Immunohistochemical Study of Survivin in Psoriasis

Fatma A. Abd Rabou[1], Amal A. El- Ashmawy[*1] and Maha M. Shamloula[2]

Departments of [1]Dermatology and Venereology, and [2]Pathology, Faculty of Medicine, Tanta University
amged1963@yahoo.com gheidas@yahoo.com

Abstract: Background: Psoriasis (Ps) is one of the most frequent skin diseases world-wide, with severe impact on the quality of the patient's life. Genetics and environmental factors may play an important role in the pathogenesis of the disease. Suppression of apoptosis is generally one of the accepted pathogenetic mechanisms of Ps and any epidermal hyperproliferative states. Survivin is a unique member of the inhibitor of apoptosis (IAP) family proteins, it mediates its apoptosis suppressive function by the inhibition of caspase pathway. It is a bifunctional protein that functions as a key regulator of mitosis and inhibitor of programmed cell death. Aim of the Work: The aim of this study was to explore the role could be played by survivin in the pathogenesis of Ps. Patients and Methods: 10 cases of plaque Ps, 10 cases of erythrodermic Ps, (in both types lesional, prelesional skin were taken), and10 control specimens from normal skin were studied by immunohistochemical method for expression of survivin. Results: Survivin was detected in psoriatic lesions both plaque and erythrodermic distributed in epidermis, endothelium, and inflammatory cells with different levels. Also it was detected in the prelesional skin in both plaque and erythrodermic Ps, also in the epidermis, endothelium, inflammatory cells, but with lesser ratios, and focal expression. In the control specimens, survivin was confined to basal layer of epidermis, and significantly up regulated in Ps in comparison with the prelesional, and the control skin, but there was no significant difference between different types of Ps.

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1. Introduction

Psoriasis is a chronic disease that affects 2–3% of the world's population [(1)] . It is characterized by keratinocyte (KC) hyperproliferation and abnormal differentiation, in addition to, both dermal and epidermal inflammatory cellular infiltrate and capillaries alteration [(2)] . The growth of KC is regulated by a delicate balance between molecules that control cell survival and cell death [(3)] . Thus, the thickness of human epidermis remains relatively constant throughout life. This regulation is disturbed in KC hyperproliferation as in Ps with the net result of an increase in the volume of cell mass [(3)]. Keratinocytic apoptosis plays a fundamental part in the control of epidermal morphogenesis and homeostasis [(4)]. Suppression of apoptosis has been proposed as a mechanism responsible for epidermal thickness in diseases like Ps [(4)]. Besides B-cell lymphoma 2 (BCL-2) family of apoptosis suppressors, another gene family of IAP has been identified [(5)]. Survivin is one of the IAP that have been discovered, it is a bifunctional protein that regulates cell division and suppresses apoptosis [(6)]. This function is mediated by the inhibition of the caspase pathway [(7)]. Survivin was abundantly expressed in fetal tissues but completely down regulated in most normal terminally differentiated adult tissues, in addition to its up regulation in most human cancers[(8)].

The aim of this work was to study the possible involvement of survivin in the pathomechanism of different types of Ps.

2. Patients and Methods

The study was conducted on 20 patients with 2 different clinical varieties of Ps; plaque and erythrodermic, 10 normal persons served as controls. They were recruited from the Out-Patient Clinic of Dermatology and Venereology Department, Tanta University Hospitals during the period from December 2009 to December 2010.The studied persons were subjected to: Detailed history taking including age , gender , duration of the disease , family history, any associated skin disease, and any previous treatment. The studied persons were divided into the following groups: GI: - included 10 patients with chronic plaque P, GII included 10 patients with erythrodermic Ps and GIII:-included 10 healthy persons as a (control group).

Skin biopsy:

After informed written consent, punch biopsies of 4 mm were taken after sterilization , under local anaesthesia from the lesional, and prelesional biopsies were taken 2 cm away from the lesion, and the normal skin biopsies were obtained during plastic operations, then they were prepared for :-

* Routine hematoxylin and eosin (H&E) staining to be studied by ordinary light microscope to assess the general histopathological picture of Ps.
* Tissue sections were done on adhesive positively charged glass slides, and were prepared for immunohistochemical staining using rabbit anti-human polyclonal anti-survivin that shows positive cytoplasmic staining in all skin layers.
*Skin biopsies from controls to determine immunohistochemical expression of survivin, and to compare the results with that obtained from psoriatic patients.

Evaluation of immunostaining: [7]

- Any number of true cytoplasmic positively stained cells is considered positive.
- Diffuse pattern: staining is considered when the whole layers of epidermis are positively stained.
- Basal pattern: staining confined to basal layer of epidermis.
- Focal pattern: staining that is not confined to the basal layer or diffusely stains the whole epidermal layers.

3. Results

Clinical results:

The mean age (year) of the studied groups were 37.400 ± 17.443 in GI, 49.900 ± 10.311 in GII, and 31.800 ± 9.390 in GIII. The difference between the studied groups revealed a significant changes (P=0.013*) and there was no significant differences between patients ≤ 40 years and patients > 40 years were seen .The male to female ratio was 43.4: 56.6. Positive family history was seen in (60%) in GI, and (30%) in GII. The mean duration (months) of the psoriatic groups was 52.700 ± 29.265, 24.100 ± 8.900 months in GI, GII respectively. Comparison between the duration of the disease in the studied groups revealed significant differences between GI, GII (P= 0.008*)

Histopathological results (H &E): As seen from **Fig (1 A &B) and Fig (2 A&B)**.

Immunohistochemical results:

- The expression of survivin in the psoriatic groups were illustrated from ***Fig (3-8***)
- Survivin was expressed only in the basal layer of the epidermis in normal skin.
- Lesional, prelesional psoriatic tissue of both GI, GII expressed survivin in variable levels including (epidermal, endothelial, inflammatory cells) Tables 1 , 2 and 3. Comparison between lesional survivin expression in both GI, GII, and the control group revealed significant changes in case of endothelial (P < 0.001*) and inflammatory cell expression(P < 0.001*), the same result was seen in case of prelesional survivin expression in GI and GII when compared with the control group(P =0.0390*) and (P =0.0392*) .
- Comparison between survivin expression in lesional, and prelesional skin of GI and the control group revealed significant difference in case of endothelial(P = 0.006*) and inflammatory cells (P < 0.020*) expression **(**Tables 5,6). The same result was seen when comparing the lesional, prelesional expression between GII and the control group (P < 0.001*) (Tables 5,6).
- Comparing of survivin expression between lesional and prelesional psoriatic skin revealed no significant differences between different two clinical varieties of Ps.
- Correlation between the expression of survivin and the concerned clinical parameters revealed no significant changes except in case of gender and distribution of the disease.
- Survivin also was expressed in the hair follicles, sweat glands of plaque, erythrodermic skin (lesional, prelesional) but was not expressed in the control group skin **Fig (7).**

Table (1): Survivin expression in the epidermis of lesional, prelesional psoriatic groups and controls

		Epidermal expression					
		Lesional			Prelesional		
		G I	G II	G III	G I	G II	G III
Negative	N	1	0	0	2	1	0
	%	10.00	0.00	0.00	20.00	10.00	0.00
Positive	N	9	10	10	8	9	10
	%	90.00	100.00	100.00	80.00	90.00	100.00
Chi-square	X^2	2.096			2.222		
	P-value	0.355			0.329		

Table (2): Survivin expression in the endothelium of lesional, prelesional psoriatic groups and controls

		Endothelial expression					
		Lesional			Pre lesional		
		G I	GII	GIII	G I	G II	G III
Negative	N	3	1	10	7	5	10
	%	30.00	10.00	100.00	70.00	50.00	100.00
Positive	N	7	9	0	3	5	0
	%	70.00	90.00	0.00	30.00	50.00	0.00
Chi-square	X^2	17.946			6.477		
	P-value	<0.001*			0.039*		

Table (3): Survivin expression in the inflammatory cells of lesional, prelesional psoriatic groups and controls

		Inflammatory cell expression					
		Lesional			Pre lesional		
		G I	GII	G III	G I	G II	G III
Negative	N	1	8	10	7	5	10
	%	10.00	80.00	100.00	70.00	50.00	100.00
Positive	N	9	2	0	3	5	0
	%	90.00	20.00	0.00	30.00	50.00	0.00
Chi-square	X^2	19.234			6.477		
	P-value	<0.001*			0.0392*		

Table (4): Epidermal expression of survivin in lesional, prelesional skin in GI, GII compared to the control group.

		Epidermal expression				
		G I		G II		GIII
		L	PL	L	PL	
Negative	N	1	2	0	1	0
	%	10.00	20.00	0.00	10.00	0.00
Positive	N	9	8	10	9	10
	%	90.00	80.00	100.00	90.00	100.00
Chi-square	X^2	1.667		0.517		
	P-value	0.197		0.472		

PL= prelesional •L= lesional

Table (5): Endothelial expression of survivin in lesional, prelesional skin in GI, GII compared to control group.

		Endothelial expression				
		G I		G II		G III
		L	PL	L	PL	
Negative	N	3	7	1	5	10
	%	30.00	70.00	10.00	50.00	100.00
Positive	N	7	3	9	5	0
	%	70.00	30.00	90.00	50.00	0.00
Chi-square	X^2	7.500		13.125		
	P-value	0.006*		<0.001*		

PL= prelesional •L= lesional

Table (6): Inflammatory cell expression of survivin in lesional, prelesional skin in GI, GII compared to the control group .

		Inflammatory cell expression				
		G I		G II		G III
		L	PL	L	PL	
Negative	N	1	7	8	5	10
	%	10.00	70.00	80.00	50.00	100.00
Positive	N	9	3	2	5	0
	%	90.00	30.00	20.00	50.00	0.00
Chi-square	X^2	5.455		11.471		
	P-value	0.020*		<0.001*		

PL= prelesional •L= lesional

4. Discussion

Psoriasis is a chronic, hyperproliferative, systemic, inflammatory disease of the skin and joints [9]. It is characterized by erythematous, and scaly plaques [10]. Ps has a major impact on health related quality of life; that is comparable to other major medical diseases such as cancer, arthritis, hypertension, heart disease, diabetes, depression and excess mortality in severe cases [11].Although altered T-cell activity is certainly involved in eliciting and maintaining inflammation, advances in molecular medicine have also suggested possible homeostatic defects in signaling pathways in KCs in Ps [12],[13].

Apoptosis has become increasingly recognized as a key mechanism involved in the maintenance of tissue homeostasis, growth and development [14]. Keratinocytic apoptosis plays a fundamental part in the control of epidermal morphogenesis and homeostasis [7]. In Ps, keratinocytic differentiation and proliferation could be modulated and regulated by many cytokines, transcription factors and inflammatory mediators released from chronic inflammatory cells which accompany this lesion. Suppression of apoptosis has been proposed as a mechanism responsible for epidermal thickness in diseases like Ps[7]. Survivin, a member of the IAP family, has unique properties and it is the only IAP involved in mitosis and cell cycle progression [15]. Recent studies have defined a role for survivin in many different cell types including endothelial cells (ECs), T cells, neutrophils, fibroblasts, epithelial cells and melanocytes[16]. Interestingly, human skin seems to be the only normal adult tissue expressing survivin [17]. Human epidermis is characterized by a constant turnover of cells based on the presence of a population of stem cells in the basal layer, that retains a high capacity of self-renewal throughout life. One could speculate that survivin, similarly to BCL -2 protects the cell viability [7]. Survivin is responsible for inhibition of the intrinsic pathway of apoptosis by blocking both mitochondrial pathway and the death receptor pathway, by directly inhibiting terminal effector caspase-3,7 and interact with SMAC / DIABLO proteins that activate caspase-9, so it interacts with these proteins in order to antagonize their proapoptotic properties [15].

In the current study, expression of the apoptosis inhibitor survivin was studied in normal and psoriatic skin. The expression of survivin in normal adult skin was observed as a cytoplasmic staining in the epidermal basal layer of normal human skin, the intensity ranging from faint to moderate (focally) ; according to Chiodino ***et al.,*** [18] . No Survivin immunoreactivity was detected in the suprabasal layers of the normal skin [19]. Epithelial cells of the adenxia (sebaceous and sweat glands, follicular outer root sheath) were also stained [18], in contrast to the current study which showed that survivin expression of normal skin was restricted to the basal epidermal layer . Immunohistochemical staining of the current study revealed that cytoplasmic survivin expression was significantly higher in lesional psoriatic tissue compared with controls. It worth noting that , the expression of survivin is significantly higher in prelesional psoriatic skin than the controls .The current study go with previous studies who mentioned that; an increased level of survivin in psoriatic tissue is an established phenomenon such as the study of Bowen *et al.,* 2004; where the positive cases for survivin were (88%) of the cases [20] , the study of Abdou & Hanout, 2008; where the positive cases were (73 %) showed the cytoplasmic positivity for survivin [7] and according to the study of Markham *et al.,* 2006 ,which showed that ; survivin was strongly expressed in the dermal perivascular regions and the basal layer of the epidermis of involved skin [21] . In the current study, expression of survivin in lesional psoriatic skin was detected in the epidermis of 95% of patients , distributed diffusely in the (epidermis, endothelium , and inflammatory cells in) without being restricted to a certain layer of them , some lesions showed a survivin expression in the hair follicles, sweat glands which was not detected in the normal skin opposite to what was mentioned in Chiodino, ***et al.*** [18] and Abdou & Hanout [7]**.** Survivin expression was often restricted to the upper third of the epidermis in Ps as mentioned in Bowen ***et al.*** [20], Raj ***et al.*** [22]**.** In the contrast , the current study showed that survivin expression in most of the lesional psoriatic skin was in the lower third of the epidermis.

The prelesional survivin expression was also detected in 85% of patients ; 60% from them were focally distributed , 40% were diffusely distributed, that was opposite to what was mentioned in the study of Abdou & Hanout 2008,as the prelesional survivin expression in that study was restricted only to the basal epidermal layer .Several studies have demonstrated that angiogenic cytokines, vascular endothelial growth factor (VEGF) and angiopoietins modulate survivin expression in ECs, disruption of which results in dysregulation of EC integrity and survival [15], demonstrated that by resistance mediated by VEGF and represents a novel therapeutic target in angiogenic processes[23]. In the study of O'Connor ***et al.*** **2000** , survivin was massively up regulated in the newly formed blood vessels of granulation tissue *in vivo*[24]., while it is minimally detected in non-proliferating capillaries of normal skin and in the study of Markham, et al.

2006 **,** survivin was strongly expressed in the dermal perivascular regions [(21)], the current study coincided with the previous study as survivin was detected also around the blood vessels. In the current study no significant differences could be found in survivin expression between the two studied clinical varieties of Ps; plaque and erythrodermic, also there was no significant correlation between the expression of survivin and the concerned clinical parameters in lesional and prelesional psoriatic skin except for gender and distribution of the disease; perhaps, this may be due to relatively small numbers of the studied cases . However, no available references in the literature deal with these variables. However, in this study over expression of survivin in psoriatic lesions in comparison with normal and prelesional skin was evident, suggesting its important role in the pathogenesis of Ps.

Conclusion:

- Keratinocytic apoptosis plays a fundamental part in the control of epidermal morphogenesis and homeostasis and suppression of apoptosis has been proposed as a mechanism responsible for epidermal thickness in diseases like Ps.
- Survivin is detected postnatally in normal skin.
- Lesional, and prelesional psoriatic skin of both plaque and erythrodermic Ps expressed high levels of survivin when compared with normal skin suggesting its role in the pathogenesis of Ps.
- Expression of survivin in prelesional psoriatic skin shows focal positivity in more than half of the cases both plaque and erythrodermic, when compared with the lesional skin as the expression was diffuse in all cases, that may need further studies to elucidate its significance.
- No significant difference was detected in survivin expression between plaque and erythrodermic Ps, which means that apoptosis suppression affects Ps as a disease whatever its clinical type.
- Over expression of survivin in Ps denoting its important role in the apoptosis suppression as a possible mechanism in the pathogenesis of Ps besides the hyperproliferative mechanism.
- The anti-apoptotic role of survivin not only came from its expression in epidermis but also in endothelial cells of the proliferating capillaries of the papillary dermis. Further genetic studies will be needed to confirm these findings and get benefit from survivin targeted therapies in the treatment of Ps.

Recommendation:

Study of survivin expression in Ps may open a new knowledge about the pathogenesis and the treatment of Ps, so the current study recommends further studies for survivin expression in different types of Ps, on large population scale to elucidate its possible role.

Corresponding author
Amal Ahmad El-Ashmawy,
Dermatology and Venereology Department, Tanta University, Egypt
amged1963@yahoo.com gheidas@yahoo.com

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