Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

ISSN NO. 2320-5407

RESEARCH ARTICLE

CDCP1, A NOVEL CUTANEOUS MARKER, EXPRESSION IN PSORIASIS VULGARIS.

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Manuscript Info

Manuscript History

Received: 6 September2015
Final Accepted: 23 September 2015
Published Online: October 2015

Key words:-
Psoriasis vulgaris,CDCP1, immunohistochemistry, image analysis

Abstract

Background: Psoriasis is a common inflammatory skin disease**,** CDCP1 is a cell-surface integral glycoprotein**.** This study assesses the presence and distribution of CDCP1 in psoriasis vulgaris.

Materials and Methods: Thirty paraffin blocks from lesions of patients with psoriasis and twenty paraffin blocks as a control group were collected. Histopathological grading was performed for all cases.Immunohistochemical staining and image analysis was performed to detect CDCP1 expression.

Results: our cases ranged from 9 to 18 out of 19 total points of the used grading system. The intensity immunostaining of CDCP1 in all biopsies of the control group (n=20) was low (+1), while in cases group (n=30) was moderate (+2) in 8 cases (26.7%) and high (+3) in 22 cases (73.3%).There were a significant difference(P value <0.001) between cases and control group regarding to immunostaining of CDCP1, and a significant positive relation (P value < 0.001) between the histological grading score and immunostaining of CDCP1 and positive relation (p=0.002) between the duration of disease and the immunostaining ofCDCP1.

Conclusion: CDCP1 was overexpressed by keratinocytes in lesional psoriasis compared with non-lesionalskin and normal skin which points to a possible role of CDCP1 in the pathogenesis of psoriasis.

Introduction:-

Psoriasis is a common skin disease characterized by abnormal keratinocyte proliferation and differentiation.[1, 2]There is accumulating evidence that proteins which mediate intercellular adhesions are involved in cellular differentiation.[3,4]

CUB-domain containing protein (CDCP)1 is a transmembrane glycoprotein, high expression levels of which have been associated with cancer of different organs.[5] It also may play a role in the regulation of anchorage versus migration or proliferation versus differentiation,[6] also, in vivo findings have suggested that CDCP1 might function as an anti-apoptotic molecule facilitating survival of tumor cells.[7, 8]

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In psoriasis; keratinocyte proliferation suggests an alteration in cell-cell interaction.[10]The mechanisms underlying the perturbed state of proliferation and differentiation in psoriatic epidermis remain poorly understood, so, we assumed that CDCP1 may have a role so we examined its expression in the psoriatic and normal skin.

Materials and methods:-

Case selection:-

Thirty paraffin blocks of patients with psoriasis collected from the archives of pathology department, Faculty of medicine, Cairo University. The available data from the patient sheet such as name of the patient, age and the duration of the disease were collected.We included in the study only patients with clear history that they hadn't received ultraviolet radiation therapy previously, and none had received topical or systemic treatment within three weeks prior to the biopsy. Ten paraffin blocks of non-lesional skin of some of our included patients were collected also from the same archive (were taken for previous research work) and other ten of normal skin were collected from surgical specimens delivered to the pathology department, Faculty of medicine, Cairo University.As all our biopsies werearchival we did not take patient consents whoever previous informed, written consentswere obtained by the dermatology Department, Faculty of Medicine, CairoUniversity that studies have been performed according to the Declaration of Helsinki,and that the procedures have been approved by a local ethics committee andapproval of the Institutional Review Board (IRB).

Histopathology:

The paraffin blocks recut at 5 microns thickness and stained with hematoxlin and eosin for routine histopathological examination.Every slide was examined by light microscopy. A grading system and check list made by Trozak[11] with a numerical value assigned to each microscopic criterion for each biopsy was taken from all cases and the histological features of psoriasis were listed and assigned value scores as in **Table 1**.

Table 1. Histologic grading system check list by Trozak(11)**:**

Microscopic Criteria		**Value**	**Score**
1-Regular elongation of rete ridges		**1**	
2-Club shaped rete ridges		**2**	
3-Elongation & edema of dermal papillae		**1**	
4-Perivascular mononuclear infiltrates in upper dermis of the papillae		**1**	
5- Absent granular layer	a-focal	**1**	
	b-total	**2**	
6- Parakeratosis	a-focal	**1**	
	b-total	**2**	
7-Suprapapillary plate thinning		**2**	
8-Mitosis above basal cell layer		**2**	
9-Munro microabscesses		**3**	
10-Spongiform pustule		**3**	
Score Total		**19**	

Immunohistochemical staining and analysis:-

CDCP1 monoclonal mouse antibody ((1C5) [NBP2-03413]Novus Biologicals USA) was used.Prostatic carcinoma was used as a positivecontrol. The first step was the deparaffinization and rehydration of the sections. Slides were then placed in 1.5% hydrogen peroxidein methanol for 15 minutes. After tapping off excess reagent the slides were rinsed for 5 minutes in phosphate buffer saline (PBS) for 5 minutes. The slides were placed in sodium citrate buffer and placed in a microwave oven 20 minutes. Blocking reagent (Ultra V Block) was placed on each slide and incubated for 10 minutes at room temperature in the humidity chamber. Excess serum was shacked off without washing. CDCP1monoclonal mouse antihuman antibody (dilution1:100) was placed on the slides. The slides were incubated overnight at room temperature in humid chamber. The slides were rinsed for 5 minutes. 2-3 drops of secondary antibody (biotinylated polyvalent) diluted in this buffer solution TBS, were placed on each slide. The slides were incubated in a humidity chamber at room temperature for 15 minutes. After tapping off excess buffer the slides were rinsed for 5 minutes, twice, in PBS and were incubated in Streptavidin enzyme for 15 minutes at room temperature. The slides then rinsed for 5 minutes, twice, in PBS.WorkingDiaminobenzidinetetrhychochloride (DAB) reagent was placed on each slide (approximately 500 ml per section). The slides were incubated for 4 minutes at room temperature in the humidity chamber and then washed thoroughly in distilled water.Counter stain

with Mayer's hematoxyline for 1 minute was done then the slides were washed. The slides were placed in two changes of 95% ethyl alcohol, then two changes of absolute alcohol, each for two minutes.Finally, the slides were cleared in xylene and cover slips were fixed using mounting reagent.
Each slide was evaluated by 2 of the authers. Positive staining is indicated by the presence of a brown cytoplasmic color. Immunoreactivity was scored accordingto the intensity of the cytoplasmic immunostaining of CDCP1 in biopsies of the control and cases scored as low (+1), moderate (+2) and high (+3).[12]

Image analysis:-
Ten CDCP1 immunohistochemical stained slides were selected from the group of cases five of them shoed moderate CDCP1 expression and the other five slides showed high expression and ten slides of the control group (5 non-lesional skin and 5 normal skin) were subjected to the morphometric analysis that was performed using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, England,) which consists of Leica DM-LB microscope with JVC color video camera attached to a computer system Leica Q 500IW. Global scale of the image analysis was set as 2,950 pixels = 1 mm, in a pixel ratio of 1. The Grey level values were similar and close to 256. Selection of the area (region of interest [ROI]), in this case, the positively immunohistochemically stained cells, was performed using the cursor (scale bar = 20 μm). The ROI file was then saved and stored in a file (ROI Manager) in the computer hard disk. From this image the software calculated the area in pixels, the mean, the maximal & minimal Grey (intensity of immunohistochemical staining) ranging from 0 (black) to 256 (total white). The final optical density was calculated according to the software ranges from 0 (zero = deep brown, highest expression), to 256 (total white). With illustrating histogram describe the number of cells take which optical density.

Statistical analysis:-
Data were collected and statistically analyzed using Statistical Package for Social Sciences SPSS version 17. Descriptive Statistics were used as arithmetic mean for central tendency, standard deviation (SD) for measuring dispersion, median and range for non-normally distributed data and percentage (%). Also, analytic Statistics were used including: non-parametric t-test (Mann Whitney test)for comparison of means of two independent groups, spearman rank coefficient as a measure of association of quantitative variables. A*P*value of less than 0.05 wasconsidered to be statistically significant.

Results:-

Clinical variables among cases and control groups:-
The age of the patients ranged from20 to65 years with a mean age 38.7± 11.97 years and that of the normal skin control group ranged from 25 to 60 years with a mean age36.8± 14.42 years (age matched groups as *P= 0.68*)**.** Among 30 patientsthere were17 male representing 56.7%and 13 female representing43.3%, while in the normal skin control group (10 individuals) there were5 males representing50% and 5 females representing50% (sex matched as *P= 0.73*)**.** The duration of the disease varied from 1year to 18 years.

Histopathology and immunohistochemical staining:-
According to the grading system used in this study our cases of psoriasis ranged from 9 to 18 out of 19 total points, with a mean of 12.7±2.8.

The intensity of the cytoplasmic immunostaining of CDCP1 in all biopsies of the control group (n=20) was low (+1) and it stains all skin layers except the horny layer in 5 of normal skin control and all the skin layers except the horny and granular layers in the other 5 while in all ten biopsies of non-lesional skin CDCP1 stained all layers except the horny one **(FIG 1 and 2)**. In cases group CDCP1 stains the whole skin layers except the horny layer and the intensity of the cytoplasmic immunostaining of CDCP1 in psoriatic skin biopsies (n=30) was moderate (+2) in 8 cases (26.7%) and high (+3) in 22 cases (73.3%) **(FIG 3)**.So, there was a significant difference *(P value <0.001)*between cases and control group regarding to immunostaining of CDCP1 **(Table 2)**.

Table 2. Comparison of immunohistochemical expression of CDCP1 between cases and control groups:

CDCP1 Expression	Study groups				P-value
	Cases		Controls		
	Count	%	Count	%	
Low	0	0	20	100%	< 0.001
Moderate	8	26.7%	0	0	
High	22	**73.3**	0	**0**	
Group Total	30	100%	20	100%	

There was no sex significant difference in immunohistochemical stainingofCDCP1 *(p value = 1)* among the cases. But there was a significant positive relation between the duration of the disease and the intensity of CDCP1 staining of the psoriatic skin *(p =0.002)*, also, there wasa significant positive relation *(P value < 0.001)*between the used histological grading score and CDCP1 immunohistochemical staining.

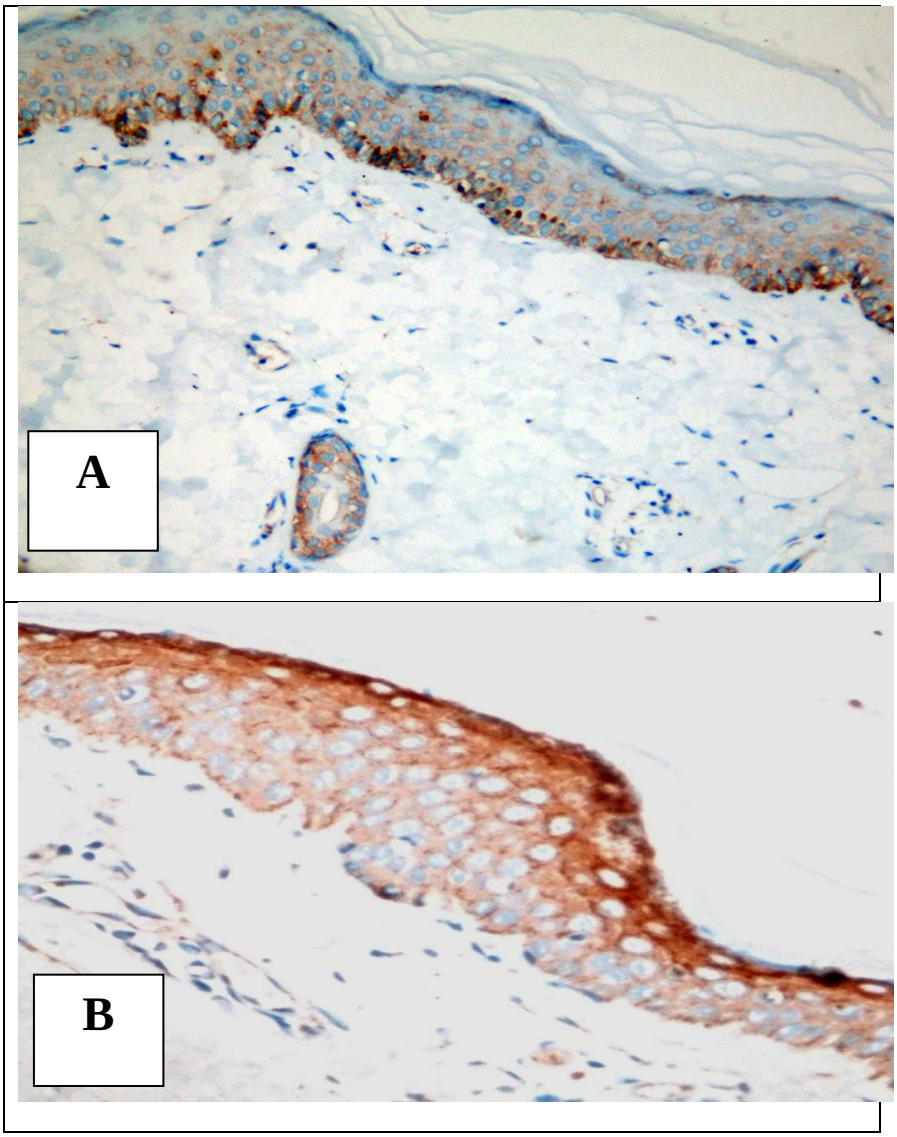

FIGURE 1. CDCP1expression in the normal skin showing low grade (+1) cytoplasmic staining (X 200). Cytoplasmic staining is seen in the basal and spinous cell layers, and absent staining in granular and horny layers (A), and absent only in the horny layer (B).

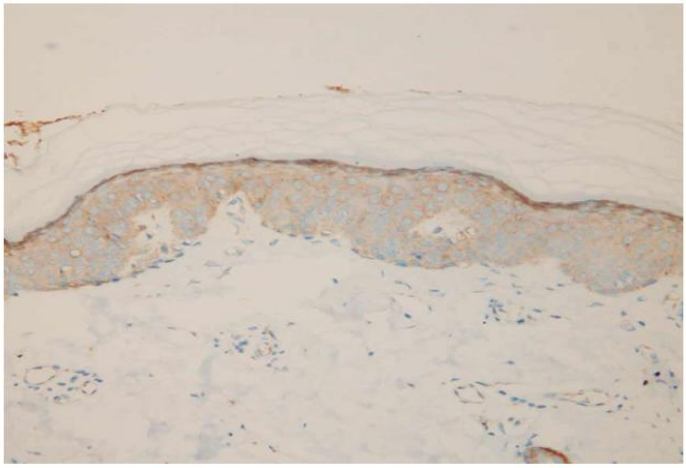

FIGURE 2. CDCP1 expression in non-lesional skin of psoriatic patients showing low grade (+1) cytoplasmic staining (X 200). Cytoplasmic staining is absent only in the horny layer.

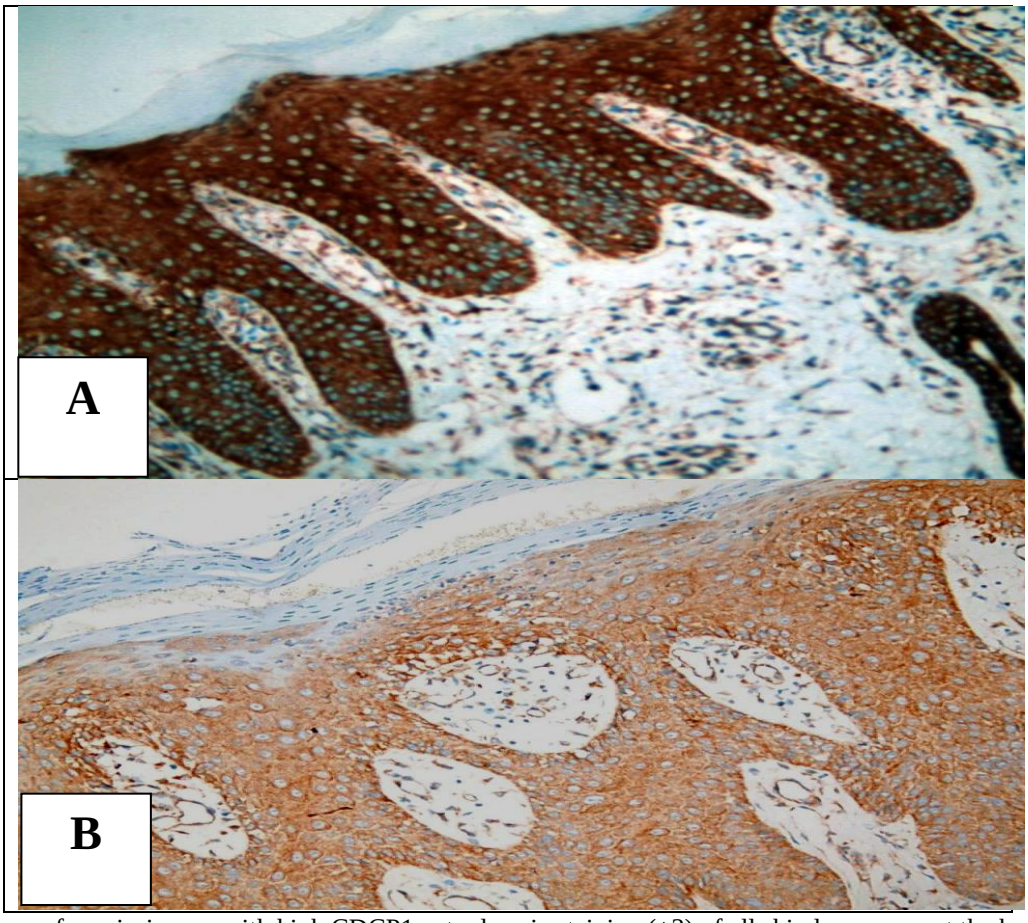

FIGURE3. 2 case of psoriasis; one with high CDCP1 cytoplasmic staining (+3) of all skin layers except the horny layer(X 200) (A), and the other with moderate CDCP1 cytoplasmic staining (+2) of all skin layers except the horny parakeratotic layer(X 400)**.**

Image analysis:-

The optical densityamong the control ranged from 210 to 216 with a mean value of 213.9 while in the 10 selected psoriatic cases it ranged from 163 to 192 with a mean value of 182.37 **(FIG 4)**. It was a statistically significant difference between cases and control *(p value= 0)* regarding the immunohistochemical staining using CDCP1.

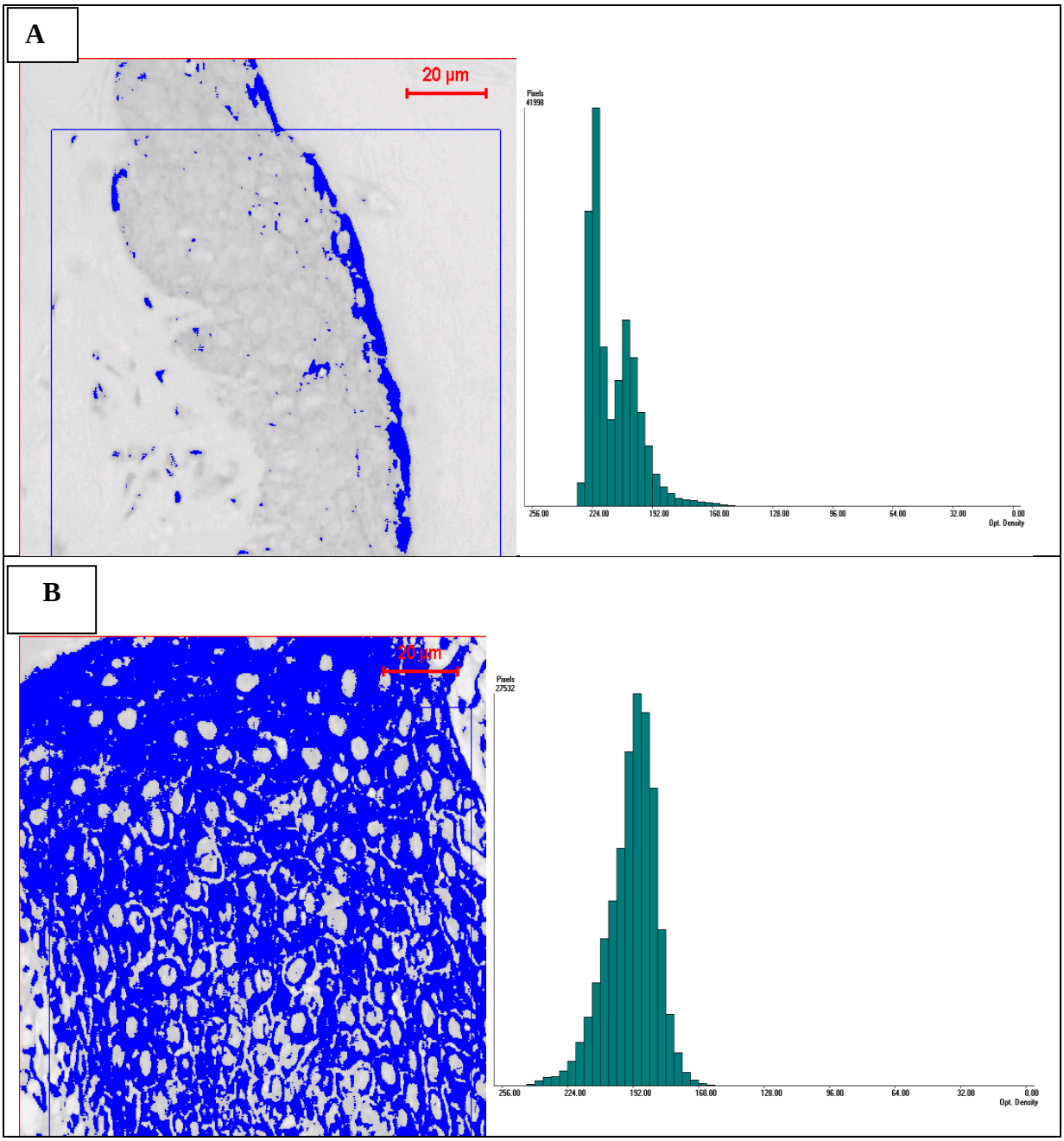

FIGURE 4. Optical density using image analyser in normal (A) and psoriatic skin (B): The histogram in normal skin the shifted to the left (as black=0& white= 256) while in psoriatic skin shifted to the right (as it goes to zero; the highest optical density)

Discussion:-

Psoriasis vulgaris is a common, chronic skin disease. Keratinocytes have a significant role in the formation of psoriasis plaque. Various types ofalterations can be seen in the keratinocytes in the plaques when compared tokeratinocytes in healthy epidermis. Proliferation of keratinocytes is highly increasedbut the factors causing the increase are unknown.[13]

To the best of our knowledge there is only single work by McGovern et al.[9] that studied theimmunohistochemical expression of CDCP1 in skin, and it used normal skin biopsies. In the current study we demonstrated for the first time that CDCP1 was overexpressed by keratinocytes in psoriasis vulgaris lesions compared with non-lesional skin and normal skin, and it is the first time to use it in dermatoses. This was evaluated by 2 ways; using an immunohistochemical scoring for the staining intensity and image analysis (optical density).

However, our work may be related to a study that used the immunohistochemical expression of activated Src-family kinases (SFKs) in 6 biopsies of normal skin and and compared it to 14 psoriatic skin biopsies and found that all psoriasis specimens displayed significantly greater staining for activated SFKs when compared to sections of normal skin that showed low level of staining and so they concluded that there is a direct implication of SFKs activation in the pathogenesis of psoriasis.[14] Hence CDCP1 is a substrate of Src and its phosphorylation is linked with SFKs [15,16]in addition over expression of CDCP1 increases the activity of SFKs and induces the formation of metastases in melanoma,[17] so the up-regulation of CDCP1 expression in psoriatic skin in our study may be related to or correlated with this increased expression of activated Src-family kinases in psoriasis.

The present results have also shown that; among the cases, there wasa highly significant positive relation *(P value < 0.001)*between the used histological grading score and CDCP1 immunohistochemicalstaining.This mostly refers to the presence of a strong relation between the up regulation of CDCP1 in psoriasis and its histopathologic picture, and that the more fully developed the lesion the more it will be likely to express CDCP1 in the skin layers.

Several reports have demonstrated that CDCP1 interacts with a number of proteins involved in cell adhesion**.**[8,15,18]Also, it was proposed that the clustering of CDCP1 and signaling components in membrane microdomains in cell-cell contacts contributes to changes in cell behavior.[19]In addition, in vivo findings have suggested that CDCP1 might function as an anti-apoptotic molecule facilitating survival of tumor cells [20]andpsoriatic keratinocytes possess an enhanced ability to resist apoptosis, which might be one of the key pathogenetic mechanisms in psoriasis.[21] However, limited data exist regarding the underlying mechanisms of this defect in the apoptosis control mechanisms of psoriatic keratinocytes, so novel apoptosis-based therapies could be directed towards enhancement of apoptotic process in psoriasis.[22]

It is hoped that an improved understanding of the pathogenesis of psoriasis will aid in providing novel treatment options directed to improving or enhancing the defect in CDCP1 and thereby leading to an amelioration of the tissue pathology and in turn the clinical presentation. So, further studies are required to investigate the role CDCP1in psoriasis.

Conclusion:-

In psoriasis there is increased expression of the cell surface glycoprotein CDCP1, and we assume that this alteration may have a role in the etiopathogenes of psoriasis and this suggestion needed to be confirmed and deeply explained in further studies.

Abbreviations:-

CDCP1: CUB-domain containing protein
PBS: phosphate buffer saline
DAB:Diaminobenzidinetetrhychochloride
ROI: Region of interest
SPSS: Statistical Package for Social Sciences
SD: standard deviation
SFKs:Src-family kinases

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