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Evaluation of immunohistochemical markers of p16 and p53 in patients with pathological diagnoses of actinic keratosis, Bowen's disease, and seborrheic keratosis; a cross-sectional study from Alzahra hospital, Isfahan, Iran (2018-2022)

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Abstract

Introduction: While Bowen's disease (BD) and actinic keratosis (AK) are premalignant conditions, seborrheic keratosis (SK) is a benign lesion; therefore, the differentiation of these processes is of clinical importance.
Objectives: This study aimed to assess the P16 and P53 markers expression in patients with pathological diagnoses of AK, BD, and SK.
Materials and Methods: This cross-sectional study included all patients with the final pathological diagnosis of AK, SK, and BD between 2018 and 2022 in Al-Zahra hospital, Isfahan, Iran. Immunohistochemical staining using the EnVision method was done for all samples. The positive results of the P16 marker were interpreted as the block staining of the nucleus and cytoplasm, and in the case of the P53 marker, the positive results were interpreted as the block staining of the nucleus. Finally, we investigated and compared the expression pattern of P53 and P16 markers in three groups of BDs, SK, and AK.
Results: The expression patterns of p16 and p53 varied notably among patients with AK, SK, and BD. In cases of AK and SK, p16 predominantly exhibited null or mosaic patterns, while BD was characterized by a block pattern. For p53, both AK and BD were more likely to display null or strong patterns, whereas SK was more frequently associated with a mosaic pattern. The diagnostic value of these markers indicated that p53 and p16 patterns could effectively assist in distinguishing BD from SK and AK.
Conclusion: The distinct expression patterns of p16 and p53 in AK, SK, and BD underscore their potential as valuable diagnostic biomarkers. The differences in p16 and p53 patterns can aid in accurately differentiating between benign and precancerous lesions, supporting their incorporation into diagnostic protocols to improve patient management and treatment outcomes.

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Introduction

Skin diseases encompass a wide range of conditions affecting the skin, and can significantly impact individuals' health and quality of life. The epidemiology of skin diseases indicates a substantial global burden, with over 4.8 billion new cases reported, primarily due to fungal (34%) and bacterial (23%) infections, alongside conditions like dermatitis and acne that contribute to significant disability-adjusted life years (1). Skin diseases can be broadly categorized into infectious and non-infectious types, with the former including bacterial infections like cellulitis and impetigo, viral infections like herpes and warts, and fungal infections like ringworm and candidiasis, while the latter encompasses allergic reactions such as eczema and hives, autoimmune diseases like psoriasis, parasitic infestations like scabies, and skin cancers (2).

Actinic keratosis (AK), seborrheic keratosis (SK), and Bowen's disease (BD) are three types of skin disease (3); AK is a precancerous skin condition caused by prolonged sun exposure that appears as rough, scaly patches on sun-exposed areas (4,5), while SK is a common, noncancerous skin growth that often appears as people age, varying in color from light tan to black with a waxy, "stuck-on" appearance (6).

Key point

The implications of these results are significant for clinical practice, as the distinct expression patterns of p16 and p53 can enhance the diagnostic accuracy for differentiating between actinic keratosis (AK), seborrheic keratosis (SK), and Bowen's disease (BD). By integrating immunohistochemical analysis of these markers into routine diagnostic protocols, healthcare providers can improve the early identification of precancerous lesions, leading to timely interventions and better patient outcomes. Furthermore, this approach may facilitate more tailored treatment strategies, reducing the risk of misdiagnosis and ensuring that patients receive appropriate management based on the specific nature of their skin condition. Overall, the findings support the potential for p16 and p53 as reliable biomarkers in dermatopathology, ultimately contributing to improved care for patients at risk of skin cancer.

Bowen's disease, also known as squamous cell carcinoma in situ, is a precancerous skin condition that can develop into invasive squamous cell carcinoma if left untreated, appearing as a red, scaly patch or plaque (7).

P16 and P53 are critical immunohistochemical markers used in the diagnosis and prognosis of various cancers, particularly in assessing the presence of malignancies and their potential for recurrence (8,9). p16, a cyclin-dependent kinase inhibitor, is often overexpressed in tumors associated with high-risk human papillomavirus infections, making it a valuable marker for cervical and oropharyngeal cancers (10). In contrast, p53, a tumor suppressor protein, is involved in regulating the cell cycle and preventing tumor formation; its abnormal expression is frequently linked to various malignancies, including breast and lung cancers, and serves as an indicator of poor prognosis in head and neck squamous cell carcinoma and other cancers such as skin neoplasm (11). Studies have shown that while both markers can provide insights into tumor behavior, their sensitivity and specificity can vary, necessitating their use in conjunction with other diagnostic tools to improve accuracy in clinical practice (12). Therefore, in this study, we aimed to assess and compare the expression profiles of the immunohistochemical markers p16 and p53 in patients diagnosed with AK, BD, and SK. By analyzing the frequency distribution of p16 and p53 expression levels in these skin conditions, we seek to determine the potential utility of these markers in differentiating between precancerous (AK and BD) and benign (SK) lesions. The findings from this investigation may contribute to enhancing the diagnostic accuracy and management strategies for these skin diseases.

Objectives

The objective of this study was to evaluate and compare the expression patterns of immunohistochemical markers p16 and p53 in patients diagnosed with AK, BD, and SK. By analyzing the frequency distribution of p16 and p53 expression, the study aimed to investigate the potential association between these markers and the different types of skin diseases.

Method and Materials

Study design and participants

This cross-sectional study aimed to evaluate the expression patterns of immunohistochemical markers p16 and p53 in patients diagnosed with AK, BD, and SK at Alzahra hospital, Isfahan, Iran, from 2018 to 2022. The study population included patients with pathological diagnoses of AK, BD, and SK who underwent treatment at Alzahra hospital during the specified period. All patients with available paraffin-embedded tissue blocks and complete medical records were eligible for inclusion in the study.

Sample size

A total of 150 patients were included in the study, with 50 patients in each disease group (AK, BD, and SK patients). The sample size was determined based on the availability of tissue samples and medical records during the study period.

Data collection

Patient data, including demographic characteristics (age and gender), sample slides, and immunohistochemical staining results for p16 and p53, were collected from medical records and pathology samples. The examination was performed by two pathologists. Paraffin cast samples were stained and analyzed by immunohistochemistry. Six-micron sections were prepared from the desired blocks, and immunohistochemical staining (Master Diagnostica, Spain) was performed using the EnVision method for each of these sections. Then, the prepared slides were examined using an optical microscope with a magnification of 400. The positive results of the P16 marker were interpreted as the brown staining of the nucleus and cytoplasm, and in the case of the P53 marker, the positive results were interpreted as the brown staining of the nucleus. The severity of AK was classified into three groups, KIN I, KIN II, and KIN III, depending on the degree of involvement of the epidermis by atypical keratinocyte cells. In this study, KIN II and KIN III cases were compared with BD by the mentioned markers due to their histological similarity. Finally, the expression patterns of p16 (Null, Mosaic, and Block) and p53 (Null/Strong and Mosaic) were evaluated and compared among the three disease groups.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences) version 27 (IBM Corp, USA). Descriptive statistics were used to summarize the demographic characteristics and expression patterns of p16 and p53. Chi-square tests were conducted to compare the frequency distribution of gender and p16/p53 expression patterns among the disease groups. Additionally, ANOVA was employed to compare the mean age between the groups. A P value of less than 0.05 was considered statistically significant.

Results

Demographic characteristics frequency distribution including age and gender showed no statistically significant difference between the three groups of patients with AK, SK, and BD ($P > 0.05$). For gender, among females, there are 18 patients (36%) with AK, 15 patients (30%) with SK, and 14 patients (28%) with BD. Among males, there are 32 patients (64%) with AK, 35 patients (70%) with SK, and 36 patients (72%) with BD. Regarding age, the mean age for AK patients was 70.28 years (SD; 14.02), for SK patients was 67.66 years (SD; 12.23), and for BD patients was 66.00 years (SD; 17.62) (Table 1).

The frequency distribution of p16 and p53 patterns differed significantly among AK, SK, and BD patients. For p16, the majority of AK and SK cases exhibited null or mosaic patterns, while BD predominantly showed a block pattern. Regarding p53, AK, and BD were more likely to have null/strong patterns, whereas SK was more often associated with a mosaic pattern (Table 2).

Figure 1 illustrates the frequency distribution of p53 expression patterns among patients diagnosed with AK, SK, and BD. It shows that the expression of p53 (null/strong) is significantly higher in patients with BD compared to those with SK and AK. Specifically, the frequency of null/strong p53 expression is observed in 43 patients with BD, while it was presented in 28 of those with AK and only 13 of SK patients. Conversely, weak p53 (mosaic) expression was found in BD cases (n = 7), compared to high expression in SK and AK with 37 and 22 cases, respectively.

Figure 2 depicts the expression patterns of P16 (null, mosaic, block) among patients with AK, SK, and BD. In patients with AK, 8 cases exhibited block expression, and 21 patients showed mosaic and null expression, equally. For SK disease, 17 cases demonstrated null expression, 30 mosaic expression, and only three patients displayed block expression. In BD patients, the majority of patients demonstrated block expression (n= 37), while only 5 and 8 patients showed mosaic and null expression.

In Table 3 the diagnostic value of p16 and p53 patterns in distinguishing between AK, BD, and SK was assessed

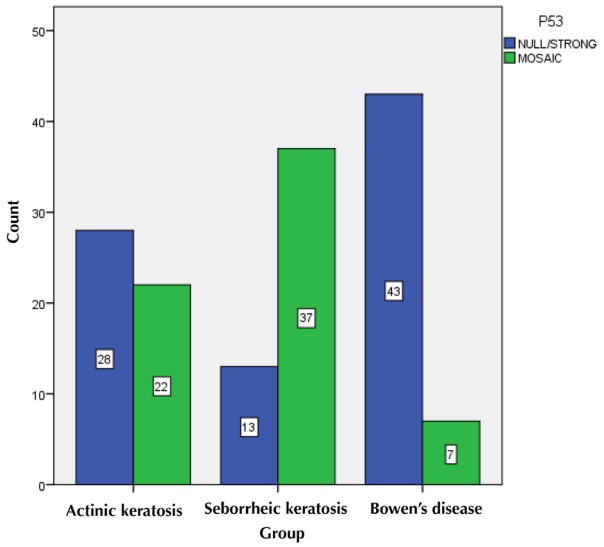

Figure 1. Comparison of p53 patterns frequency distribution between patients with actinic keratosis, seborrheic keratosis, and Bowen's disease

Table 1. Demographic frequency distribution in three groups of patients

Variable			Disease			*P* value
			AK	SK	BD	
Gender	Female	No.	18	15	14	0.668*
		%	36	30	28	
	Male	No.	32	35	36	
		%	64	70	72	
Age (year)		Mean	70.28	67.66	66.00	0.348**
		SD	14.02	12.23	17.62	

SD, Standard deviation; BD, Bowen's disease; AK, Actinic keratosis; SK, Seborrheic keratosis.
*Chi-square test; ** ANOVA test.

Table 2. Frequency distribution of p16 and p53 patterns in three groups of patients

Variable		AK (n=50)		SK (n=50)		BD (n=50)		*P* value
		No.	%	No.	%	No.	%	
P16	Null	21	42	17	34	8	16	<0.001*
	Mosaic	21	42	30	60	5	10	
	Block	8	16	3	6	37	74	
P53	Null/strong	28	56	13	26	43	86	<0.001*
	Mosaic	22	44	37	74	7	14	

BD, Bowen's disease; AK, Actinic keratosis; SK, Seborrheic keratosis.
*Chi-square test.

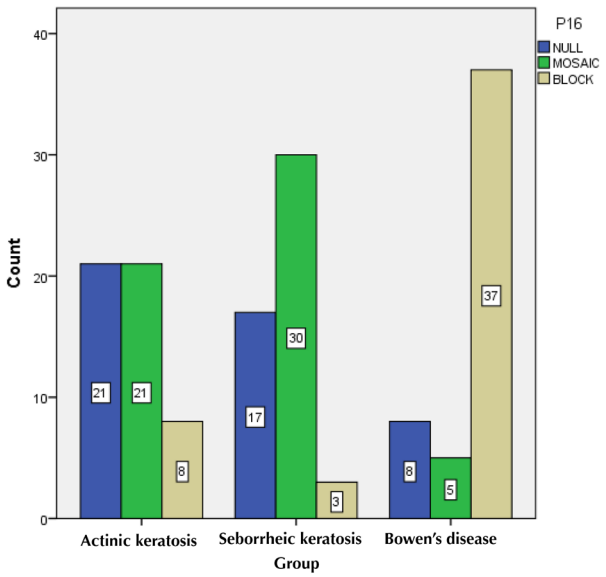

Figure 2. Comparison of p16 patterns frequency distribution between patients with actinic keratosis, seborrheic keratosis, and Bowen's disease

through various metrics. For the comparison of BD versus SK and AK, the sensitivity of p53 (null/strong) was 86%, with a specificity of 59%, a positive predictive value (PPV) of 2.1, and a negative predictive value (NPV) of 0.24. In contrast, p16 (block) demonstrated a sensitivity of 74%, a specificity of 89%, a PPV of 6.7, and an NPV of 0.29. When comparing SK versus BD and AK, p53 (mosaic) showed a sensitivity of 74%, specificity of 71%, a PPV of 2.5, and an NPV of 0.37, while p16 (mosaic) had a sensitivity of 60%, specificity of 74%, a PPV of 2.3, and an NPV of 0.54.

Discussion

The results of this study showed that the expression patterns of p16 and p53 showed significant variation among patients diagnosed with AK, SK, and BD. In AK and SK cases, p16 primarily demonstrated null or mosaic patterns, whereas BD patients was marked by a block pattern. Regarding p53, both AK and BD patients tended to exhibit null or strong patterns, while SK cases were more commonly linked to a mosaic pattern. The diagnostic significance of these markers suggests that the expression patterns of p53 and p16 can effectively aid in differentiating BD from AK and SK.

Previous studies showed that p53, a nuclear protein, acts as a guardian of the genome and inhibits cancer development by triggering programmed cell death or halting the cell cycle in response to external factors that can lead to DNA damage (13). When DNA is damaged, p53 protects the genome by coordinating multiple DNA damage response mechanisms, such as activating the expression of DNA repair proteins DDB2 and XPC. Moreover, p53 can also induce apoptosis in cells with irreparable DNA damage (14). p16, a protein that inhibits the growth of tumors, is produced by the CDKN2A gene located in the 9p21 region. It is part of the retinoblastoma (Rb) pathway and halts the cell cycle's progression in the G1 phase, preventing entrance into the S phase. Cell cycle and tumor growth occur continuously while p16 is active. UV radiation has been demonstrated to be a source of p16 mutations (13).

Few studies have examined the immunohistochemical patterns of p16 and p53 in AK, SK, and BD. However, other research has investigated additional markers, such as p63. The results indicated that a significant proportion of BD cases exhibited extensive staining with p63, while a lesser extent of staining was observed in AK cases. This variation may be due to differences in the antibodies and scoring methodologies used (15,16). Consistent with the current findings, a study by Genders et al found that p16 exhibited a block pattern in BD cases, while AK cases showed no pattern. This suggests that the p16 block pattern may be a useful marker in distinguishing BD from AK (17). In contrast, another study in 2010 by Bagazgoitia et al found that both BD and bowenoid AK (bAK) cases were positive for p16, showing a similar immunostaining pattern, while non-bowenoid AK did not exhibit p16 staining. This indicates that p16 may not be a reliable marker for differentiating BD from bAK, as the study suggests these conditions have a common pathogenic mechanism (18). Additionally, a 2023 study by Balcere et al examining the expression of p53, p63, p16, and other markers in AK and normal skin found that AK cases had weak to no staining for p53, in line with the current results. However, this study did not include BD or SK samples for comparison (19).

In summary, p53 and p16 are critical tumor suppressor proteins that work through different mechanisms to maintain genomic stability and prevent cancer development. Meanwhile, p53 coordinates DNA damage response and apoptosis, while p16 regulates cell cycle progression at the G1/S checkpoint. The diagnostic significance of p16 and p53 expression patterns is underscored by their potential to differentiate between AK, SK, and BD. The distinct expression profiles observed in this study suggest that p16 and p53 can serve as reliable biomarkers for distinguishing between these skin diseases, particularly

Table 3. The diagnostic value p16 and p53 patterns in the diagnosis of actinic keratosis, Bowen's disease, and seborrheic keratosis diseases

Variable		Sensitivity (%)	Specificity (%)	PPV	NPV
BD versus SK and AK	p53 (Null/strong)	86%	59%	2.1	0.24
	p16 (Block)	74%	89%	6.7	0.29
SK versus BD and AK	p53 (Mosaic)	74%	71%	2.5	0.37
	p16 (Mosaic)	60%	74%	2.3	0.54

PPV, Positive predictive value; NPV, Negative predictive value; AK, Actinic keratosis; SK, Keratosis diseases, BD; Bowen's disease.

in cases where clinical and histopathological features may overlap. The findings also highlight the importance of immunohistochemical analysis in the diagnostic process. By utilizing these markers, clinicians can better stratify patients based on their risk for progression to malignancy, particularly in the context of AK and BD.

In conclusion, the variations in p16 and p53 expression patterns among AK, SK, and BD patients not only contribute to our understanding of the pathogenesis of these conditions but also enhance the diagnostic capabilities available to dermatopathologists.

Overall, while the current findings align with some previous studies regarding the utility of p16 and p53 patterns in distinguishing BD from AK, there are also discrepancies in the literature. More research is needed to establish the reliability and consistency of these markers across larger sample sizes and standardized scoring methods. Incorporating additional markers, such as p63, may also improve diagnostic accuracy when differentiating between these squamous-proliferative skin disorders.

Conclusion

In conclusion, the distinct expression patterns of p16 and p53 among patients with AK, SK, and BD highlight their potential utility as diagnostic biomarkers in clinical practice. The predominant null or mosaic patterns of p16 in AK and SK, contrasted with the block pattern observed in BD, along with the varying p53 expression patterns, suggest that these markers can provide valuable insights into the differentiation of these skin conditions. Consequently, the findings support the incorporation of p16 and p53 immunohistochemical analysis into diagnostic protocols, enhancing the accuracy of distinguishing between benign and precancerous lesions, and ultimately aiding in the management and treatment of affected patients.

Limitations of the study

This is a cross-sectional study, which was conducted on a limited number of patients. We suggest further investigations on this subject with larger sample size.

Authors' contribution

Conceptualization: Maryam Derakhshan and Parto Nasri.
Data curation: Parto Nasri and Pegah Hedayat.
Formal analysis: Narges Motamedi.
Investigation: Marzieh Derakhshan and Shahnaz Eskandari.
Methodology: Narges Motamedi and Arman Goudarzi Nezhad.
Project management: Parto Nasri.
Resources: All authors.
Supervision: Maryam Derakhshan.
Validation: Arman Goudarzi Nezhad and Pegah Hedayat.
Writing–original draft: All authors.
Writing–reviewing and editing: All authors.

Conflicts of interest

The authors declare no conflict of interest.

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Ethical issues

The research was conducted in accordance with the principles of the Declaration of Helsinki.. This study resulted from the pathology residential thesis of Parto Nasri (Thesis #3401624), with the Ethical code (IR.MUI.MED.REC.1402.283; https://ethics.research.ac.ir/EthicsProposalView.php?&code=IR.MUI.MED.REC.1402.283), approved by the Isfahan University of Medical Sciences, Isfahan, Iran. Accordingly, written informed consent was taken from all participants before any intervention. Besides, the authors have ultimately observed ethical issues (including plagiarism, data fabrication, and double publication).

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