http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (12): 929-933, 2009
ISSN 1028-8880

Expression of P27, Ki67 and P53 in Squamous Cell Carcinoma, Actinic Keratosis and Bowen Disease

[1]S. Talghini, [2]M. Halimi and [2]H. Baybordi
[1]Department of Pathology, Sina Hospital, Ward of Pathology,
[2]Department of Pathology, Imam Reza Hospital, Ward of Pathology,
Tabriz University of Medical Sciences, Tabriz, Iran

Abstract: This study aims at evaluating the expression of P27, Ki67 and P53 in Squamous Cell Carcinoma (SCC), Actinic Keratosis (AK) and Bowen Disease (BD) specimens. In an analytic-descriptive setting, skin biopsy specimens of 45 patients were evaluated in three 15-case groups including BD, AK and SCC specimens. Fifteen normal skin biopsy specimens were obtained and used as the control group. Immunohistochemical staining was performed in all the specimens and the expression rates and patterns of Ki67, P27 and P53 were determined. The results were compared between the four groups. Ki67 was expressed in 0.8, 23.7, 12.3 and 19.3% of the cells in the normal skin, AK, BD and SCC groups, respectively. No significant difference was seen between the three pathological conditions regarding the expression rate of Ki67. P27 was positive in 23.4, 26.2, 25.9 and 4.5% of specimens in the normal skin, AK, BD and SCC groups, respectively. This rate was significantly the lowest in the SCC group. P53 expression was detected in 26.6, 41.8 and 54.6% of the assessed cells in the AK, BD and SCC groups, respectively. There was no expression of P53 in the normal skin specimens. This rate was significantly the highest again in the SCC group. Based on these results, the quantitative and qualitative (pattern of distribution) evaluation of the expressions of Ki67, P27 and P53 may be helpful in differentiating malignant and premalignant epidermal lesions, particularly in unsatisfactory or fragmented specimens.

Key words: Squamous cell carcinoma, actinic keratosis, bowen disease, Ki67, P27, P53

INTRODUCTION

Squamous Cell Carcinoma (SCC) is the second most common skin cancer all over the world which usually arises in sun-exposed areas of skin. This is a malignant tumor of keratinocytes in the epidermis that may occur either primarily or complicate previously presented premalignant lesions like Actinic Keratosis (AK) (Takata and Saida, 2005; Fernández-Figueras *et al*., 2005). Actinic Keratosis is the most common premalignant skin lesion induced by sun exposure (Cockerell, 2000; Salasche, 2000). Bowen Disease (BD) was at first considered as a type of *in-situ* SCC with a potential ability to spread locally. Modern science has rejected this hypothesis (Arlette and Trotter, 2004). In keratinocytic atypias, protrusion of abnormal cells into the reticular dermis and the apparent detachment of individual nests of keratinocytes from the lower layers of the epidermis are generally considered as invasion criteria (Weedon, 2002). Traditional approach of considering the passing of malignant cells through the basement membrane as a distinctive criterion of SCC from AK is not applicable in some conditions in which the basement membrane is not simply definable. A fragmented tissue, presence of severe inflammatory infiltration or a small size specimen may cause difficulty in the interpretations (Lo-Muzio *et al*., 2003). Several cyclin-dependant kinase complexes have important roles in cell-cycle regulation. P27 can inhibit some of these cyclin-dependant kinase complexes. So it may suspend the eukaryotic cells in the G0 phase. On the other hand, monoclonal antibodies against Ki67 have introduced a nuclear antigen known as Ki67 which is in close relation with the activity of cellular proliferation. This antigen is demonstrable during all phases of cellular proliferation (G1, S, G2, M) but not in the resting cells (at G0 phase) (Weedon, 2002). In some studies, assessment of P27 as an index of a dormant cell and Ki67 as an indicator of cell proliferation has been proposed for clarifying true entity of some epidermal atypias with vague diagnosis. Likewise, abnormal or distorted function of P53, a tumor-suppressor gene has been proposed as one of main underlying etiologies of cell cycle abnormality

Corresponding Author: S. Talghini, Department of Pathology, Sina Hospital, Ward of Pathology,
Tabriz University of Medical Sciences, Tabriz, Iran

in many human malignancies (Fernández-Figueras *et al*., 2001). There is not yet a study about simultaneous evaluation of changes related to these three genes in these dermatological conditions (SCC, AK and BD). The goal of this study was investigating the expression of P27, Ki67 and P53 in SCC, AK and BD.

MATERIALS AND METHODS

In this descriptive-analytic study, skin biopsy specimens obtained from 45 patients were evaluated in three groups each one with 15 biopsy specimens with either diagnosis of SCC, AK or BD. Fifteen normal skin biopsy specimens were employed as the controls. Diagnosis of disease was confirmed by two different pathologists. This study was performed in Tabriz Sina Hospital, Iran during a 13-month period (March 1st 2008 to April 1st 2009). In each group the cells were evaluated for positive expression of Ki67 and P27 and presence of immunoreactivity for P53. Paraffin embedded blocks were cut at 6-micron thick slices by microtome and immunohistochemical staining technique (avidine biotin peroxidase) was performed. The prepared specimens were evaluated by light microscope (400X magnification). A positive finding for Ki67 expression was categorized in three patterns: the peripheral pattern in which the positive nuclear staining was seen in the basal and parabasal layers with the granular area spared; the diffused pattern which was defined as the positive nuclear staining in majority of cells in the full-thickness of epithelium; and the confluent or scattered pattern (Weedon, 2002). Labeling index of P27 was calculated by counting the stained and non-stained cells by grid-shaped eyepiece micrometer (Oh and Penneys, 2004). The immunohystochemical staining was performed by applying murine monoclonal antibodies against P53 protein and after that, the percentage of positively stained cells was determined. Accordingly, a dark brown nucleus proposed a P53+cell (Fernández-Figueras *et al*., 2001). In AK, BD and normal skin biopsy specimens, immunohistochemical evaluation of cells were performed separately in the upper two third (surface) and the lower one third (deep) of the epidermis. This approach was employed based on the traditional categorizing of AK in three severity subgroups: mild disease in which the involved area is restricted to the lower one third part of the epidermis; moderate disease in which the lower two third part of the epidermis is involved; and severe disease in which the full-thickness of epidermis is involved (Weedon, 2002). A similar approach was not applicable for the SCC specimens, because clear definition of the mentioned boundaries was not feasible. This study was approved by the ethical committee of Tabriz University of Medical Sciences. The data has shown as Mean±SD deviation and frequency (percent). The SPSS software version 15 was used for statistical comparisons. The One Way ANOVA test or the Contingency tables (Chi-square or Fisher's Exact tests, where appropriate) were used for analyzing the data. The $p \leq 0.05$ was considered statistically significant.

RESULTS

Accordingly, there was not any significant difference between the four groups for the data shown in Table 1.

Ki67: In the surface part of the epidermis, Ki67 was expressed in 73 cells out of 864 in the AK group and in 572 cells out of 4410 in the BD group. No Ki67 expression was documented in the normal skin specimens (Fig. 1). The percentage of Ki67+cells was significantly the highest in the BD group and the lowest in the normal skin biopsy specimens ($p<0.001$). In the deep part of the epidermis, there were 757 cell out of 2632 in the AK group,1511 cells out of 12552 in the BD group and 62 cells out of 6261 in the control group with positive expression for Ki67 (Fig. 1). The percentage of the cells positively stained for Ki67 was the highest in the AK group and the lowest in the control group ($p<0.001$). Comparing the rate of expression of Ki67 in the surface and deep portion of the epidermis in each group showed that this rate was significantly higher in the deep epidermis comparing with the rate in the surface epidermis in the AK and the normal skin specimens ($p \leq 0.001$). There was not a significant difference between the surface and the deep parts of the epidermis for the expression rate of Ki67 in the BD group ($p = 0.15$). In the full thickness of the epidermis of the SCC, AK, BD and normal skin groups there were 942 cells out of 4781, 830 cells out of 3498, 2083 cells out of 16962 and 62 cells out of 7386 positive for

Table 1: Characteristics and general data of patients

Characteristics	SCC	AK	BD	Normal skin	p-value
Gender					
Male	6 (40)	7 (46.7)	10 (66.7)	3 (20)	0.079
Female	9 (60)	8 (53.3)	5 (33.3)	12 (80)	
Age (year)	52.87±7.05	57.33±9.23	59.53±14.28	51.40±16.90	0.258
Disease onset (year)	1.80±0.99	2.53±3.04	2.63±2.29	-	0.553

SCC: Squamous cell carcinoma, AK: Actinic keratosis, BD: Bowen disease. Values In brakets are percentage

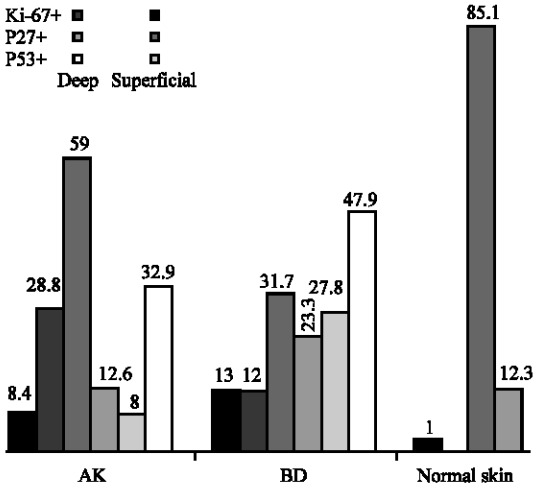

Fig. 1: Percentage of Ki67+, P27+ and P53+cells in one-third superficial and two-third deep layer of epidermis in the studied specimens

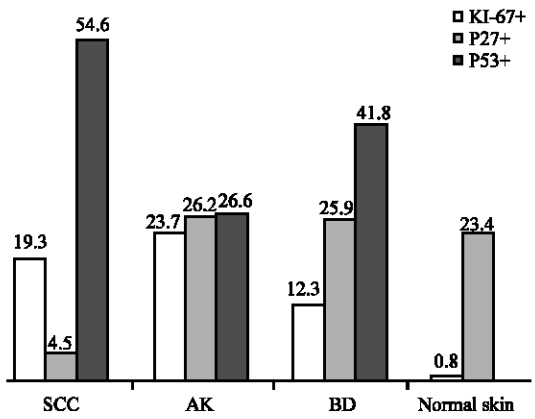

Fig. 2: Percentage of Ki67+, P27+ and P53+cells in epidermis of the studied specimens

Ki67, respectively (Fig. 2). The percentage of Ki67+cells was significantly the highest in the AK group and lowest in the control group ($p<0.001$). The percentage of Ki67+cells was significantly the highest in the AK group comparing with that in the SCC group and the lowest in the deep portions of the epidermis of the BD and the control groups comparing with that in the SCC group ($p<0.001$ for all comparisons). According to mentioned findings, the expression pattern of Ki67 was peripheral, diffused and confluent in the AK, BD and SCC specimens, respectively.

P27: In the surface layer of the epidermis, P27 was expressed in 655 cells out of 1111 in the AK group, 2134 cells out of 6723 in the BD group and 957 cells out of 1125 in the control group (Fig. 1). The percentage of P27+cells was significantly the highest in the normal skin group and the lowest in the BD group ($p<0.001$). In the deep layer of the epidermis, there were 340 cell out of 2691 in the AK group, 3460 cells out of 14841 in the BD group and 772 cells out of 6261 in the control group positively expressed P27 (Fig. 1). The percentage of the cells positive for P27 was significantly the highest in the BD group ($p<0.001$). In all groups of AK, BD and normal skin, the percentages of P27+cells were significantly higher in the superficial portion of the epidermis comparing with those in the deep areas of the epidermis ($p<0.001$ for the AK and BD groups, $p = 0.001$ for the normal skin group). In the full thickness epidermis of the SCC, AK, BD and control groups, there were 355 cells out of 7943, 995 cells out of 3802, 5594 cells out of 21564 and 1729 cells out of 7386 positive for P27 expression, respectively (Fig. 2). The rate of P27+cells was significantly the lowest in the SCC group ($p<0.001$). The percentages of P27+cells were significantly higher in the deep area of the epidermis in the AK, BD and control groups comparing with that in the SCC specimens ($p<0.001$ for all comparisons).

P53: In the surface portion of the epidermis, P53 was expressed in 69 cells out of 861 in the AK group and in 212 cells out of 762 in the BD group. There was not any P53+cell in the normal skin group (Fig. 1). The percentage of P53+cells was significantly the highest in the BD group and the lowest in the control group ($p<0.001$). In the deep layer of the epidermis, there were 839 cell out of 2550 in the AK group and 823 cells out of 1717 in the BD group with positive expression for P53. In the control group, none of the 6261 evaluated cells were positive for P53 expression (Fig. 1). The percentages of the P53+cells were significantly the highest in the BD group and the lowest in the control group ($p<0.001$). In the AK and BD groups, the percentages of P53+cells were significantly higher in the deep portion of epidermis comparing with those in the superficial area (both $p<0.001$). In full thickness epidermis of the SCC, AK and BD groups, there were 3120 cells out of 5712, 908 cells out of 3411 and 1035 cells out of 2479 positive for P53, respectively. There was not any P53+cell among the 7386 cells evaluated in the normal skin specimens (Fig. 2). The percentage of P53+cells was significantly the highest in the SCC group and the lowest in the control group ($p<0.001$). The percentages of P53+cells in the deep portions of the AK, BD and control groups were significantly lower than that in the SCC group ($p<0.001$ for all comparisons). The expression rates of the Ki67, P27 and P53 in the normal skin and the normal lateral surgical margins are summarized in Table 2. Accordingly, there was not any significant difference between the normal skin and the normal lateral surgical

Table 2: Expression rates of P27, Ki67 and P53 in the normal skin and the unaffected lateral surgical margins

Parameter	Normal skin	BD	AK	SCC	P1	P2	P3
P27	20.6	22.2	24	26.9	0.165	0.075	<0.001
Ki67	0.8	1.6	9.2	7	<0.001	<0.001	<0.001
P53	0.0	0.0	0	0	1	1	1

SCC: Squamous cell carcinoma, AK: Actinic keratosis, BD: Bowen disease, 1: Normal*BD, 2:Normal*AK, 3:Normal*SCC

margins in the SCC, AK and BD groups regarding the rates of expression of P27 and P53; however, the rate of expression of Ki67 was significantly higher in the normal lateral surgical comparing with the specimens obtained from healthy cases. The rates of expression of P27 were 10% (308 cells out of 3072) and 0.3% (8 cells out of 3072) in the well differentiated areas of the SCC specimens. These rates were significantly higher in the deep portions of the epidermis in the AK ($p = 0.002$ for P27 and <0.001 for Ki67), BD ($p<0.001$ for P27 and Ki67) and normal skin groups ($p<0.001$ for P27 and Ki67) comparing with those in the well differentiated areas of the SCC specimen.

DISCUSSION

In this study, the expression rates of Ki67, P27 and P53 were evaluated in the AK, BD, SCC and normal skin specimens. Ki67 was positive in 0.8, 23.7, 12.3 and 19.3% of the studied specimens, respectively. Accordingly, it seems that the quantitative evaluation of this parameter may not be usefull for differentiating the malignant and premalignant conditions. In a study by Tilli *et al.* (2003), the expression rate of Ki67 was evaluated in the normal skin, AK and SCC specimens. The related percents were 11, 31 and 18%, respectively. In another series by Hirai *et al.* (2001), the expression rate of Ki67 was not different between the BD and SCC specimens. Likewise, the expression rate of Ki67 was lower in the normal skin. Oh and Penneys (2004) evaluated the rate and the pattern of Ki67 expression in the specimens of BD, AK and SCC. It was concluded that the distribution pattern of Ki67+cells may distinguish these three entities. The Ki67+cells were reported to be scattered in the full-thickness of epidermis in the BD specimens. On the other hand, the whole epidermis was not involved in the SCC and AK specimens. Bordbar *et al.* (2007) evaluated 14 specimens of normal skin, 15 specimens of AK, 10 specimens of BD and 7 specimens of SCC for the distribution pattern of Ki67+cells in the epidermis. Aggregation of Ki67+cells was reported in the basal and parabasal areas in the normal skin, in the basal, suprabasal and mid-epidermis in the AK specimens, the full-thickness epidermis in the BD specimens and discretely in the SCC specimens. In the current study, the pattern of Ki67 expression was the same reported in the mentioned studies in three pathological conditions. The specimens were separately evaluated in superficial and deep areas of the epidermis in AK, BD and normal skin specimens. In the superficial area, the density of Ki67+cells was significantly higher in the BD group comparing with the AK group and in the deep areas, vice versa. This means that a higher aggregation of Ki67+cells in the upper layer of epidermis is in favor of diagnosis of BD. So, the qualitative assessment (distribution pattern) of Ki67 is useful for diagnosis of BD, in particular. Likewise, higher rate of Ki67 expression in the cells of basal and parabasal areas of the epidermis is in favor of diagnosis of AK. Further studies are recommended, particularly for the quantitative use of Ki67+cells in this regard. In the current study, P27 was positive in 23.4, 26.2, 25.9 and 4.5% of the cells in normal skin, AK, BD and SCC specimens, respectively. In other words, quantitative evaluation of P27 expression may clearly distinguish between SCC and other premalignant conditions of skin (the lower rate of P27+cells, the higher probability of malignancy). Oh and Penneys (2004) reached the same conclusion, as well. Comparing the superficial and deep areas of the epidermis, the aggregation of P27+cells was significantly higher in the deep area of BD and the surface area of normal skin specimens. Further evaluation showed that the rate of P27+cells is significantly higher in any given layer of the epidermis in AK, BD and normal skin groups comparing with that in SCC specimen. So, without considering the vertical level of epidermis, quantitative assessment of P27+cells can be beneficial for distinguishing between malignancy and nonmalignant conditions. Further studies with larger sample sizes are needed for determining the optimal cut-off point of the number of P27+cells in this regard. In the current study, P53 was positive in 26.6, 41.8 and 54.6% of cells in AK, BD and SCC groups, respectively. This parameter was not detectable in the normal skin. Based on these findings, P53+cells were significantly more prevalent in SCC group. Park *et al.* (2004) evaluated the rate of P53+cells in SCC and AK specimens. In this study, all the cells of SCC specimen were positive for P53, while the P53+cells were discrete in AK group. Lo-Muzio *et al.* (2001) showed that the rate of P53+cells was significantly higher in SCC and AK specimens comparing with other skin lesions. The results of the current study are also in line with the others; i.e. higher rates of P53+cells is possibly an indicator of skin malignancy. The P53+cells were detected more commonly in surface and deep layers of the epidermis in BD group comparing with that in AK and normal skin specimens. There is not a similar study about the pattern of distribution of P53+cells in these conditions. In conclusion, this study showed that quantitative measuring of P53+ and P27+cells can frankly help in distinguishing SCC from other premalignant conditions of

the skin. The distribution pattern of the Ki67+cells is also helpful for this purpose; however it may not be helpful in the fragmented or insufficiently obtained specimens without the basement membrane. So, in the later conditions, evaluating cells for P53 and P27 expression in the first steps of histopathological study may be of great value for planning the next approach. This guideline could eliminate the need of further biopsies.

REFERENCES

Arlette, J.P. and M.J. Trotter, 2004. Squamous cell carcinoma in situ of the skin: History, presentation, biology and treatment. Aust. J. Dermatol., 45: 1-9.

Bordbar, A., D. Dias, A. Cabral, S. Beck and M.E. Boon, 2007. Assessment of cell proliferation in benign, premalignant and malignant skin lesions. Applied Immunohistochem. Mol. Morphol., 15: 229-235.

Cockerell, C.J., 2000. Histopathology of incipient intraepidermal squamous cell carcinoma ("actinic keratosis"). J. Am. Acad. Dermatol., 42: 11-17.

Fernández-Figueras, MT., A. Casalots, L. Puig, R. Llatjós, C. Ferrándiz and A. Ariza, 2001. Proliferating trichilemmal tumour: p53 immunoreactivity in association with p27Kip1 over-expression indicates a low-grade carcinoma profile. Histopathology, 38: 454-457.

Fernández-Figueras, M.T., L. Puig, E. Musulen, M. Gilaberte, C. Ferrándiz, E. Lerma and A. Ariza, 2005. Prognostic significance of p27Kip1, p45Skp2 and Ki67 expression profiles in Merkel cell carcinoma, extracutaneous small cell carcinoma and cutaneous squamous cell carcinoma. Histopathology, 46: 614-621.

Hirai, K., M. Kumakiri, S. Fujieda, H. Sunaga and L.M. Lao, 2001. Expression of granulocyte colony-stimulating factor and its receptor in epithelial skin tumors. J. Dermatol. Sci., 25: 179-188.

Lo-Muzio, L., S. Staibano, G. Pannone, M.D. Mignogna and A. Mariggiò *et al*., 2001. Expression of the apoptosis inhibitor survivin in aggressive squamous cell carcinoma. Exp. Mol. Pathol., 70: 249-254.

Lo-Muzio, L., G. Pannone, R. Leonardi, S. Staibano and M.D. Mignogna, 2003. Survivin, a potential early predictor of tumor progression in the oral mucosa. J. Dent. Res., 82: 923-928.

Oh, C.W. and N. Penneys, 2004. P27 and mib1 expression in actinic keratosis, Bowen disease and squamous cell carcinoma. Am. J. Dermatopathol., 26: 22-26.

Park, H.R., S.K. Min, H.D. Cho, K.H. Kim, H.S. Shin and Y.E. Park, 2004. Expression profiles of p63, p53, survivin and hTERT in skin tumors. J. Cutan. Pathol., 31: 544-549.

Salasche, S.J., 2000. Epidemiology of actinic keratoses and squamous cell carcinoma. J. Am. Acad. Dermatol., 42: 4-7.

Takata, M. and T. Saida, 2005. Early cancers of the skin: clinical, histopathological and molecular characteristics. Int. J. Clin. Oncol., 10: 391-397.

Tilli, C.M., F.C. Ramaekers, J.L. Broers, C.J. Hutchison and H.A. Neumann, 2003. Lamin expression in normal human skin, actinic keratosis, squamous cell carcinoma and basal cell carcinoma. Br. J. Dermatol., 148: 102-109.

Weedon, D., 2002. Skin Pathology. 2nd Edn., Churchill Livingstone, New York, ISBN: -13: 978-0443070693.