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Original Article

Pathology Section

Immunohistochemical Evaluation of Role of Serotonin in Pathogenesis of Psoriasis

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ABSTRACT

Introduction: Psoriasis is a common skin disorder characterized by erythaematosquamous papules and plaques. It is known to be associated with stressful and depressive disorders. Serotonin is a neurotransmitter that plays a role in the pathogenesis of inflammatory skin disorders.

Aim: To evaluate the role of serotonin in pathogenesis of psoriasis.

Materials and Methods: Using standard immunohistochemical techniques, 24 biopsies from patients with chronic plaque psoriasis were examined together with 12 biopsies from age and gender-matched healthy subjects as a control group.

Results: Both the percentage of positive cells (p=0.018) and H-score values (p=0.015) of serotonin expression were significantly higher in psoriasis compared to normal skin. H score of serotonin expression was significantly higher in cases with totally absent Granular Cell Layer (GCL) as opposed to those with thin/focally absent GCL (p=0.011), and in cases with moderate/strong epidermal inflammation compared to cases with mild inflammation (p=0.035). No significant correlation was detected between H score of cases and age, disease duration or Psoriasis Area and Severity Index (PASI) score.

Conclusion: Serotonin might play a role in development of psoriasis through its role as a growth factor promoting keratinocyte proliferation, and as mediator of inflammation and stimulant of T cell activation. It recruits T cells to sites of cutaneous inflammation and potentiate macrophage accessory function for T cell activation. Its expression is not related to the disease severity. Future large-scaled research on population of different ethnicities including other disease variants is needed. The use of serotonin receptor antagonists and serotonin reuptake inhibitors may be evaluated on wide-based studies to put the current observation into action.

Keywords: Erythrosquamous skin disorders, Immunohistochemistry, Neurotransmitter, Psychocutaneous disorders

INTRODUCTION

Psoriasis is a common chronic, immunologically-mediated, relapsing papulosquamous dermatosis, characterized by abnormal proliferation and altered maturation of epidermal keratinocytes. Patients with psoriasis present typically with chronic erythaematous papules and plaques that are sharply demarcated and covered by silvery white scales, that appear commonly on the elbows, knees and scalp [1].

There is a crosstalk between both neuroendocrine and immune systems especially described in dermatologic diseases [2]. The relationship between psoriasis and different psychological disorders was addressed in a number of studies. Psoriasis is believed to be related to stress and mood disorders [3,4].

Serotonin{5-Hydroxytryptamine (5-HT)} is a neurotransmitter, that plays an important role in stress. In addition, stress-related hormones lead to increased 5-HT synthesis. It is a vasoactive amine, which is stored in the blood by platelets and released at sites of inflammation [5].

Serotonin expression was reported previously to be related to inflammatory dermatologic diseases. It was found to be significantly higher in patients with eczema than in normal controls [6]. It was also found to be increased in epidermis and inflammatory cells in cases of contact allergy [7]. Rasul et al., have found that some serotonergic markers are more expressed in atopic dermatitis more than controls. They have also found this expression to be significantly related to extent of disease, anxiety traits, and to depression [8].

This study aimed to address the role of serotonin in pathogenesis of psoriasis by assessing its Immunohistochemical (IHC) expression in skin biopsies of this disease in comparison to normal skin, and correlating it with clinicopathologic features of studied cases.

MATERIALS AND METHODS

This prospective study was carried out on 24 patients who presented with chronic plaque psoriasis. Cases were selected from Dermatology outpatient clinic, Menoufia University Hospital spanning the period between January 2010 and December 2011. All selected cases had stable disease and had no history of systemic therapy or phototherapy. For those on topical therapy, treatment was stopped for at least 4 weeks before taking biopsy. Twelve additional biopsies were obtained from age, and gender-matched apparently healthy individuals without past or family history of psoriasis during plastic surgery to be used as a control group. All selected cases and control subjects had sedentary life style. A written informed consent was obtained from both patients and control groups after the procedure had been fully explained. This was also in accordance with the Helsinki Declaration of 1975 (revised in 2000).

Clinical data describing patients' demographics (age and gender) as well as the clinical variables (disease duration, nail involvement and scalp involvement) were all documented. The severity of the disease was assessed by the Psoriasis Area and Severity Index (PASI) score [9]. Dermatologic diseases other than psoriasis and the presence of other acute or chronic systemic or cutaneous inflammatory and/or autoimmune diseases were excluded. Patients with known history of psychiatric disorders or those under antidepressant therapy were also excluded.

Skin biopsies were taken under 2% lignocaine local anaesthesia. Biopsies from cases and control subjects were site-matched (taken from sun-protected areas). Formalin 10% was used for fixation of specimens, followed by dehydration using ascending grades of ethanol. Specimens were immersed in xylene before impregnation in paraffin. Several 5μm thick sections from each block were taken.

One slide was stained by Haematoxylin and Eosin (H&E) for routine histopathological examination. Other sections were mounted on positively charged slides and stored at room temperature for IHC staining.

Histopathological Examination

H&E-stained sections were examined microscopically to confirm the diagnosis and to evaluate variable epidermal and dermal changes. Evaluated epidermal changes included degree of acanthosis, parakeratosis, Granular Cell Layer (GCL) attenuation/ loss, epidermal inflammation and microabcesses formation. Dermal changes included degree of dermal inflammation and dilated/ tortuous blood vessels.

IHC Staining of Serotonin

5 µm thick sections, were cut from the paraffin-embedded blocks with subsequent steps of deparaffinization and rehydration in xylene and graded series of alcohol, respectively. Antigen retrieval was performed by boiling in 10 ml citrate buffer (pH 6.0) for 20 min, followed by cooling at room temperature. The slides were incubated overnight at room temperature with: Mouse Monoclonal Antibody raised against serotonin (clone 5HT-H209). The antibody was provided by Thermo Fisher Scientific Anatomical Pathology (Tudor Road, Manor Park, Runcorn, Cheshire WA71 TA, UK) in a single vial containing 1 ml of antibody (Cat. No. #MS-1431-S0) that was diluted by using Lab Vision Antibody Diluent (Cat. No. #MS-1431-S0).

Endogenous peroxidase activity inhibition (hydrogen peroxidase for 15 min) was done followed by microwave antigen retrieval (20 min; 10 mmol/citrate buffer, pH 6.0). Overnight, at room temperature, incubation of the slides with primary antibody applied to them was done in humidity chambers. Afterwards, incubation with the secondary antibody for 15 minutes was done preceded and followed by Phosphate Buffer Saline (PBS) wash. The last step was the antibody detection using a modified Labeled Avidin–Biotin (LAB) reagent (20minutes), followed by PBS wash, then diaminobenzidine (DAB) was used as a chromogen (0.1% solution, 5minutes). Mayer's haematoxylin (5–10min) was used for counterstaining.

Interpretation of IHC-Stained Slides

Assessment of serotonin staining was done in the available specimens in epidermis (keratinocytes) and dermis (inflammatory cells, adnexa, and blood vessels). Any number of cells with cytoplasmic immunoreactivity for serotonin was considered positive. Positive cases were further categorized into mild (faint staining), moderate (chestnut brown), and strongly positive (deep brown). Assessment of epidermal serotonin staining localization was done as well and cases were categorized accordingly into suprabasal staining, basal staining or both.

Semiquantitative Histo-score (H-score) was calculated in the epidermis also combining both the intensity and the percentage of imunoreactivity using the formula: H-score = Σ (intensity of staining X percentage of stained area), where 0; negative, 1; mild, 2; moderate, and 3; strong staining intensity. The H-score ranged between 0-300 [10]. The percentage of positive cells was calculated at 100X magnification [11] H-score assessment was done to have more accurate assessment, to avoid type I and type II statistical errors.

STATISTICAL ANALYSIS

Data were collected, tabulated, and statistically analyzed using a personal computer with SPSS version 22 program (Armonk, NY: IBM corp.2013). Values were expressed in number, percentage, mean ± standard deviation (X±SD) when appropriate. Chi-square test was used to compare qualitative data. Yate's correction for continuity was applied whenever indicated for small numbers. Mann-Whitney *U* and Kruskal-Wallis tests were used in comparing quantitative variables since the data were not normally distributed. Spearman's correlation was used to measure the association between two quantitative variables. Differences were considered statistically significant with $p< 0.05$.

RESULTS

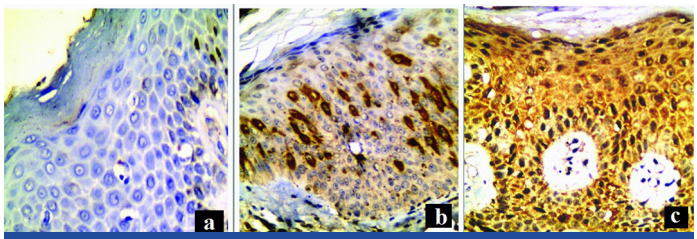
Clinical data: The study population included 9 (37.5%) females and 15 (62.5%) males. Age ranged from 18 to 56 years with mean ±SD age of 20.5±9.55 years. All cases had trunk lesions with involvement of extremities in 20 (83.3%) cases. Scaly scalp was detected in 7 (29.1%) cases and nail was affected in 8 (33.3%) cases. Disease duration ranged from 2 to 12 months with mean ±SD value of 5.3±1.9 months. PASI score ranged from 15 to 52 with mean ±SD value of 21.8±6.9. Control group included 5 (41.6%) females and 7 (58.4%) males. Their ages ranged from 15 to 52 years with mean±SD age of 21.4± 6.5 years.

Serotonin expression in psoriasis and control groups [Table/ Fig-1-4]: Serotonin expression in the epidermis of psoriatic skin was significantly higher than that of control skin. The percentage of positive cells was 79.2% in psoriasis, compared to 33.3% of control cases (p=0.019). H-score was significantly higher in psoriatic epidermis (X±SD: 70.8±79.7), and it was significantly lower in normal skin (X±SD: 29.16±49.81). This result was statistically significant (p=0.015).

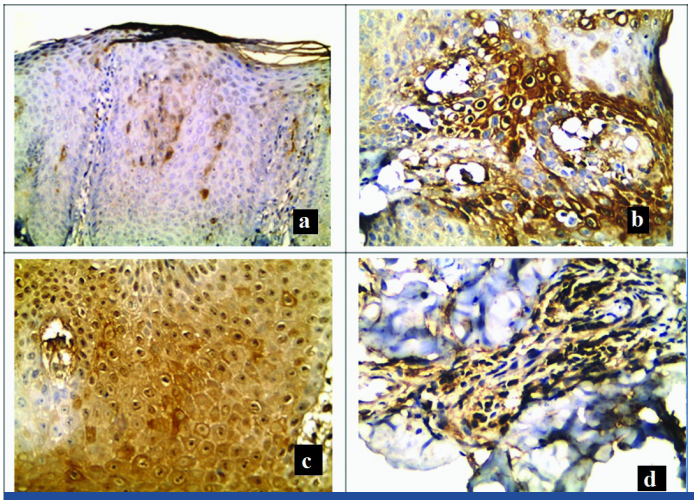
Serotonin expression in psoriasis in relation to clinicopathologic features: H score of serotonin expression was significantly higher in cases with totally absent GCL as opposed to those with thin/ focally absent GCL (p=0.011). This result was supported by the intensity of staining as well. Most cases with moderate-strong serotonin expression showed absence of GCL (p= 0.044). Higher H-score was also significantly associated with moderate/strong

Variable	Control subjects (12) No (%)	Psoriasis cases (24) No (%)	Test of significance and p-value
Epidermal Staining percentage			
Positive	4(33.3%)	19(79.2%)	$\chi^2 = 5.43$
Negative	8(66.7%)	5(20.8%)	p = 0.019*
H score in positive cells			
Range	0-150	0-300	U = 2.7
X±SD	29.16±49.81	70.8±79.7	p = 0.015*
Blood vessel staining			
Positive	4(33.3%)	8(33.3%)	$\chi^2 = 0.00$
Negative	8(66.7%)	16(66.7%)	p = 1.00
Adnexal staining			
Positive	4(33.3%)	6(25%)	$\chi^2 = 0.17$
Negative	8(66.7%)	18(75%)	p = 0.89
Inflammatory cells			
Positive	4(33.3%)	12(50%)	$\chi^2 = 0.35$
Negative	8(66.7%)	12 (50%)	p = 0.55

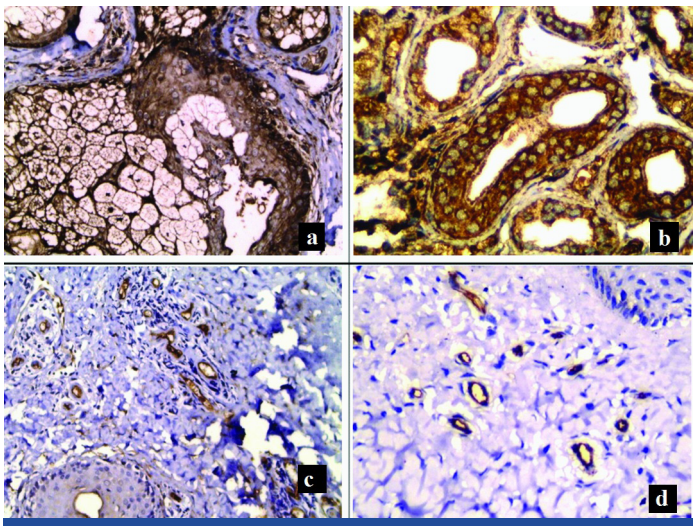
[Table/Fig-1]: Immunohistochemical staining of serotonin in studied groups. χ^2: chi square test" with yate's correction"; *U*: Mann Whitney test; X±SD: mean±Standard deviation; *: Significant



[Table/Fig-2]: Variable expression of serotonin in control skin: (a) Negative for serotonin (40 X); (b&c) Positive for cytoplasmic serotonin staining (10 X).



[Table/Fig-3]: Expression of serotonin in keratinocytes and inflammatory cells: (a&b) focal cytoplasmic staining of keratinocytes (10X & 40X respectively); (c) Diffuse cytoplasmic staining of serotonin in keratinocytes (40X); and (d) serotonin positivity in dermal inflammatory cells (40X).



[Table/Fig-4]: Expression of serotonin in sebaceous and sweat glands (a&b) (Original magnification 40X); and in blood vessels (c& d) [10X & 20X respectively].

epidermal inflammation compared to cases with mild inflammation (p=0.035). The localization of staining of serotonin showed no relation to any of the studied clinicopathologic variables. [Table/Fig-5,6]. No significant correlation was detected between H score of cases and age, disease duration or PASI score. [Table/Fig-7].

DISCUSSION

The current study aimed to address the role of serotonin in pathogenesis of psoriasis by investigating its immunohistochemical expression. Expression of serotonin was also studied in relation to clinicopathologic features of studied cases. In the present study, serotonin showed positive epidermal expression in about 33% of examined control specimens. Positive immunoreactivity was also detected in dermal blood vessels, adnexa, and inflammatory cells.

As demonstrated in the present study, serotonin epidermal expression was significantly higher in patients with psoriasis as opposed to controls, using both qualitative and H score methods of staining assessment. This finding may suggest the role of serotonin in pathogenesis of psoriasis. Huang et al., found that serotonin was not expressed at all in normal skin biopsies but was expressed in psoriasis, more in progressive stage of disease [12]. An earlier study showed that serotonin was expressed in 2 out of 10 psoriasis skin biopsies, and weakly in 1 out of 10 normal skin biopsy specimens [13].

Psoriasis is an immunologically mediated disease that is characterized by keratinocyte proliferation and altered epidermal

Variable	Serotonin staining intensity				Test of significance and p-value
	Negative	Mild	Moderate	Strong	
Age (ys)					
X±SD	40±14.08	37.2±12.39	35.3±12.45	36.5±12.2	K= 0.4
Median	39	35	35	36	p= 0.93
Mean rank	13.8	13.3	11.95	11.25	
Gender					
Male	4(80%)	3(60%)	5(50%)	3(75%)	χ^2 = 1.6
Female	1(20%)	2(40%)	5(50%)	1(25%)	p= 0.65
Duration (months)					
X±SD	4± 2.64	5.2± 2.8	5.1± 4.4	4.5± 3.38	K= 1.6
Median	3.66	4.5	3.66	3	p= 0.6
Mean rank	12.1	15.4	12.5	4.38	
PASI score					
X±SD	83.33±5.7	35.4±12.8	32±12.9	31.2±7.5	K= 1.5
Median	38	35	30	31.66	p= 0.6
Mean rank	15.5	13.4	11.2	10.88	
Nail affection					
Present	4(80%)	0(0%)	3(30%)	1(25%)	χ^2 = 7.75
Absent	1(20%)	5(100%)	7(70%)	3(75%)	p= 0.055*
Scaly scalp					
Present	1(20%)	2(40%)	3(30%)	1(25%)	χ^2 = 0.52
Absent	4(80%)	3(60%)	7(70%)	3(75%)	p= 0.91
Parakeratosis					
Present	2(40%)	2(40%)	5(50%)	2(50%)	χ^2 = 0.23
Absent	3(60%)	3(60%)	5(50%)	2(50%)	p= 0.97
GCL					
Thin/focally absent	4(80%)	4(80%)	2(20%)	3(75%)	χ^2 = 8.9
Totally absent	1(20%)	1(20%)	8(80%)	1(25%)	p= 0.044*
Acanthosis					
Mild/sparse	2(40%)	4(80%)	8(80%)	2(50%)	χ^2 = 3.3
Marked	3(60%)	1(20%)	2(20%)	2(50%)	p= 0.34
Epidermal inflammation					
Mild/sparse	3(60%)	3(60%)	1(10%)	2(50%)	χ^2 = 5.6
Marked	2(40%)	2(40%)	9(90%)	2(50%)	p= 0.13
Microabcesses					
Present	1(20%)	2(40%)	2(20%)	1(25%)	χ^2 = 0.8
Absent	4(80%)	3(60%)	8(80%)	3(75%)	p= 0.85
Dermal inflammation					
Mild/sparse	3(60%)	2(40%)	6(60%)	0(0%)	χ^2 = 4.64
Marked	2(40%)	3(60%)	4(40%)	4(100%)	p= 0.19
Dilated capillaries/tortous BVs					
Present	2(40%)	4(80%)	5(50%)	4(100%)	χ^2 = 4.8
Absent	3(60%)	1(20%)	5(50%)	0(0%)	p= 0.18

[Table/Fig-5]: Serotonin staining intensity in relation to clinicopathologic features of psoriasis cases.
χ^2: chi square test; K: Kruskal Wallis test; X±SD: mean±Standard deviation; GCL: granular cell layer.*: significant

cell maturation. Serotonin may be involved in the pathogenesis of psoriasis by stimulating the proliferation of keratinocytes. It plays a role in proliferation of keratinocytes and in regulation of epidermal cell turnover as well [14].

Serotonin mediates inflammation, and it is well known that cutaneous serotonin expression is altered by inflammatory conditions [15]. Nordlind et al., demonstrated expression of serotonin in both epithelial and adnexal structures in chronic eczema [16]. It is thought to play a role in modulation of skin inflammatory response [17]. Nagy et al., found that serotonin induces secretion

Variable	Serotonin staining localization				Test of significance and p-value
	Negative	Suprabasal	Basal	Both	
Age (yrs)					
X±SD	40± 14.09	38.8±10.3	48	33.46± 12.12	K= 2.51
Median	39	40	48	31	p= 0.47
Mean rank	13.8	14.4	20	10.69	
Gender					
Male	4(80%)	3(60%)	1(100%)	7(53.8%)	χ^2 = 1.68
Female	1(20%)	2(40%)	0(0%)	6(46.2%)	p= 0.64
Duration (months)					
X±SD	4±2.6	6.4±5.37	5	4±2.8	K= 0.87
Median	3	4	5	3	p= 0.83
Mean rank	12.1	14.4	20	10.69	
PASI score					
X±SD	38± 5.7	35± 12.7	52	30.4± 10.3	K= 5
Median	40	35	52	30	p= 0.172
Mean rank	15.5	13.1	24	10.23	
Nail affection					
Present	3(60%)	1(20%)	0(0%)	4(30.8%)	χ^2 = 2.53
Absent	2(40%)	4(80%)	1(100%)	9(69.2%)	p= 0.47
Scaly Scalp					
Present	1(20%)	2(40%)	0(0%)	4(30.8%)	χ^2 = 0.92
Absent	4(80%)	3(60%)	1(100%)	9(69.2%)	p= 0.82
Parakeratosis					
Present	2(40%)	2(40%)	1(100%)	6(46.2%)	χ^2 = 1.32
Absent	3(60%)	3(60%)	0(0%)	7(53.8%)	p= 0.72
GCL					
Thin/focally absent	4(80%)	1(20%)	1(100%)	7(53.8%)	χ^2 = 4.5
Totally absent	1(20%)	4(80%)	0(0%)	6(46.2%)	p= 0.21
Acanthosis					
Mild/sparse	2(40%)	5(100%)	0(0%)	9(69.2%)	χ^2 = 6.13
Marked	3(60%)	0(0%)	1(100%)	4(30.8%)	p= 0.11
Epidermal inflammation					
Mild/sparse	3(60%)	1(20%)	1(100%)	4(30.8%)	χ^2 = 3.65
Marked	2(40%)	4 (80%)	0(0%)	9(69.2%)	p= 0.3
Microabcesses					
Present	1(20%)	1(20%)	1(100%)	3(23.1%)	χ^2 = 3.15
Absent	4(80%)	4(80%)	0(0%)	10(76.9%)	p= 0.36
Dermal inflammation					
Mild/sparse	3(60%)	2(40%)	1(100%)	5(38.5%)	χ^2 = 1.9
Marked	2(40%)	3(60%)	0(0%)	8(61.5%)	p= 0.58
Dilated capillaries/tortous BVs					
Present	2(40%)	4(80%)	1(100%)	8(61.5%)	χ^2 = 2.3
Absent	3(60%)	1(20%)	0(0%)	5(38.5%)	p= 0.5

[Table/Fig-6]: Serotonin staining localization in relation to clinicopathologic features of psoriasis cases.
χ^2: chi square test; K: Kruskal Wallis test; X±SD: mean±standard deviation

Variable	X±SD	Mean rank	Test of significance and p-value
Gender			
Male	80± 81.59	11.63	U= 54.5
Female	112± 101.1	13.94	p= 0.43
Nail affection			
Present	75±103.59	10.62	U= 49
Absent	100.62± 82.5	13.44	p= 0.35
Scaly Scalp			
Present	108.57± 108.07	13.64	U= 51
Absent	85.29± 28.16	12.03	p= 0.62
Parakeratosis			
Present	72.72±55.69	11.86	U= 62.5
Absent	108.46±108.84	13.19	p= 0.61
GCL			
Thin/focally absent	58.46±80.29	9.15	U= 28
Totally absent	131.81±84.47	16.45	p= 0.011*
Acanthosis			
Mild/sparse	100±84.93	13.34	U= 50.5
Marked	76.25±99.7	10.81	p= 0.42
Epidermal inflammation			
Mild/sparse	57.78±94.31	8.61	U= 32.5
Marked	112.67±81.37	14.83	p= 0.035*
Microabcesses			
Present	150±122.47	14.42	U= 42.5
Absent	78.3±76.25	11.86	p= 0.45
Dermal inflammation			
Mild/sparse	86.36±80.9	12	U= 66
Marked	96.9±97.75	12.92	p= 0.78
Dilated capillaries/tortous BVs			
Present	94±91.32	12.9	U= 61.5
Absent	88.89±89.36	11.83	p= 0.7

[Table/Fig-7]: H-score of serotonin expression in relation to clinicopathologic features.
χ^2:: chi square test; U: Mann Whitney test; GCL:granular cell layer; BVs: Blood vessels; X±SD: mean±standard deviation; *: Significant

of IL-16 from CD8+ cells, which is an immunomodulatory and proinflammatory cytokine, and thought to play role in inflammatory skin disease as atopic dermatitis [18], a mechanism that needs to be investigated particularly in psoriasis. In addition, serotonin, via serotonin type 2 receptors, may promote the recruitment of CD4+ T lymphocytes into inflammatory focus. Therefore, it influences CD4+ T lymphocytes activity [19]. The importance of serotonin in macrophage accessory function for T-cell activation was also suggested previously [20]. This effect on T cells may explain the positive immunoreactivity of inflammatory cells, detected in the current work in psoriatic lesions.

The relationship between antidepressants and psoriasis was addressed previously by Young et al., who showed that the selective serotonin reuptake inhibitor antidepressants may be beneficial in the treatment of psoriasis [21]. This further enhances the suggested role of serotonin in the pathogenesis of psoriasis. More recently, it has been found that the use of serotonin reuptake inhibitors in patients with psoriasis is associated with a decreased need for systemic psoriasis treatment [22].

Psoriasis is associated with an increased risk of stressful and depressive disorders. It is associated with social stigmatization, discomfort, physical disability and emotional distress [23]. It is well known that stress and stress-related hormones lead to increased serotonin synthesis [5]. However, to the best of our knowledge, no studies have investigated the association between psychological stress and serotonin expression in this disease entity; this is an area that requires future clarification.

In the current work, serotonin was expressed in suprabasal cell layers in 26.3% of positive psoriatic lesions and in both basal and suprabasal layers in 68.4% of positive cases. It showed basal and suprabasal expression in all positive skin biopsies. The suprabasal immunoreactivity may provide evidence about its role in keratinocyte differentiation which adds to its known role in proliferation, an observation which was not previously recorded in similar studies. However, Nordlind et al., demonstrated positive 5-HT_{1A}R immunoreactivity to the upper part of the epidermis

suggesting the importance of this serotonin receptor for a normal differentiation process of the keratinocytes [16]. Further studies are needed for firmer conclusion.

Serotonin was expressed in adnexal structures, as well, in the present study, which did not show significant differences between normal and psoriatic skin. Huang et al., was able to detect staining for serotonin in adnexae from psoriatic skin and explained it by the fact that adnexae are derived from keratinocytes [12].

In the current study, semiquantitative H-score and staining intensity analysis showed that significantly higher expression of serotonin was found in psoriasis cases with absent GCL. H-score was also significantly higher in cases with moderate/marked epidermal inflammation. This finding may underscore its role in disease pathogenesis. A similar finding was reported previously [24]. The positive serotonin vascular immunoreactivity, noted in this group of cases, was not demonstrated before. Serotonin was reported to induce arteriolar vasoconstriction in the skin of hypertensive individuals [21].

Huang et al., showed positive expression of 5-HT2A receptor in the vessel walls in psoriatic dermis, which suggests a role of this receptor in the inflammatory process and inflammatory cell migration [12]. Whether this is applicable for serotonin or not, requires further molecular investigation.

In the present work, no significant association was found between serotonin intensity or H scoring values and PASI scores. Therefore, serotonin expression has no relation to the disease severity.

LIMITATION

The limitations of this study were that a small number of cases who were of the same ethnic background were included. Also, only one clinical type of psoriasis was studied. Future large-scaled research on population of different ethnicities including other disease variants is needed. The use of serotonin receptor antagonists and serotonin reuptake inhibitors may be evaluated on wide based studies to put the current observation into action.

CONCLUSION

We conclude that serotonin might play a role in development of psoriasis through its role as a growth factor promoting keratinocyte proliferation, and as mediator of inflammation and stimulant of T cell activation. It recruits T cells to sites of cutaneous inflammation and potentiate macrophage accessory function for T cell activation. Its expression is not related to the disease severity. Further investigation is warranted to prove or deny current observations.

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