that the intensity of uremic pruritus was related to the duration of hemodialysis[16]. There were some limitations in this study. First, the basic investigation concerning the pathogenesis of how uremia induces PAR-2 expression was not performed. Inflammatory cytokines that could increase protease activities should have been done. Moreover, the evaluation of increased expression of PAR2 in the pathogenesis of CKD pruritus would be better if there was a third group of non-pruritic ESRD patients.

Conclusion

In this study, epidermal PAR-2 expression was increased in chronic kidney disease patients with pruritus suggesting that PAR-2 might be a new therapeutic target for the treatment of itching in those patients.

Ethical Considerations

The study was approved by the Research Ethical Committee of the Faculty of Medicine, Suez Canal University (No. 3928).

Abbreviations

PAR2: Protease-activated receptor 2; CKD: Chronic kidney disease; H&E: Hematoxylin and Eosin; ESRD: End-stage renal disease; SCU: Suez Canal university; HCV: Hepatitis c virus; DM: Diabetes mellitus; AD: Atopic dermatitis, ESRD: end-stage renal disease.

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