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Study of skin expression of thrombomodulin in the Psoriatic Plaques

Running title: Thrombomodulin in psoriasis

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Abstract

Background: Psoriasis is a chronic relapsing, immune-mediated inflammatory skin disease of unknown etiology. The role of thrombomodulin (TM) in different autoimmune inflammatory diseases was explored previously in some studies. Thus; the aim of this study was to assess TM expression in the affected skin of patients with psoriasis.

Methods: This prospective case-control study was carried out on 30 patients with chronic plaque psoriasis and 30 age and gender matched apparently normal subjects as a control group. Skin biopsies were taken for immunohistochemical evaluation of TM expression.

Results: There was a highly significant difference between the two studied groups regarding the expression of TM in the skin. Patients group had a higher expression of TM in both epidermis and dermis compared to controls with a highly statistically significant difference, P-value< 0.01.

Conclusions: These findings might provide a new sight of psoriasis pathogenesis in which TM appears to be implicated but its specific function remains unclear. Therefore, further

studies are required to indicate the role of the TM in the pathogenesis of psoriasis and resolve the controversy about TM expression in the disease.

Keywords: Psoriasis, immunohistochemistry, thrombomodulin.

Introduction

Psoriasis is a long-lasting inflammatory and immune-mediated skin disease. [1] Its prevalence in adults ranged from 2 to 4%. [2] Its pathophysiology is a complex process [1] and its exact cause is not yet fully known. [3] [4]

It has been hypothesized that microcirculation may play a role in the pathogenesis of psoriasis. Studies suggested that endothelial dysfunction leads to local procoagulation which can in turn increase inflammation. [5]

Thrombomodulin (TM), an anticoagulant protein, has been known to have an important role in inflammation through several pathways. [6] Some studies [7] [8] showed the role of TM in different autoimmune inflammatory diseases.

So this study was designed to assess the thrombomodulin level in psoriasis through its immunohistochemical (IHC) expression in involved skin in psoriatic cases compared to healthy controls.

Patients and methods

This prospective case-control study included 30 patients with chronic plaque psoriasis and 30 age and gender matched apparently normal subjects as a control group. Patients were collected randomly from the outpatient clinic of Dermatology Department of Al-Zahraa University Hospital in the period between from April 2018 to October 2019. Control subjects were selected from apparently healthy persons attending Plastic Surgery Department. A Written consent form, approved by the research ethics committee of the Faculty of Medicine, Al-Azhar University Hospitals, was obtained from every participant prior to study initiation.

This study included patients with chronic plaque psoriasis at least a one year history of the disease and not received any specific systemic treatment for at least 4 weeks before the study.

We excluded newly diagnosed cases of psoriasis (less than1 year) (to ensure well-established pathogenesis of the disease), Patients with PASI score < 5 and cases of other types of psoriasis (guttate, erythrodermic, pustular, etc.). Patients who

had received any topical or systemic therapy for at least 4 weeks were also excluded.

We also excluded pregnant and lactating patients, who were younger than15 years old, patients with any dermatological diseases (except chronic plaque psoriasis), any associated inflammatory or autoimmune disease and patients with history of any bleeding/clotting disorder or using anticoagulants e.g warfarin or NSAIDs e.g. aspirin.

All patients were subjected to detailed history taking, complete general and dermatological examination. The severity of the disease was assessed by Psoriasis Area and Severity Index (PASI) score and body surface area (BSA). [9]

Punch skin biopsy was taken from the psoriatic lesion of the patients as well as from the skin of healthy individuals.

Skin biopsy

The site of biopsy was sterilized by alcohol 75% where local anesthesia was applied. 4mm punch biopsy from the psoriatic lesion of the patients in the investigated group as well as from the skin of healthy individuals was taken. The biopsies were preserved at 10% neutral buffered formalin.

All biopsies were submitted to histology Department. They were dehydrated in ascending grades of ethanol followed by immersion in xylene then impregnated in paraffin. Several 5 micron (5um) thick sections from each block were taken. [10] One slide was stained for routine histopathological examination using Hematoxylin and Eosin (H&E). Other sections were mounted on positive charged slides and stored at room temperature for immunohistochemical staining for TM antibody.

Immunohistochemistry

Immunohistochemical (IHC) staining was performed using mouse monoclonal anti-thrombomodulin (catalogue number Mc0974, dilution 1:100). Medaysis Co. Ltd. (139 E Airway Blvd. Livermore, CA 94551, USA). It was supplied as a liquid Phosphate Buffered Saline (PBS), containing BSA and ≤ 0.09% sodium azide (NaN3). It was received in a single vial containing 1 ml of concentrated antibody. Concentration was 1 ml, concentrated and diluted 1: 100 in EDTA.

IHC staining was performed according to the manufacturer's recommendations. Immunostaining required pretreatment by boiling in 10Mm citrate buffer (catalogue number AP 9003) Ph

6 for antigen retrieval. The pretreatment by citrate buffer was done using Pressure Cooker for 15 minutes and left to cool for 30 minutes at room temperature.

Ultravision detection system (catalogue number TP-015-HD) was used to complete the immunostaining. Slides were counter-stained for 5–10 min with Mayer's hematoxylin (catalogue number TA-060- MH). Negative control slides were prepared by omitting the primary antibodies from the staining procedure. Positive control was human placenta.

Positive immunoreactivity appeared as brown deposits. Citrate buffer, ultravision detection system and Mayer's hematoxylin were purchased from Richard-Allan Scientific Subsidiary of Thermo fisher Scientific, UK.

Morphometric study:

This included measurement of the mean area percent of positive immune staining for TM in the epidermis and dermis for all specimens from all subjects of the study.

This was done in five non overlapping fields at X200 magnification for every subject using Leica Qwin 500C image analyzer computer system (England) present in Histology Department, Faculty of Medicine, Cairo University.

Images were captured live on the screen from sections under a light microscope (Olympus BX-40, Olympus Optical Co. Ltd. Japan) with affixed video camera (Pansonic Color CCTV camera, Matsushita Communication Industrial Co. Ltd., Japan).

The video images were digitized using a Leica Qwin which is a Leica's windows based image analysis tool kit fitted to an IBM compatible personal computer with a color monitor. The positive immunoreactivity for TM appeared as brown membrane deposits.

Binary images were generated by color thresholding for the brown color then the area of these binaries was measured by the Leica Qwin 500 software. This was done for every field of the five fields for each subject to obtain a mean area for each. Mean area percent is the relation between the areas of the positivity marked by the binary images to the field area. Results obtained were subjected for Statistical analysis.

Statistical analysis

It was conducted using Statistical Package for Social Science (IBM SPSS) version 23. Chi-square test and/or Fisher exact test, Independent t-test, Mann-Whitney test and Spearman correlation coefficients were used. P value up to 0.05

is considered statistically significant while P value less than 0.001 is considered highly significant.

Results

Descriptive data of the studied groups in our study showed no statistically significant difference between the patient group and control group regarding age, sex, body mass index (BMI), waist circumference and associated comorbidities (P-value >0.05), **(Table 1).**

Disease characteristics among the patients group showed that the duration of disease ranged from 1 – 40 years with median =10.00 (6 - 19) years. Regarding PASI score in patients, it ranged from 6 – 41.7 with mean ± SD= 16.74 ± 8.32. Their body surface area (BSA) ranged from 10 – 69 with mean ± SD= 26.16 ± 15.47. Accordingly, patients were classified into 3 groups, 11 patients (36.7%) with moderate disease, 11 patients (36.7%) with severe disease and 8 patients (26.7%) with very severe disease.

Regarding the expression of TM in the skin of the studied groups, in patients group, the epidermis had Median (IQR) = 23.66 (18.03 - 29.68) and dermis had Median (IQR) = 7.89 (4.92 - 10.84). While in the control group, the epidermis had Median (IQR) = 8.89 (6.65 - 10.57) and dermis had Median (IQR) = 0.31 (0.16 - 0.74). There was a highly statistically significant difference between the studied groups, P-value< 0.01**, (Figure 1&2).**

Our study showed that patients group had a higher expression of TM in both epidermis and dermis **(Figure 3&4)** compared to the control group **(Figure5&6).**

In the current study, no significant correlation was found between the expression of TM in skin in the studied groups (patients and controls) either in the epidermis or dermis and sex, age, BMI, the waist circumference and risk factors (including DM, HTN and obesity).

There was also no statistically significant correlation between the expression of TM in the skin in patients group and disease duration or disease severity by measured by PASI and BSA.

Discussion

Psoriasis is a chronic immune-mediated inflammatory disease (IMID) of the skin. [1] Although, its pathophysiology is

a complex process[1] and its exact cause is not yet fully known, [3] [4] Persistent inflammation is considered the most important event in psoriasis and causes uncontrolled keratinocyte proliferation and dysfunctional differentiation. [11] It may be due to interaction between environmental and genetic factors that cause defects in the innate and adaptive immune responses which in turn are responsible for the development and maintenance of psoriatic inflammation. [12]

Inflammation is a complex pathological process that is caused by different mediators secreted from inflammatory cells. Coagulation cascade is one of the systems that have a role in the inflammatory events. [13]

TM and Protein C are one of natural anticoagulant systems. In addition to their role in hemostasis, TM has been shown to mediate anti-inflammatory activities using activated protein C (APC) dependent and APC-independent mechanisms. [13]

On the other hand, some studies recognized TM as a cofactor in different autoimmune inflammatory diseases in previous studies. [7][8]

No much data are available evaluating TM expression in psoriasis. According to our knowledge, only one study evaluated the role of TM in psoriasis. Based on these facts, we aimed to assess the level of thrombomodulin in psoriasis through its immunohistochemical (IHC) expression in involved skin in psoriatic cases compared to healthy controls.

In the current study, we found that patients group had a statistically significant higher expression of TM in both epidermis and dermis than in controls.

In contrast with current study, ***Gębska et al.,*** study, [14] found a significantly lower TM expression in the epidermis of the psoriatic plaques than the normal skin sections. while, they did not find any significant differences in microvascular TM expression between the psoriasis and the control (NS).

Gębska et al., [14] explained that possible decrease of TM was due to tumor necrosis factor (TNF) which has a central role in pathogenesis of psoriasis and can cause a reduction in expression of functional TM on cell surfaces. [15]

Gębska et al., [14] results are in accordance with the previous study by ***Osorio and Sousa,*** [16] who hypothesized that TM as a cofactor for thrombin was decreased in the chronic inflammatory conditions including psoriasis due to proinflammatory cytokines (such as TNF) which are responsible for the down-regulation of TM expression.

In disagreement with previous studies, [[14] [16] ***Van der Poll et al.,*** [17] showed that TNF caused reduction of thrombomodulin activity and gene expression in endothelial cell cultures but did not affect thrombomodulin expression keratinocytes.

Gębska et al., [14] also postulated that possible decrease of TM was accompanied by induction of tissue factor (TF) on the skin capillary microvessels and this may lead to local procoagulability in patients with psoriasis.

Marzano et al., [18] study contradicted ***Gębska et al.,*** [14] regarding TF expression in psoriasis, who did not observe TF expression in lesional skin of patients with psoriasis vulgaris, despite its extensive clinical presentation and high PASI index, nor in the healthy skin specimens.

This discrepancy among the studies can be explained by different sample size and methodological pitfalls including, the use of paraffin or cryostat sections.

Our results showed a higher expression of TM in both epidermis and dermis in the skin of patients group compared to controls with a highly statistically significant difference between the studied groups, P-value< 0.01.

Our results are in accordance with the previous study by ***Yehia et al.,*** [7] who evaluated and investigated a soluble form of TM (sTM) as a parameter of disease activity in children and adolescents with systemic lupus erythematosus (SLE) and juvenile idiopathic arthritis (JIA). ***Hanyu et al.,*** [8] also showed increase of sTM level in the urine and joint fluid from the patients with rheumatoid arthritis.

Our results could be explained by increased vascular endothelial growth factor (VEGF) in psoraisis that transcriptionally upregulate expression of TM or its mRNA. [13]

VEGF has an important role in the induction of angiogenesis of psoriasis. The psoriatic patients have high serum levels of VEGF correlated with the severity of disease. [19] Moreover, vascular endothelial growth factor receptors (VEGFRs) were strongly expressed in non-lesional, perilesional, and lesional psoriatic epidermis in vivo. [20]

Pruritus affects a bout 70–90% of psoriatic patients. The pathogenesis of pruritus in psoriasis is still not fully known. [21] Although, the role of histamin in psoriasis is in a controversy, ***Harvima et al.*** [22] found that the number of mast cells were

increased in the upper dermis and in epidermis of psoriasis while ***Schubert and Christophers*** found that degranulated mast cells are found in the psoriatic skin at the very early stages. [23] Using microdialysis technique, histamine level, in the lesional psoriatic skin, was found to be increased compared to nonlesional psoriatic skin and the normal skin in the controls. [24]

The previous studies, regarding the role of histamine in psoriasis, could support our results , According to ***Conway,*** [13] who indicated that histamine can upregulate TM activity resulting from increased TM mRNA levels.

Moreover, Psychological or emotional stress is one of the main triggers for the psoriasis. [25] Stress also reported to cause upregulation of TM, which may be important to protect the vasculature and underlying tissue during inflammation and ischemia–reperfusion. [13]

Trauma, whether it is physical, chemical, infective, electrical, surgical, or even inflammatory, all can trigger the psoriasis (Koebner phenomenon). [26] The RNA released from damaged tissue may be one of the important factors in the upregulation of TM, [27] and it was regulated by RNA/Toll-like receptor 3 signaling pathways. [28]

Angiogenesis is both an important event in psoriasis and an important key in its pathogenesis. The mechanisms responsible for it are complex and affected by interaction between the secretion by keratinocytes, leukocytes and possibly even neurons. [29]

Interestingly, ***Shi et al.,*** [30] found that the domins of TM 2 and 3 (TMD23) can induce both angiogenesis and vasculogenesis. Depending on this fact studies suggested the TM ectodomain appears to be an autocrine/paracrine factor in wound healing as it was increased during the early phase of wound healing. [27]

In the current study, no significant correlation was found between the expression of TM in skin in patients and controls either in the epidermis or dermis and sex, age, BMI, the waist circumference and risk factors (including DM, HTN and obesity). We also found no significant correlation between the expression of TM in skin in patients and disease duration or severity of disease measured by PASI and BSA. However,

further studies are recommended to confirm or refused these findings.

Collectively, these findings might provide a new sight of psoriasis pathogenesis in which TM appears to be implicated but its specific function remains unclear. Therefore, further studies are required to indicate the role of the TM in the pathogenesis of psoriasis and resolve the controversy about TM expression in the disease. TM also could be potential target for a therapeutic approach in patients with psoriasis in the future.

Conclusion: TM in psoriatic skin might be implicated in the pathogenesis of psoriasis and could be a good future target for a therapeutic approach in patients with psoriasis.

Limitation

- No much data are available evaluating TM expression in psoriasis. This makes our results difficult to clearly explained.
- Different techniques including, the use of paraffin or cryostat sections.
- Small sample size was another limitation of our study

Conflict of interest

There is no direct or indirect conflict of interest.

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Table (1): Descriptive data of the studied groups:

		Control group	Patients group	Test value	P-value	Sig.
		No. = 30	No. = 30			
Age	Mean ± SD	42.20 ± 13.63	46.07 ± 15.02	-1.044 •	0.301	NS
	Range	21 – 65	19 – 70			
Sex	Female	16 (53.3%)	11 (36.7%)	1.684 *	0.194	NS
	Male	14 (46.7%)	19 (63.3%)			
DM	No	28 (93.3%)	25 (83.3%)	1.456 *	0.228	NS
	Yes	2 (6.7%)	5 (16.7%)			
HTN	No	20 (66.7%)	23 (76.7%)	0.739 *	0.390	NS
	Yes	10 (33.3%)	7 (23.3%)			
BMI	Mean ± SD	30.09 ± 4.97	28.86 ± 5.98	0.862 •	0.392	NS
	Range	23.2 – 42.2	16.99 – 44.9			
Obesity	Normal	5 (16.7%)	8 (26.7%)	2.492 *	0.477	NS
	Under weight	0 (0.0%)	1 (3.3%)			
	Over weight	12 (40.0%)	8 (26.7%)			
	Obese	13 (43.3%)	13 (43.3%)			
Waist circumference	Mean ± SD	100.6010.28	101.00 ± 15.18	-0.119 •	0.905	NS
	Range	80 – 122	75 – 132			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

*:Chi-square test; •: Independent t-test