Egyptian Journal of Surgery Vol. (20), No. (4), Oct., 2001

A COMPARATIVE STUDY OF THE PROLIFERATIVE MARKERS IN DIFFERENT PROLIFERATIVE SKIN CONDITIONS COMPARED TO NORMAL HUMAN SKIN

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The present study was designed to evaluate the biological and clinical significance of the expression of two different proliferation markers used for assessment of the proliferative response in different proliferative skin conditions, in comparison to normal skin.

42 specimens were collected including 7 specimens of normal skin and 35 specimens representing different skin lesions diagnosed as verrucous vulgaris, chronic suppurative dermatitis, foreign body granuloma, squamous papilloma and basal cell carcinoma (7 specimens each). Prepared paraffin sections were subjected to Hx. and E and immunostaining to detect PCNA and a nuclear antigen reacting with Ki-67 monoclonal antibody. Morphometric assessment of the thickness of the epidermis and percent positive nuclear area using image analysis was performed and followed by statistical analysis of the data. A highly significant increase in the thickness of the epidermis was found in all proliferative conditions in comparison to normal skin. PCNA immunoreactivity was highly significantly increased in all proliferative conditions except in foreign body granuloma when compared to normal skin. Concerning Ki-67 immunoreactivity, a significant increase in the mean area percent of the positive cells was detected in skin affected by verrucous vulgaris and squamous papilloma and highly significantly increased in cases of basal cell carcinoma, when compared to normal skin. A highly significant difference between PCNA and Ki-67 immuno-reactivity was estimated in normal skin and in the different proliferative conditions except in basal cell carcinoma, where no significant difference was recorded. We concluded that, Ki-67 detecting a nuclear antigen might be more specific for evaluation of the proliferation rate in neoplastic lesions particularly malignant conditions.

Key words: *Ki 67 – PCNA - skin.*

INTRODUCTION

Labile cells like those of the epidermis of the skin undergo continuous division throughout life[1]. Dermatitis classified as atopic, contact or dermatitis of unknown aetiology comprises the vast majority of non-neoplastic disease[2]. Warty lesions of the skin include virally induced proliferation of keratinocytes. Verrucous vulgaris as an example of viral infections of the skin may exhibit a proliferative tendency sufficient to suggest a benign tumour[3]. Chronic inflammatory lesions including foreign body granulomas, which are, also termed low turnover granulomas are traditionally considered proliferative lesions[4]. Neoplastic conditions of the skin are characterized by proliferation of abnormal behaviour, resulting in abnormal appearance[5].

Immunohistochemical determination of two established cell proliferation markers is required to assess cellular proliferation and not a non-specific response[6]. Proliferating cell nuclear antigen (PCNA), a 36 kilodalton (KD) nuclear protein expressed by cycling cells, but not by resting cells was established using Ki-67 monoclonal antibody[7].

The present study was designed to evaluate the biological and clinical significance of the expression of two different proliferation markers used for the assessment of

the epithelial proliferative response in different proliferative skin conditions, in comparison to normal skin.

PATIENTS AND METHODS

This study was performed at general surgery department at Theodor Bilharz Research institute & Histology department Cairo University in the period between April 2000 & April 2001. Seven specimens of normal skin obtained from patients underwent abdominoplasty and 35 specimens of different skin lesions (Table I) were used. A representative portion of each surgical specimen was obtained and sent to the Pathology Department at theodor Bilharz Research Institute

Histology Study:

A second representative portion of each surgical sample was obtained fresh, fixed in formol saline and processed to paraffin. Sections were prepared at 5 µm thickness and stained with Hx. and E.

Immunohistochemical Study:

Serial sections were taken onto polylysine coated slides and incubated with Ki-67 (IgG_1 subclass) monoclonal antibodies[8]. PCNA was detected using the monoclonal PC_{10} (Dako, Carpentia, California, USA)[9].

Sites of antibody binding were localized using the substrate diaminobenzidine (DAB) and hydrogen peroxide (0.01%)[10].

Immunohistochemical Controls:

Substitution of the primary antibody by normal serum was used as a negative control. Paraffin sections of human tonsil were used as the positive control[11].

Morphometric Study:

Using 500 LTD Qwin image analysis system, the thickness of the skin was assessed in Hx. and E. stained sections[12] to establish proliferation (Figs. A, B). The distribution of the nuclear antigen reacting with PC_{10} and the nuclear protein reacting with Ki-67 monoclonal antibodies[13] was assessed and expressed as the percent positive nuclear area (Figs. C, D, E F). Statistical analysis of the data was performed using Anova and Student's t-tests[14].

RESULTS

Hx. and E. Stained Sections:

Normal skin sections revealed normal architecture and thickness of the epidermis and the dermis (Fig. 1). In cases of verrucous vulgaris obvious thickening of the epidermis was observed (Fig. 2). Chronic suppurative lesions revealed an increase in the thickness of the epidermis at the edges of ulcers (Fig. 3). Thickened epidermis with thickened stratum spinosum was seen in cases of foreign body granuloma (Fig. 4). Squamous cell papilloma demonstrated an exophytic growth pattern raised above the surface of the epidermis (Fig. 5). Basal cell carcinoma revealed epidermal thickening at the edges of the ulcer with an increase in the thickness of the prickle cell layer (Fig.6).

Immunohistochemical Results:

Ki-67 or PCNA positive, immuno-reactivity was restricted to the nucleus.

In normal skin, expression of PCNA in the nuclei of the basal and prickle layers and occasional nuclei of the surface cells . Low level expression of Ki-67 was noticed, where some nuclei of the stratum germinativum appeared positive (Fig. 7, 8).

In sections of verruca, PCNA immunoreactive cells were detected in all layers of the epidermis. Ki-67 reactive cells were occasionally detected in the middle and upper layers of the epidermis (Figs. 9, 10).

In chronic suppurative dermatitis PCNA nuclear reaction appeared strongly in the basal and prickle cell layers and less in the surface cells. Ki-67 positive nuclei were detected in some cells of the basal and prickle cell layers (Fig. 11, 12). In granulomas PCNA immunoreactivity appeared mainly in the basal and intermediate cells, while Ki-67 positive cells were not detected in the epidermis except in occasional cells of the basal cell layers. Few positive PCNA reactive cells and no Ki-67 reactivity were seen in the granuloma masses (Fig. 13, 14, 15, 16).

Sections of papilloma demonstrated positive nuclei mainly in the basal and lower prickle layers, in addition to occasional reactivity of the upper prickle and surface cells of the exophytic growth on PCNA immunostaining. (Fig. 17, 18). Sections of basal cell carcinoma showed strong expression of PCNA as well as Ki-67 in the nuclei of the basal cell layer, less immunoreactivity was detected in the prickle cell layer. The marginal and central cells of the dermal masses revealed strong PCNA and Ki-67 reactivity (Fig. 19, 20).

Table I: *Clinical characteristics and pathological diagnosis of skin lesions.*

Sex	*Age range*	*Skin lesion*	*Diagnosis*
4 males / 3 females	7-30 years	Multiple warts of the foot	Verrucous vulgaris
3 males / 4 females	40-60 years	Multiple inflammatory lesions of the head	Chronic suppurative dermatitis
4 males / 3 females	40-60 years	Nodular pigmented lesions of the head	Foreign body granuloma
7 males	60-80 years	Pigmented wart of the face	Squamous papilloma
7 males	50-60 years	Ulcer of the scalp or face	Basal cell carcinoma

Morphometric Results:

Table (II): *Mean thickness of epidermis and mean area percent of PCNA and nuclear antigen reacting with Ki-67 ± S.D. in normal skin and different proliferative conditions (Figs. 21, 22, 23, 24)*

Skin specimen	*Epidermal thickness (micron)*	*PCNA*	*Ki-67*
Normal	108.61±30.51	1.08±0.38	0.14±0.04
Verrucous vulgaris	487.37±123.87**	1.8±0.64**	0.28±0.1*
Chronic suppurative	298.07±100.24**	1.70±0.32**	0.17±0.06
F.B. granuloma	274.57±70.23**	0.93±0.27	0.11±0.04
Squamous papilloma	396.15±114.05**	1.88±0.56**	0.24±0.09*
Basal cell carcinoma	356.62±105.62**	2.77±0.98**	2.75±0.91**

** Highly significant (P<0.001).

* Significant (P<0.05).

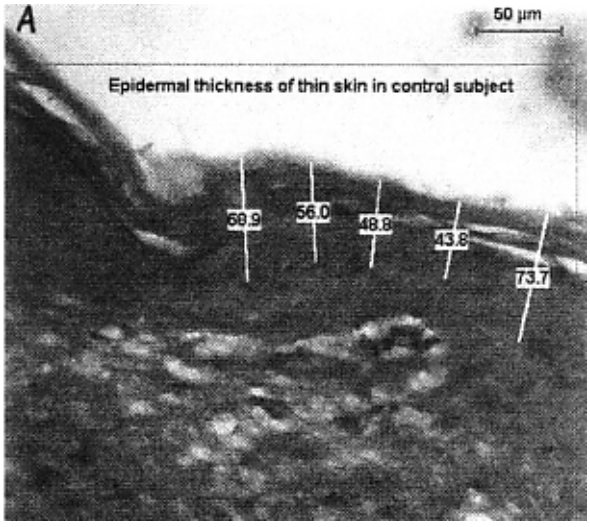


Fig. (A): A copy of display seen on the monitor's screen of the image analyzer showing epidermal thickness of thin skin in control subject.

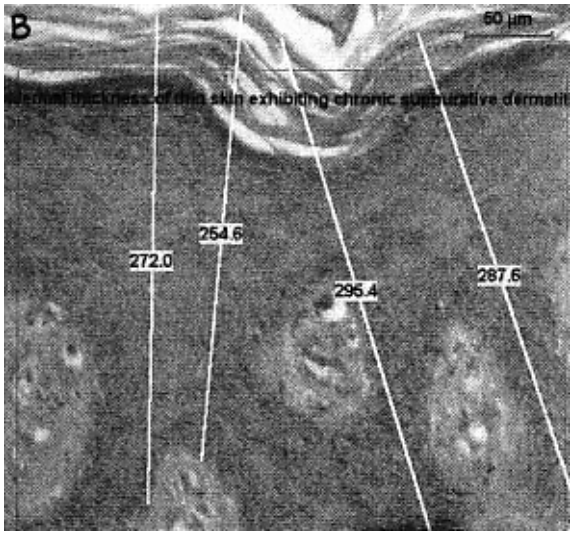


Fig. (B): A copy of display seen on the monitor's screen of the image analyzer showing epidermal thickness of thin skin exhibiting chronic suppurative dermatitis.

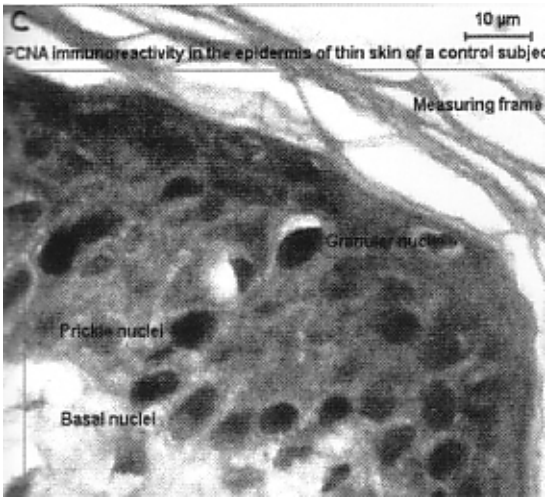


Fig. (C): A copy of display seen on the monitor's screen of the image analyzer showing PCNA immunoreactivity in the epidermis of thin skin of a control subject.

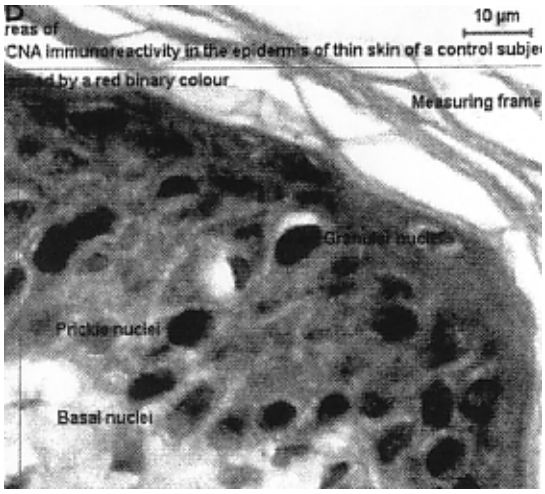


Fig. (D): A copy of display seen on the monitor's screen of the image analyzer showing area of PCNA immunoreactivity in the epidermis of thin skin of a control subject masked by a red binary colour.

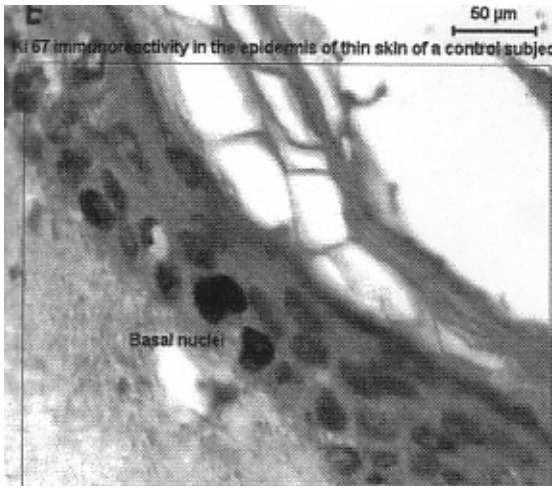


Fig. (E): A copy of display seen on the monitor's screen of the image analyzer showing Ki-67 immunoreactivity in the epidermis of thin skin of a control subject.

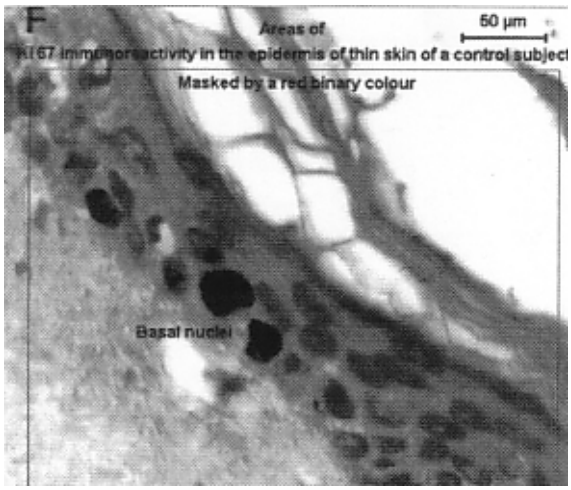


Fig. (F): A copy of display seen on the monitor's screen of the image analyzer showing areas of Ki-67 immunoreactivity in the epidermis of thin skin of a control subject masked by a red binary color.

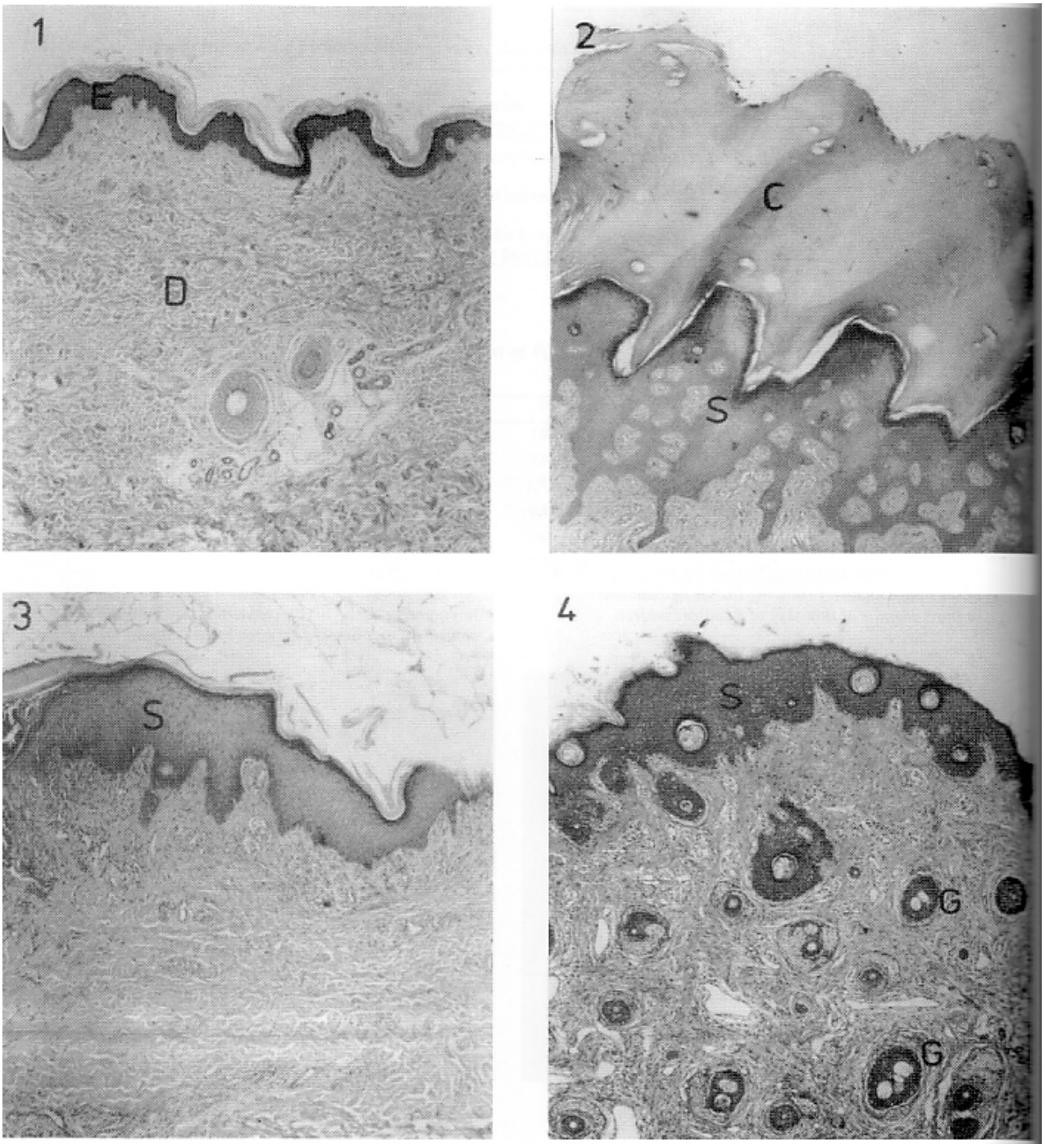


Plate I (Figs. 1, 2, 3, 4): Photomicrographs of sections in the human skin (Hx. and E., X40):

Fig. (1): Normal skin showing epidermis (E) and dermis (D).

Fig. (2): Skin affected by verrucous vulgaris showing increased thickness of stratum spinosum (S) and stratum corneum (C).

Fig. (3): Skin affected by suppurative dermatitis showing increased thickness of stratum spinosum (S).

Fig. (4): Skin affected by foreign body granuloma showing increased thickness of stratum spinosum (S) and multiple granulomas (G) in the dermis.

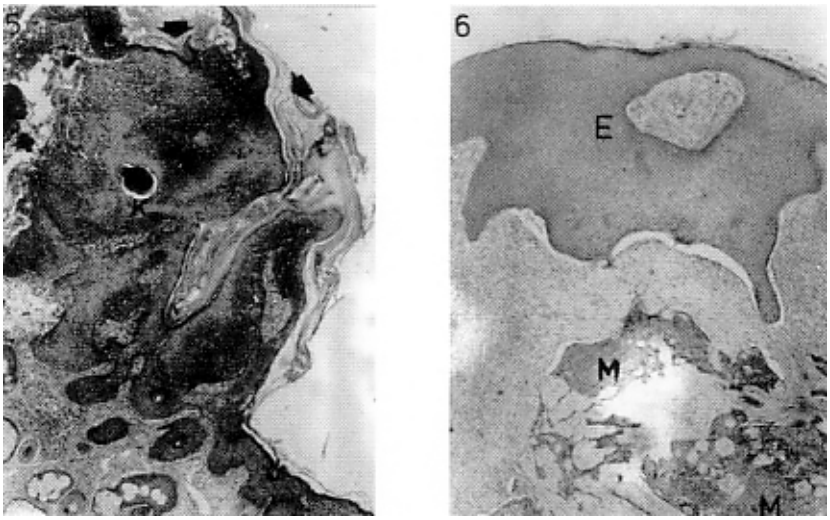


Plate II (Figs. 5, 6): Photomicrographs of sections in the human skin (Hx. and E., X40):
Fig. (5): Skin affected by squamous cell papilloma showing exophytic growth (↑), exhibiting spherical masses of keratin (K).
Fig. (6): Skin affected by basal cell carcinoma showing epidermal thickening (E) and multiple solid masses (M) in the dermis.

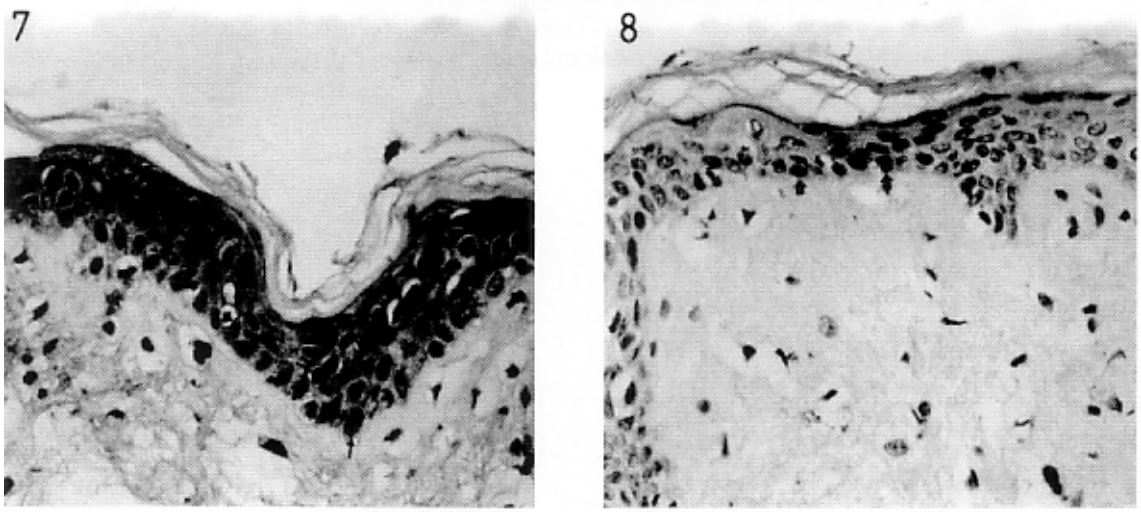


Plate III (Figs. 7, 8): Photomicrographs of sections in the normal human skin (immunostaining, X400):
Fig. (7): PCNA immunoreactivity is seen as a brown reaction in the nuclei of the basal (↑), prickle cell layers (↑↑) and occasional nuclei of the surface cells (↑↑↑).
Fig. (8): Ki-67 expression is observed as a brown reaction in some of the nuclei of the basal cell layer (↑).

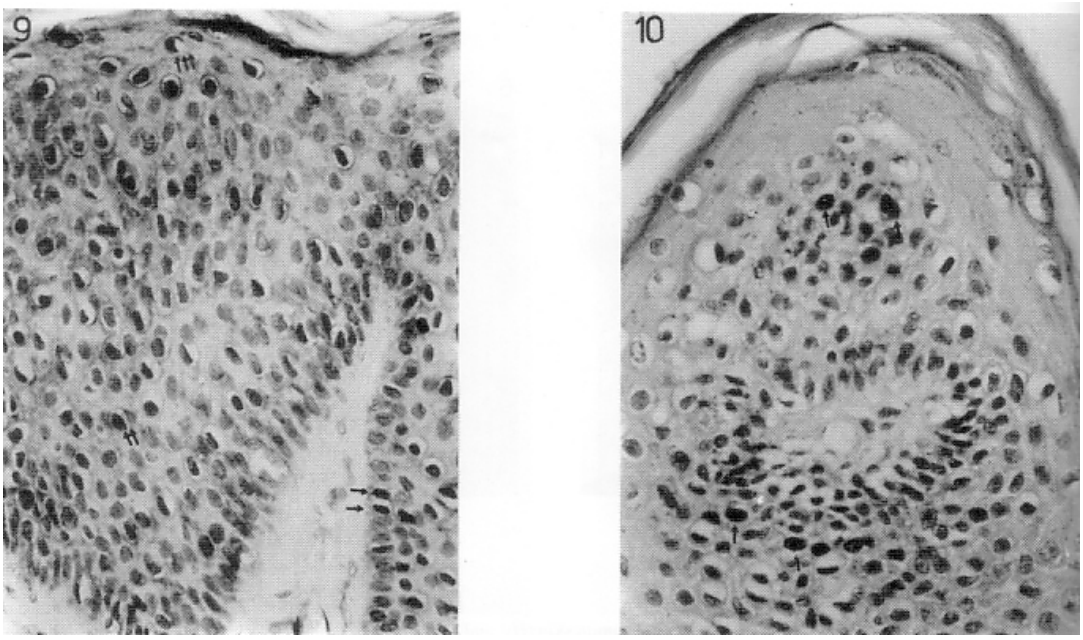


Plate IV (Figs. 9, 10): Photomicrographs of sections in the human skin affected by verrucous vulgaris (immunostaining, X400):
Fig. (9): PCNA positive nuclei are seen in the basal (↑), prickle (↑↑) and granular (↑↑↑) cell layers of the epidermis.
Fig. (10): Ki-67 expression is observed in some nuclei of the middle and upper layers of the epidermis (↑).

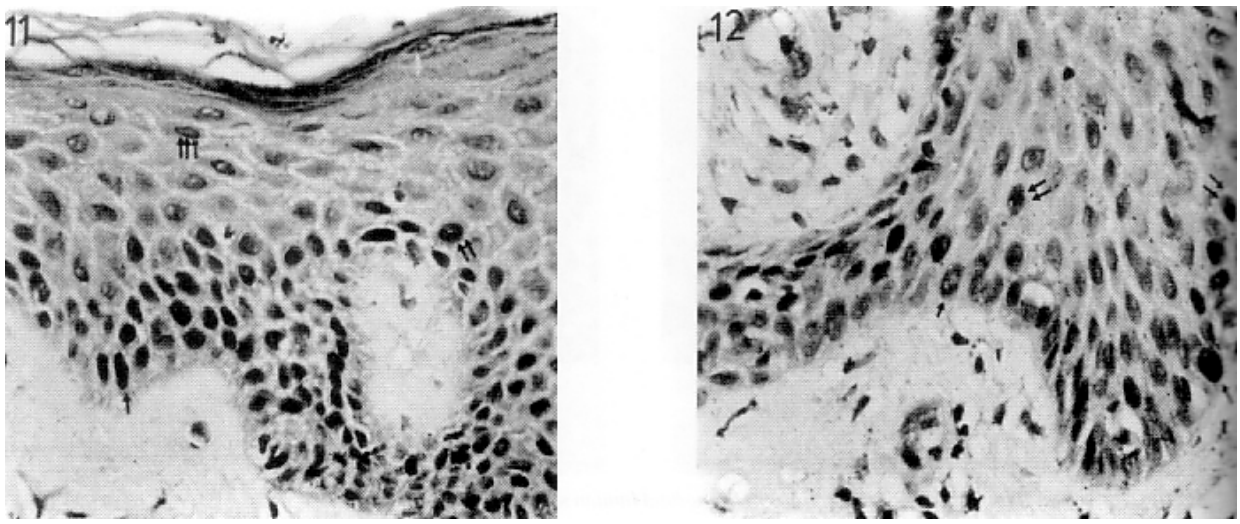


Plate V (Figs. 11, 12): Photomicrographs of sections in the human skin affected by suppurative dermatitis (immunostaining, X400):
Fig. (11): PCNA immunoreactivity is seen in the basal (↑), prickle (↑↑) cell layers and some of the surface cells (↑↑↑).
Fig. (12): Ki-67 expression is observed in some cells of the basal (↑) and prickle (↑↑) cell layers.

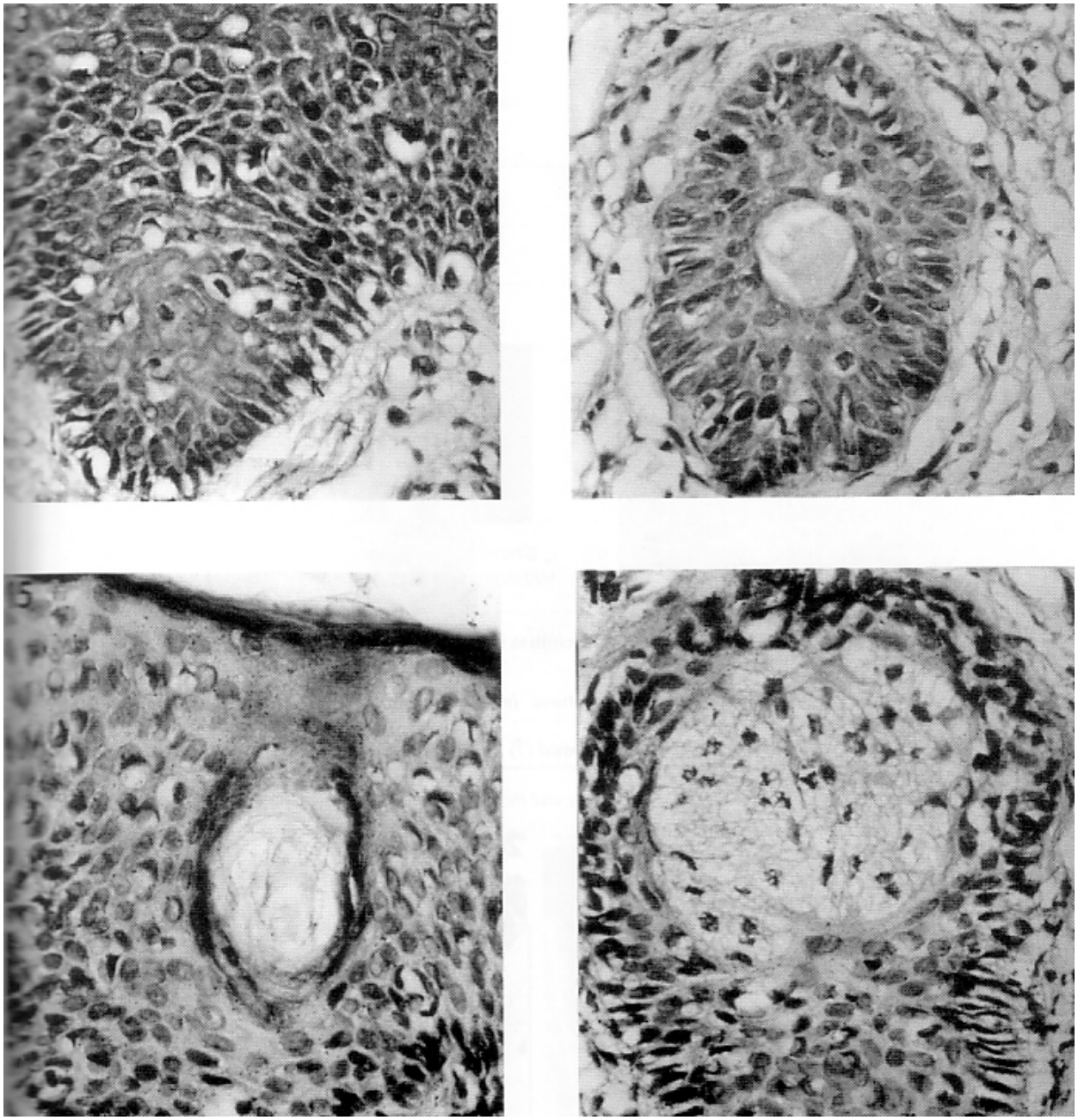


Plate VI (Figs. 13, 14, 15, 16): Photomicrographs of sections in the human skin affected by foreign body granuloma (immunostaining, X400):

Fig. (13): PCNA immunoreactivity is seen in some of the nuclei of the basal (↑) and suprabasal (↑↑) layers of the epidermis.

Fig. (14): A granuloma is seen in the dermis exhibiting few PCNA positive nuclei (↑).

Fig. (15): Ki-67 expression is seen in some nuclei of the stratum germinativum (↑).

Fig. (16): A granuloma showing negative Ki-67 immunoreactivity.

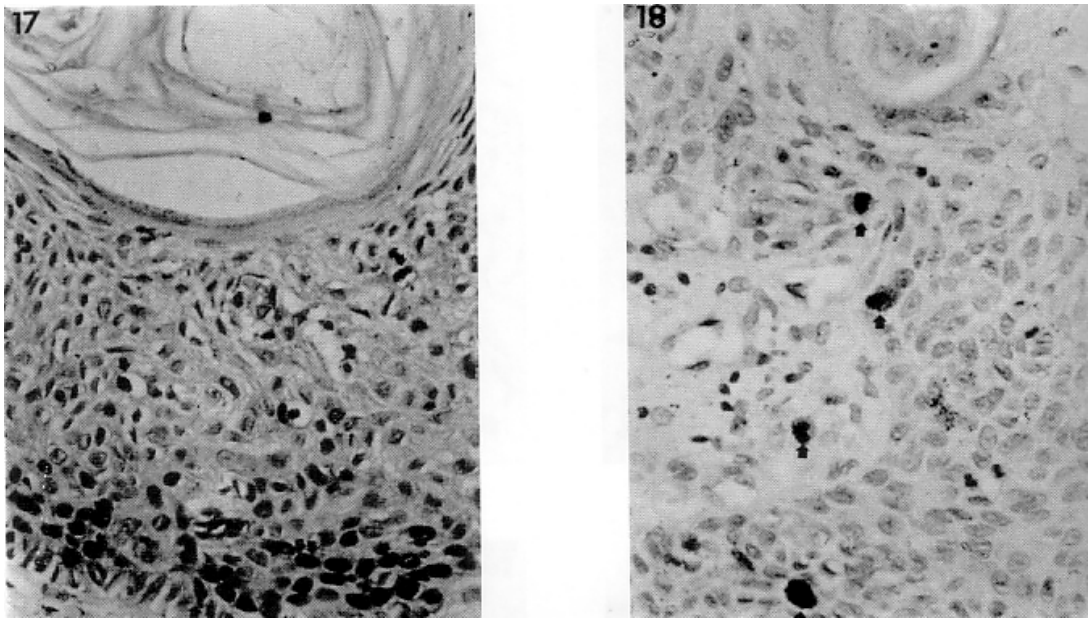


Plate VII (Figs. 17, 18): Photomicrographs of sections in the human skin affected by squamous cell papilloma (immunostaining, X400):
Fig. (17): PCNA positive nuclei are seen mainly in the basal (↑) and lower prickle (↑↑) cells and occasionally in the superficial (↑↑↑) cells.
Fig. (18): Ki-67 immunoreactive cells (↑) are seen sporadic and the positive nuclei show mitotic figures.

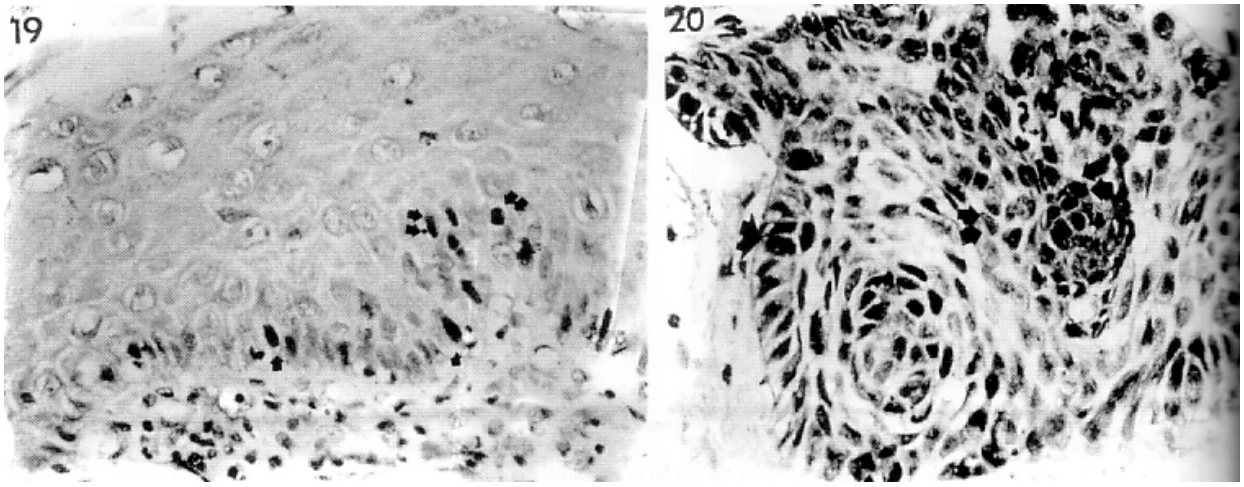


Plate VIII (Figs. 19, 20): Photomicrographs of sections in the human skin affected by basal cell carcinoma, (immunostaining, x400):
Fig. (19): Ki-67 immunoreactive nuclei are seen mainly in the basal cell layer (↑) and few are detected in the prickle cell layer (↑↑) of the epidermis.
Fig. (20): PCNA positive nuclei are strongly detected in the peripheral and central cells of the dermal masses.

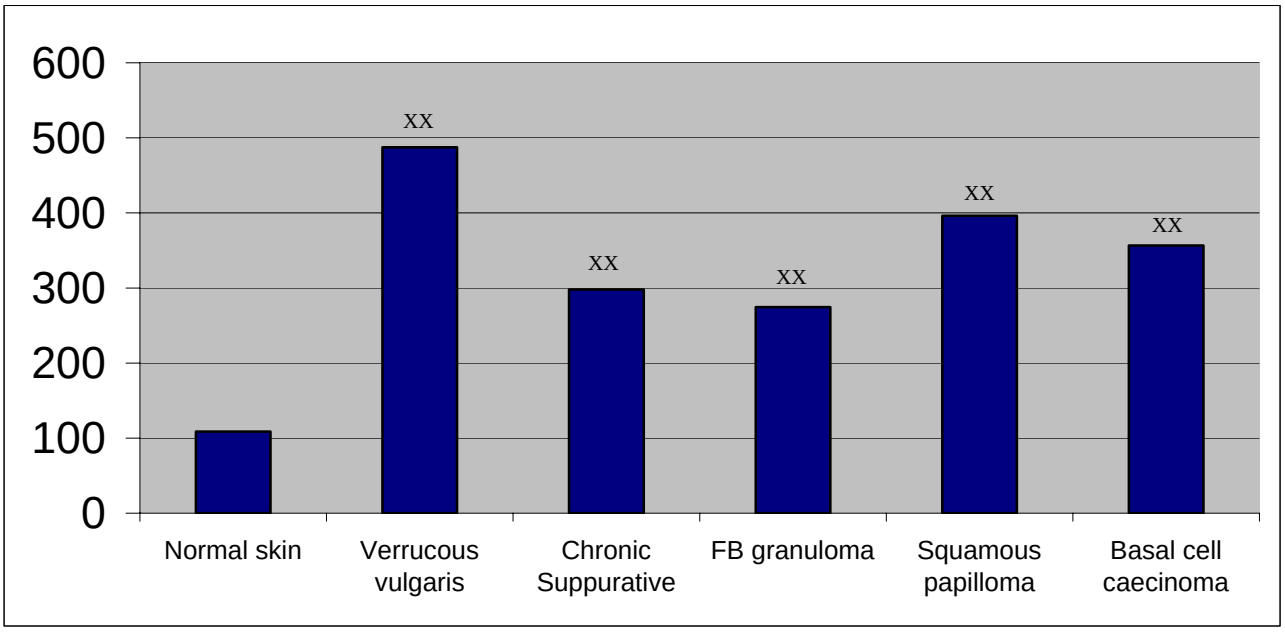


Fig. (21): Epidermal thickness in normal skin & different proliferative skin conditions (microns)

** Highly significant (p<0.001)

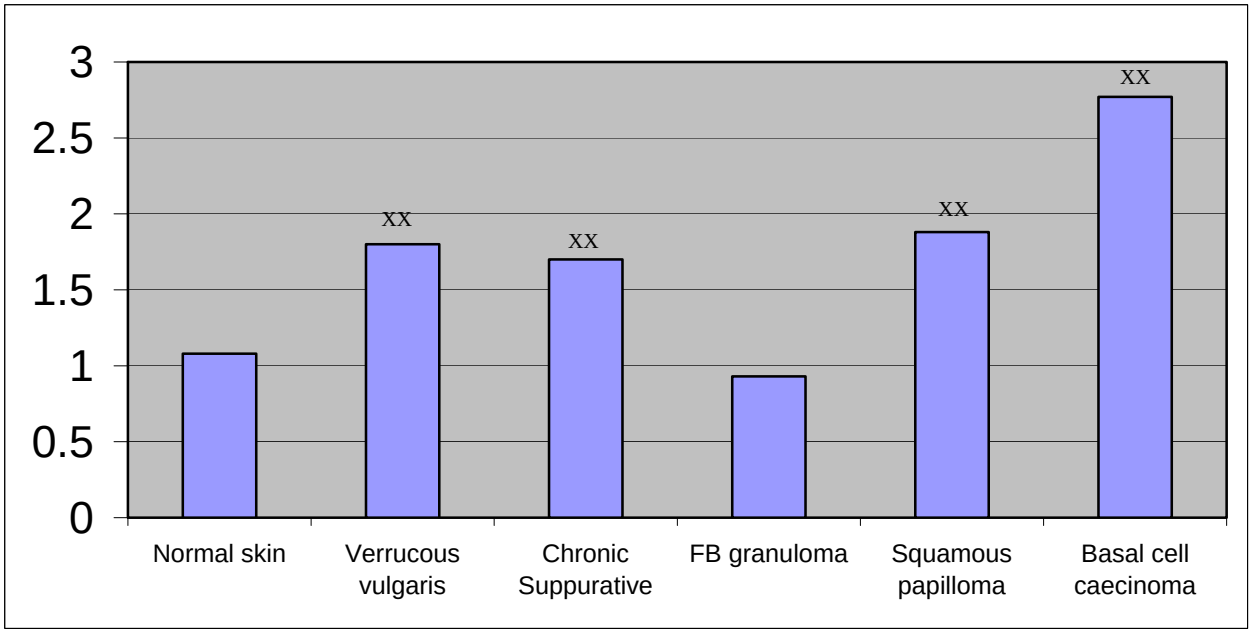


Fig (22): PCNA area % in normal skin & different proliferative skin conditions

** Highly significant (p<0.001)

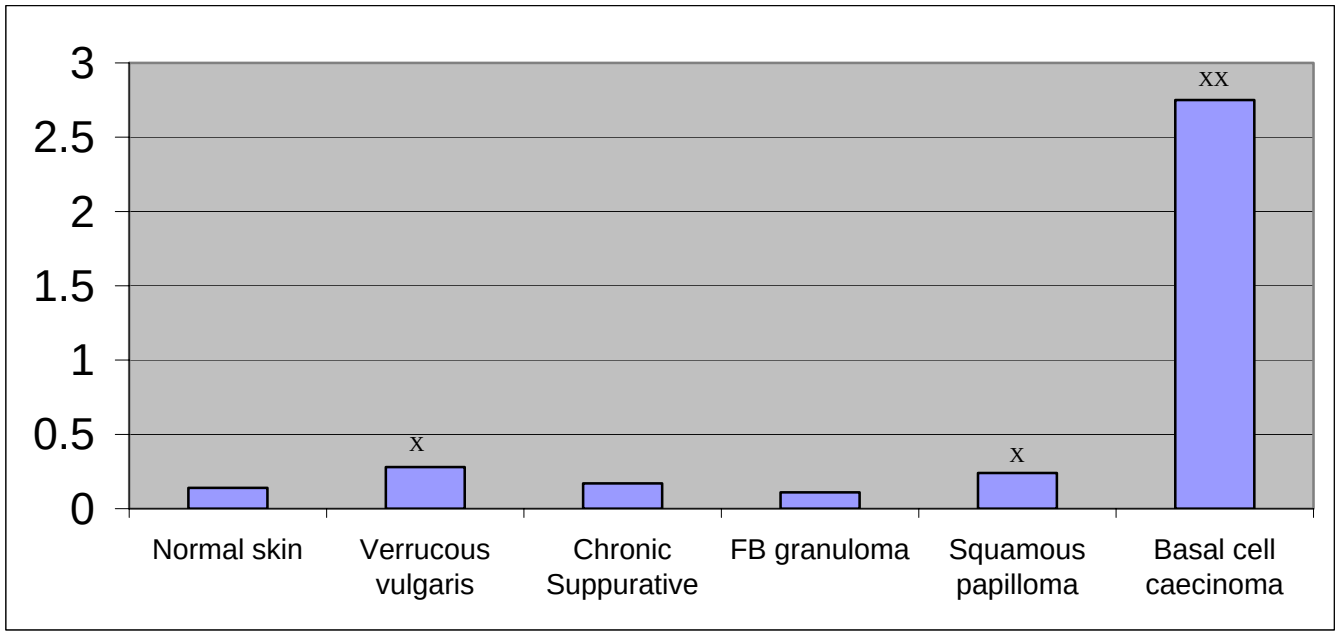
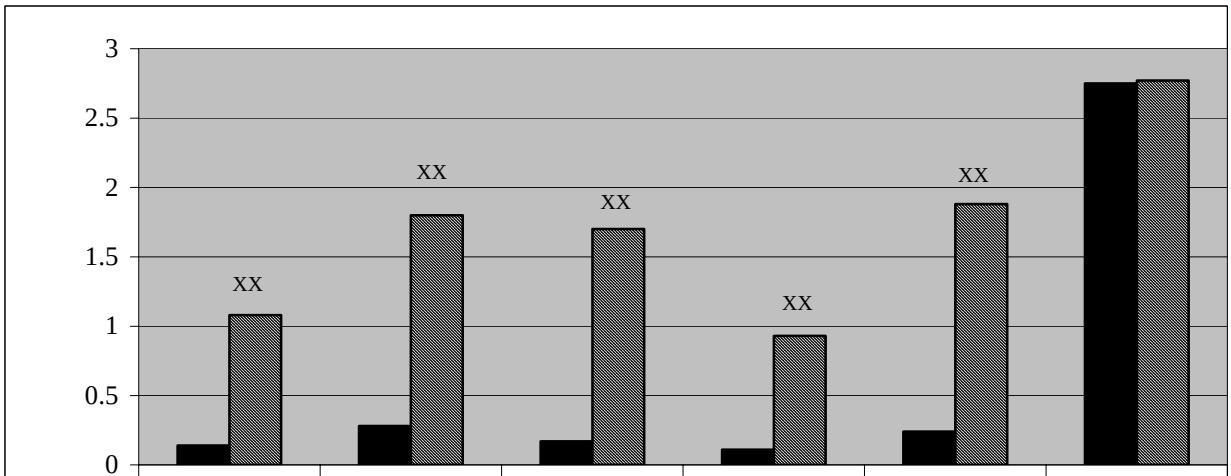


Fig (23): Ki 67 area % in normal skin & different proliferative skin conditions

** Highly significant (p<0.001)
X Significant (p<0.05)



	normal	verrucous vulgaris	chronic suppurative	F.B. granuloma	Squamous papilloma	Basal cell carcinoma
Ki67	0.14	0.28	0.17	0.11	0.24	2.75
PCNA	1.08	1.8	1.7	0.93	1.88	2.77

Fig (24) PCNA & Ki 67 area % in normal skin and different proliferative skin conditions

** Highly significant (P<0.001)

DISCUSSION

Mitosis is the visible manifestation of cell division, but there are other processes, not so easily observed with the light microscope, that play a fundamental role in the cell multiplication. No visible phenomena of cell division are observable with the microscope during the interphase. This alternation between mitosis and interphase in all tissues with cellular turnover is known as the cell cycle[15] Detection of nuclear antigens by immunocytochemistry, using antibodies and counting the number of positively stained nuclei in proportion to all nuclei, allows the possibility of evaluation of the cell proliferation index[16] This procedure might be more accurate and easier than counting mitotic figures.

In the present study, normal skin sections revealed a mean thickness of 108.61±30.51 microns. Halprin (1972)[17] stated that the turnover rate of the epithelium of the epidermis of the normal skin is rapid, because of the continuous cell division and the ongoing death of the cells. A great expression of PCNA was observed in the nuclei of the basal and prickle celll ayers with occasional reactivity at the surface cells. The mean percent area of proliferating antigen reacting with PC_{10} was 1.08±0.38. Wolfe (1993)[18], reported that the stratum basale is characterized by intense mitotic activity and is responsible in conjugation with the initial portion of the stratum spinosum for constant renewal of epidermal cells.

Ki-67 immunoreactivity was seen to be restricted to some of the nuclei of the stratum germinativum of the examined sections of normal skin. Mean area percent of nuclear antigen reacting with Ki-67 was 0.14±0.04. Cattoretti et al. (1992)[19] suggested an antigenically distinct protein, namely Ki-67 to be expressed during the S and G_2 phases of the cell cycle, which is concerned with DNA synthesis. Alberts et al. (1994)[20] described cells leaving the cell cycle following mitosis and remaining resting for a period, as having entered the G_0 phase. They may however at some subsequent time reenter the cell cycle. Gerdes et al. (1997)[21] divided the interphase into G_1 (presynthesis), S (DNA synthesis) and G_2 (post DNA replication). The authors proved Ki-67 nuclear antigen to be expressed during all phases of the cell cycle except G_0 phase.

In cases of verrucous vulgaris obvious thickening was observed and confirmed by a highly significant increase in the thickness of epidermis, in comparison to normal skin. Champion et al. (1992)[22] stated that verruca infection of the skin is accompanied by acanthosis & hyperkeratosis.

In the present study, PCNA +ve nuclei were detected in all layers of the epidermis and a highly significant increased area % was assessed, while Ki-67 +ve nuclei were observed in the middle and upper layers of the epidermis and a significantly increased percent area was noted. Zuckerman et al. (1994)[23] reported that viral DNA replication occurs in the proliferating basal cells, but structural capsid protein forms in the midepidermis and the wart virus, replicates in the nucleus. Guillou (1995)[24] recorded a high rate of proliferation in verruca cases that the condition might be mistaken for a tumour.

In chronic suppurative dermatitis a highly significant increase in the thickness of the epidermis was found. MacSween and Whaley (1992)[25] stated that chronic dermatitis is associated with acanthosis of the epidermis. PCNA immunoreactivity was highly significantly increased, while that of Ki-67 was insignificant, in comparison to normal skin. This indicated that epidermal cells leave the cell cycle following mitosis and remain resting, Ki-67 being proved not to be expressed in G_0 phase.

Granuloma cases showed a highly significant increase in the thickness of the epidermis and a non significant change in neither PCNA nor Ki-67 immunoreactivity, when compared to normal skin. Walter and Talbot (1996)[26], reported that the monocytes from the blood appear to undergo mitosis soon after leaving the circulation and then enter a resting phase before further division, indicating subsequent passage into a quiescent state lasting for several days.

Both papilloma and basal cell carcinoma showed a highly significant increase in the epidermal thickness as assessed by analysis. In inflammatory conditions, hyperplasia develops secondary to a normal stimulus whether virus, bacteria or chemical irritation. McLean and Haynes (1987)[27] stated that the growth in hyperplasia is directly related to the degree of stimulation and once the stimulus is removed the proliferation regresses. On the other hand, neoplasia (including papilloma and basal cell carcinoma) arises spontaneously or in response to a carcinogen. Lever[28] and Schaumburg-Lever[29] (1975, 1989) reported that the growth in neoplasia is autonomous. PCNA values in the examined sections were found to be highly significantly increased, while Ki-67 percent area was significantly and highly significantly increased in papilloma and basal carcinoma respectively versus normal values. Kohn et al. (1994)[30] described benign tumours as slowly proliferating growth, showing few mitosis and limited autonomy, because growth often ceases when the tumour has attained a certain size. Haber (1995)[31] mentioned a less accurate reproduction causing invasion in malignancy.

On paired comparison between the values of positive area % expressed by PCNA versus Ki-67 in normal skin and different proliferative conditions, a highly significant difference was determined except in basal cell carcinoma, where no significant difference was proved. Cells in transition from G_0 to G_1 are referred to as G_{1T} cells and according to the ribonucleic acid content of these cells, G_1 is divided into G_{1A} and G_{1B} phases (Darzynkiewicz and Traganos, 1998)[32]. Under normal conditions G_{1T} and G_{1A} cells are Ki-67 -ve, while G_{1B} cells are Ki-67 +ve. Following mitogen stimulation triggered cells were found to be constantly positive in G_1 phase. This was explained by that the Ki-67 nuclear antigen requires protein (but not DNA) for synthesis[33]. This indicated that Ki-67 nuclear

antigen might be synthesized de novo early in G_1 phase on mitogen stimulation. Gerdes et al. (1997)[21] found that the total number of G_0 cells decreases constantly in mitogen stimulated cultures and the majority of the cells remain in the cycling compartment. King et al. (1998)[34] stated that recent insights into the mechanisms of the cell cycle will be hopefully of value for designing new forms of treatment for malignant diseases. Ouchi et al. (2000)[35] considered Ki-67 a new predictor of biologic aggressiveness, expressed as actively proliferating cells.

It could be concluded that two independent proliferation markers should be incorporated to ensure reflected cell proliferation and exclude a non specific response. Image cytometric quantitation of PCNA and nuclear antigen reacting with Ki-67 was proved to be a comparable measure in different proliferative skin conditions. Ki-67 could be considered a more potent tool for easy and quick evaluation of growth fraction in proliferative neoplastic conditions affecting the skin. It could be considered a reliable method for exact determination of the proportion of proliferating neoplastic cells. This is suggested to be of prognostic value and might be consequently important in the choice of appropriate therapy.

REFERENCES

1. Millington, P.F. and Wilkinson, R. (1983): Skin. Cambridge University Press (Abstract).
2. Auger, M.J. (1989): Mononuclear phagocytes. British Medical Journal; 298:546.
3. Dinarello, C.A. and Wolff, S.M. (1993): The role of interleukin 1 in disease. New England Journal of Medicine; 328(2):106.
4. Angell, M. (1994): Do breast implants cause systemic disease? New England Journal of Medicine; 330:1748.
5. Mason, D.Y. and Gatter, K.C. (1994): Immuno-histochemistry in histological diagnosis. Recent advances in Histopathology; 16:263.
6. Goldblum, J.R. and Appelman, H.D. (1995): Stromal tumours of the duodenum. A histologic and immuno-histochemical study of 20 cases. Am. J. Surg. Pathol.; 19(1):71.
7. Fan, S.T. (1997): Better survival in women with resected hepatocellular carcinoma is not related to tumour proliferation or expression of hormone receptors. Am. J. Gastroenterol.; 92(8):1355.
8. Matthews, J.B.; Mason, G.I. and Browne, R.M. (1988): Epithelial cell markers and proliferating cells in odontogenic jaw cysts. J. Pathol.; 156(4):283.
9. Fang, J.W.S.; Bird, G.L.A.; Nakamura, T.; Davis, G.L. and Lan, T.Y.N. (1994): Hepatocyte proliferation as an indicator of outcome in acute alcoholic hepatitis. Lancet; 343:820.
10. Graham, R.C. and Karnovsky, M.J. (1967): The early stages of absorption of injected horseradish peroxidase into the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem.; 14:291.
11. Gerdes, J.; Schwab, U.; Lemke, H. and Stein, H. (1983): Production of mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int. J. Cancer; 31:13.
12. Apte, S.S. (1990): Ki-67 monoclonal antibody reacts with a proliferation associated nuclear antigen in the rabbit Oryctolagus cuniculus. Histochemistry; 94(2):201.
13. Wells, S.J.; DeRose, P.B. and Cohen, C. (1996): Image cytometric comparison of proliferating cell nuclear antigen and MIB-1 staining in hepatocellular carcinoma and adjacent liver tissue. Cytometry; 26(3):198.
14. Armitage, P. and Berry, G. (1994): Statistical Methods in Medical Research, 3rd edition, Blackwell Scientific Publications, London: 40.
15. Fantes, P. and Brooks, R. (1994): The cell cycle. A practical approach. I.R.L. Press, Oxford:55.
16. Steel, M. (1995): Telomerase that shapes our ends. Lancet; 345:935.
17. Halprin, K.M. (1972): Epidermal turnover time: A re-examination. J. Invest. Dermatol.; 86:14.
18. Wolfe, S.L. (1993): The cell cycle. In: Molecular and Cellular Biology. Wadsworth, 3rd ed.: 57.
19. Cattoretti, G.; Becker, M. and Key, G. (1992): Monoclonal antibodies against recombinant parts of the Ki-67 antigen detect proliferating cells in microwave-processed formalin-fixed paraffin sections. Journal of Pathology; 68:357.
20. Alberts, B.; Bray, D.; Lewis, J.; Raff, J. and Roberts, L. (1994): Cell cycle. In: Molecular Biology of the Cell, 3rd ed., Garland, New York:57.
21. Gerdes, J.; Lemke, H.; Heinz, B.; Hans, H.W. and Ulrich, S. (1997): Cell cycle analysis of a cell proliferating associated human nuclear antigen defined by the monoclonal antibody Ki-67. Int. J. of Immunology; 133:1710.
22. Champion, R.H.; Burton, J.L. and Ebling, F.J.G. (1992): Textbook of Dermatology, 5th ed., Boston, Mass: Blackwell Scientific Publications Inc.; 2:1384.
23. Zuckerman, A.J.; Banatvala, J.E. and Pattison, J.R. (1994): Viral infections of humans. In: Principles and Practice of Clinical Virology, Wiley, New York: 385.
24. Guillou, P.J. (1995): Adjuvant biological response modifiers after major surgery, trauma and infection. Br. J. Surg.; 85:721.

25. MacSween, R.M.N. and Whaley, K. (1992): The skin. In: Muir's Textbook of Pathology, 13th ed., Edward Arnold, Kent: 1107.

26. Walter, J.B. and Talbot, I.C. (1996): Chronic inflammation. In: General Pathology, 7th ed., Churchill Livingstone, New York: 195.

27. McLean, D.I. and Haynes, H.A. (1987): Cutaneous aspects of malignant disease. In: Dermatology General Medicine, 3rd ed., McGraw-Hill, New York: 1917.

28. Lever, W.F. and Schaumburg-Lever, G. (1975): Histo-pathology of the skin. 5th ed., Philadelphia, Pa; JB Lippincott: 409.

29. Lever, W.F. and Schaumburg-Lever, G. (1989): Histo-pathology of the Skin, 7th ed., Lippincott, Philadelphia: 505.

30. Kohn, K.W.; Jackman, J. and O'Connor, P.M. (1994): Cell cycle control and cancer. Journal of Cellular Biochemistry; 54:440.

31. Haber, D.A. (1995): Telomeres, cancer and immortality. New England Journal of Medicine; 332:955.

32. Darzynkiewicz, Z. and Traganos, F. (1998): Cycling and non-cycling cell populations. In: Genetic Expression in the Cell Cycle, G.M. Padilla, and K.S. McCarty eds., Academic Press, New York: 103.

33. Stein, H.; Bonk, A.; Tolksdorf, G.; Lennert, K. and Rodt, H. (1997): Immunohistologic analysis of the organization of normal lymphoid tissue and non-Hodgkin's lymphomas. Int. J. Cancer.; 30:445.

34. King, K.L.; Hwnag, J.J.; Chan, G.Y.; Tsay, S.H. and Chi, C.W. (1998): Ki-67 expression as a prognostic marker in patients with hepatocellular carcinoma. J. Gastroenterol. Hepatol.; 13(3):273.

35. Ouchi, K.; Sugawara, T.; Ono, H.; Fujiya, T. and Kamiyama, Y. (2000): Mitotic index is the best predictive factor for survival of patients with resected hepatocellular carcinoma. Digestive Surgery; 17:42.