Journal of Pakistan Association of Dermatologists. 2022;**32(4)**:676-682.

Original Article

Can Ki-67 immunohistochemistry marker differentiate mycosis fungoides from cutaneous lichen planus?

Kambiz Kamyab Hesari[1], Negar Ramezanizoorab[2], Mazaher Ramezani[3,4], Masoud Sadeghi[5], Mansour Rezaei[6], Sedigheh Khazaei[4]

[1] Department of Pathology, Razi Hospital, Tehran University of Medical Sciences, Tehran, Iran.
[2] Students Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[3] Molecular Pathology Research Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[4] Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[5] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran 1416753955, Iran.
[6] Departments of Biostatistics, Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Abstract

Objective The purpose of this study was to evaluate the immunohistochemical expression of Ki-67 marker in Mycosis Fungoides (MF) and Lichen Planus (LP) lesions.

Methods This cross-sectional analytical study included patients undergoing skin biopsy with their first clinical and pathologic diagnosis of MF and cutaneous LP by Ki-67 immunohistochemistry (IHC). Sixty-two patients with cutaneous LP and MF were studied in two groups of 39 and 23 patients, respectively. Linear regression was used for the possible correlation between age and Ki-67 index (%) in both LP and MF patients.

Results The results of this study found that Ki-67 was expressed in 100% of both MF and LP lesions, and no significant correlation was found between Ki-67 and either of the two types of diseases (p=0.606). The results of this study also showed that expression of Ki-67 marker was more than 25% in both types of diseases in 70% of the population, which seems not to be directly related to malignancy.

Conclusion IHC with proliferative marker Ki-67 is not a valuable technique for differentiating cutaneous LP from MF lesions alone.

Key words
Cutaneous diseases, lichen planus, mycosis fungoides, immunohistochemistry marker.

Introduction

Lichen planus (LP) is a chronic and common skin disease that is slightly more prevalent in women which was first described by Erasmus Wilson from England.[1,2] The prevalence of LP has been reported to be between 0.5 and 1% of the population.[3,4] Most patients with LP are middle-aged adults with the mean age at diagnosis of 55 years and it is rarely seen in children.[5] A recent review estimated mean age of 46.4 years at the onset.[6] Its symptomatic treatment includes topical and systemic steroids.[7-9]

Mycosis Fungoides (MF) is a malignant neoplasm of T lymphocyte origin[10,11] and has an

Address for correspondence
Mazaher Ramezani
Molecular Pathology Research Center,
Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran.
Email: mazaher_ramezani@yahoo.com

incidence of about 1 in 300,000 per year, which affects all races. MF is generally a very slow, chronic, and four-stage disease. In the early stages (patch stage), there are macular lesions or patches that can have a size of 1 to 5 cm or more. Plaque lesions are more infiltrated and may look like psoriasis, subacute dermatitis, or granulomatous skin lesions. The tumor stage is characterized by large nodules that differ in shape and size and appear on the plaque of healthy skin.[12,13] The primary pathologic feature of MF is lymphocytic infiltration in the papillary dermis. When patients progress to the plaque stage, epidermotropism appears. Occasionally, at this stage the infiltration of the histiocytes is seen, which is called the interstitial MF. At higher stages, the epidermotropic disease disappears, and large cell transformation appears which may give rise to a poor prognosis.[14]

One theory for more than 20 years in relation to MF was that the disease is associated with an antigen that causes the possible conversion of benign lymphocytes to low-grade T-cell lymphoma.[14] This is an interesting theory, but the antigen or antigens responsible have not yet been identified for this conversion. In the early stages of MF, clinical differential diagnoses include psoriasis, allergic contact dermatitis, fungal infections, and atopic dermatitis. Any disease with chronic polymorphic plaques, especially with pelvic girdle involvement, should undergo biopsy and histologic examination.[14,15] Several biopsies are needed in challenging cases for differentiation of benign conditions from malignancy.[16-18] In fact, variants and atypical forms of MF may be in differential diagnosis of many benign skin disorders, and this disease is a great imitator.[19-21] Sometimes gene analysis methods are used for differentiation.[22]

Treatment modalities in MF are different based on the stage of the disease. Either skin-directed or systemic therapy, these therapies can involve topical steroids, phototherapy, imiquimod, bexarotene, nitrogen mustard gels, and local radiation. Systemic therapy consists of methotrexate, oral bexarotene, interferons, histone deacetylase inhibitors, photopheresis, monoclonal antibodies, chemotherapy and even stem cell transplantation.[23,24] Ki-67 is a monoclonal antibody that reacts with the nuclear antigen expressed in the dividing cells. Its coloring is mainly nuclei as the Ki-67 gene is located on the long arm of chromosome 10 (10q25) in human.[25-27] The product of this gene is a non-histone protein (proliferating cell nuclear antigen: PCNA) that has a half-life of about 60 to 90 minutes.[28,29] This protein can be detected in dividing cells by immunohistochemistry (IHC). PCNA occurs in the late stages of M, G2, S, and G1 of the cell division cycle, whereas this protein is not visible in the G0 phase. Ki-67 as the most common marker indicates the cell division and is reliable.[30] The cellular proliferation rate evaluated by Ki-67 immunoreactivity has been surveyed as a prognostic marker in several malignant neoplasms and has been shown to correlate with tumor grade and clinical course.

Although Ki-67 is considered a poor prognostic factor in cancer, recent studies suggest that in patients with this protein being positive, the use of anti-inflammatory drugs is effective for their treatment and determination. This marker is recommended before chemotherapy by IHC.[31,32] Since MF is the most common form of cutaneous involvement of T-cell lymphoma, which is generally characterized by itchy skin lesions, its diagnosis is mainly by biopsy and pathologic diagnosis. Given the similarity of the early stages of the disease with other diseases such as LP, psoriasis, and eczema, it requires multiple biopsies and pathologist skills. Given the possibility of malignancy being spread to other organs and different treatments available,

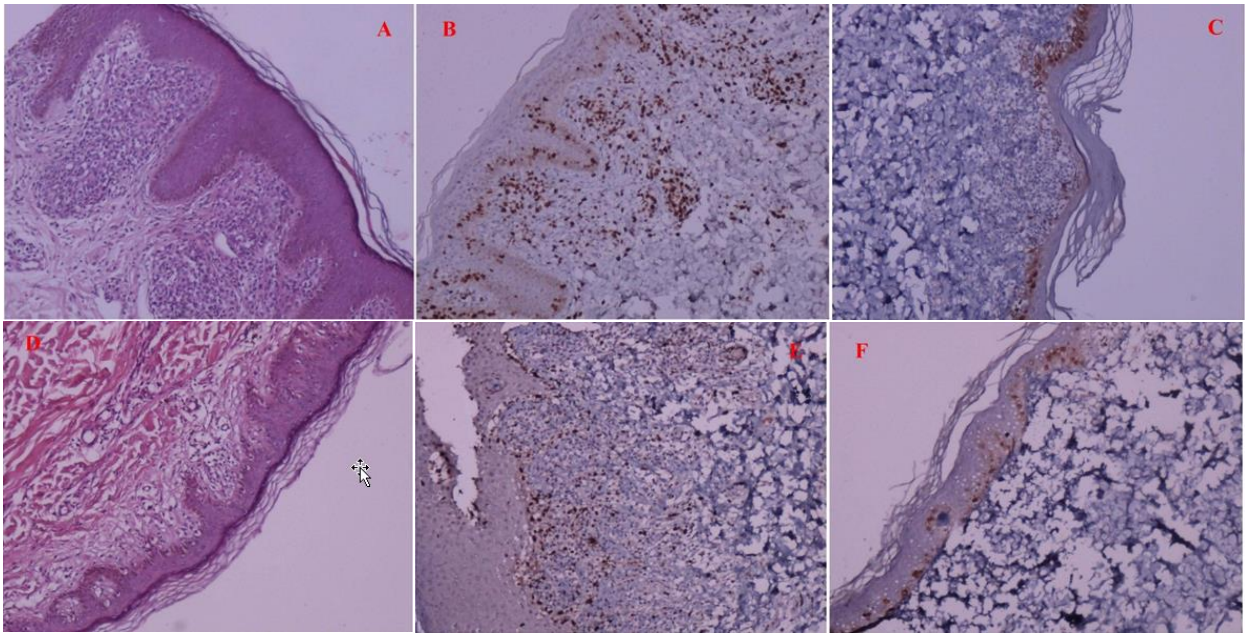

Figure 1 Hematoxylin-Eosin staining and Immunohistochemistry (IHC): A) Mycosis fungoides (MF), tumor stage; Hematoxylin-Eosin staining. B) IHC of MF, 80% nuclear positivity. C) IHC of MF, 1% nuclear positivity. D) Lichen planus (LP), Hematoxylin-Eosin. E) IHC of LP, 50% nuclear positivity. F) IHC of LP, 1% nuclear positivity. All magnifications were "x100".

the diagnosis and stage of the disease have great importance. Due to the difficulty of diagnosis and differentiation of the disease, in this study, the IHC study of Ki-67 marker was performed to find a better way to differentiate MF from LP disease and benign lesions as well as evaluating different treatments.

Materials and methods

The ethics committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (Code: IR.KUMS.REC.1396.443) approved this study. In an analytical cross-sectional study, 62 patients with cutaneous LP and MF who referred to Mahdieh Clinic, Imam Reza Hospital of Kermanshah, and Razi Hospital of Tehran were studied in two groups of 39 and 23 patients, respectively. Inclusion criteria were initial clinical diagnosis and pathology of MF and LP. The patients undergoing skin biopsy and their initial clinical diagnosis and pathology of MF or LP were reapproved by a dermatopathologist.

IHC After confirmation of the diagnosis, 4-micron cuts were prepared from paraffin blocks, and IHC staining was performed with Ki-67 marker. Rabbit Anti-Human Ki-67 Monoclonal Antibody (Clone SP6) of master diagnostic Granada was used (ready-to-use MAD-000310QD-3). IHC positive control was tonsil, and visualization was nuclear according to the manufacturer's brochure. The counts of stained lymphocytes' nuclei were then estimated in dermoepidermal junction in the whole specimen in multiple high power fields. Quantitative evaluation of positive cells for Ki-67 was performed only by the observer. Only the positive ones were distinguished from the negative ones, and no staining intensity was considered. The characteristics of round nuclei that were just below the epidermis were counted in the upper dermis and were considered as a percentage of positive brown staining. Staining cut-off was negative at a mean of 25% or less and positive above 25%[33] (**Figure 1**).

Statistical analysis A *p*-value of <0.05 showed

the values were statistically significant. SPSS 16 software was used for this purpose, and we used the Kolmogorov-Smirnov test to check the normality of the data. The null hypothesis included the non-normal distribution and the assumption that the data distribution was normal. According to the results of the test, the significant data of the test was higher than 0.05, so the null hypothesis was rejected and the data assumption was normal (p=0.302). T-test was used to compare Ki-67 protein in two groups of patients with cutaneous LP and MF. According to the results of the chi-square test, values were greater than 0.05, indicating that Ki-67 protein expression was not correlated with cutaneous LP and MF. Linear regression was used for the possible correlation between age and Ki-67 index (%) in both LP and MF patients.

Limitations Lack of records for patient information, unavailability of some paraffin blocks, low number of samples, and lack of analysis according to the disease stage in MF were the limitations of this study.

Results

In the present study, 62 cutaneous LP and MF specimens were evaluated. In the patients with cutaneous LP (39 patients), the mean age was 43 years, and 46.2% were male. In the MF patients (23 patients), the mean age was 41.8 years, and 34.8% were male (**Table 1**). Out of all cutaneous LP patients, 35.9% were Ki-67-positive, and among the MF patients 21.7% were Ki-67-positive. There was a lack of significant difference between both groups in terms of age (p=0.748), sex (p=0.434), mean Ki-67 (p=0.434), and Ki-67 status (p=0.271).

The number of patients with Ki-67 index (%) in both groups is shown in **Figure 2**. The highest Ki-67 protein expression was 50% in the LP group and 90% in the MF group.

Table 1 Comparison of patients' characteristics in two groups.

Variable	*Lichen planus*	*Mycosis fungoides*	*P-value*
Age (year)			
Mean	43.4±16.0	41.8±23.3	0.748
Range	2-76	9-72	
Sex			
Male	18 (46.2%)	8 (34.8%)	0.434
Female	21 (53.8%)	15 (65.2%)	
Ki-67 (%)			
Mean	22.9±12.6	20.0±24.8	0.434
Range	1-50	1-90	
Ki-67 status			
Positive	14 (35.9%)	5 (21.7%)	0.271
Negative	25 (64.1%)	18 (78.3%)	

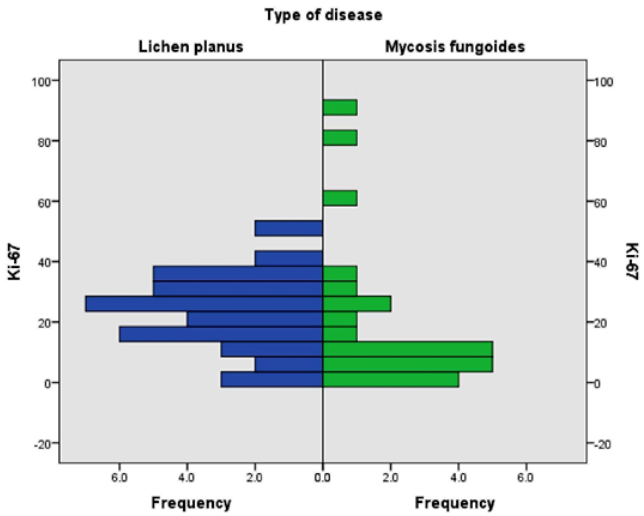

Figure 2 Comparison of patients' frequency with Ki-67 index (%) in both groups.

The correlation between age and Ki-67 index is shown in **Figure 3**. The results didn't show any correlation between the two variables in LP (p=0.768) and MF patients (p=0.107).

Discussion

The only way to diagnose MF and distinguish it from benign lesions such as LP is currently to examine the pathology of the patient's tissue specimen, which depends on the pathologist's skill. Due to the different treatment pathways, the two diseases should be diagnosed correctly through histopathology and IHC, including Ki-67 marker. There are very few studies on the

expression of Ki-67 marker in MF patients.

In a study conducted by Jankowska-Konsur *et al*.,[34] IHC analysis of Ki-67 was performed in 90 MF samples and 19 control samples of benign chronic dermatoses. The higher expression of Ki-67 was found in advanced stages of the disease. In a 2009 study by Edinger *et al*.,[35] 47 patients with MF were evaluated regarding age, sex, stage of disease at diagnosis, disease progression, treatment, and follow-up. Patients were also immunohistochemically evaluated for Ki-67 and CD30 expression. The results showed a direct correlation between the Ki-67 expression, the stage of disease at diagnosis, and the highest stage of the disease. The findings also showed the higher the expression of Ki-67, the lower the survival rate. Thus, the expression of Ki-67 is a prognostic factor. In a study by Gambichler *et al*.[36] in 2007, 51 samples of patients with parapsoriasis, MF, and lymphomatoid papulosis were immunohistochemically evaluated for PCNA, MCM7, p21, and Ki-67. More Ki-67 was found in stage IIB-IV MF in comparison with parapsoriasis and stage I-IIA MF. Therefore, Ki-67 was introduced as an appropriate parameter for determining the stage of disease progression. In a study by Soini *et al*.[37] in 1994, 62 patients with a variety of benign lesions (psoriasis, chronic dermatitis, seborrheic keratosis, and LP) and 7 normal skin samples underwent Ki-67 and P53 IHC. Ki-67 expression was shown to be associated with p53 expression .Variable expression of Ki-67 has been observed in these lesions, but generally higher expression occurs in lesions with high mitosis. In a study[38] in 1990, Ki-67 was used to evaluate the difference between epidermal and dermal T cells in MF. All 14 cases were in the patch and plaque phase. In 12 samples epidermal T-cells showed Ki-67 positivity whereas all T-cells in the dermal layer were negative.

In a study by Russian researchers,[39] 57 patients with clinical T-cell lymphoma (MF and Sezary's disease) were studied, among whom twelve showed lymphosarcoma. Clinical, histologic, biomolecular, and immunophenotypic analyses were done on these cases, and the results showed that most of them were high in the expression of Ki-67 marker (10-70%). A study conducted in Brazil[40] evaluated the survival rate and its association with a histological pattern of 52 known cases of T-cell cutaneous lymphoma, all of which were HTLV-I-positive and HIV-negative. In this study, Ki-67 marker was used as a proliferative marker. In a case-report conducted at the same university in the same year, the expression of Ki-67 marker was 70%.[41] The results of these studies indicated a higher expression of Ki-67 marker in MF than in benign lesions. The results also showed a higher expression of the marker in the advanced stages of MF than in the early stages, and Ki-67 marker was introduced as a prognostic factor. However, there was a probability of error in the differentiation of stained lymphocytes in IHC with keratinocytes.

In addition, it was found that despite the obvious difference in marker expression in the early stages of MF, such as patch and plaque with benign LP, the difference was significant in the tumor stage of the disease. For example, in one sample with tumor phase, the marker expression was 80%, to be unprecedented among LP samples. However, due to lack of registration of disease stage in most of the studied cases, it was not possible to perform statistical examination according to the stage of the disease.

Conclusions

IHC evaluation with Ki-67 factor to differentiate MF lesions from LP lesions, especially in the early and pre-malignant stages of the disease which is difficult to perform, is not useful, so

routine morphological evaluation of these lesions is still recommended.

References

1. Omal P, Jacob V, Prathap A, Thomas NG. Prevalence of oral, skin, and oral and skin lesions of lichen planus in patients visiting a dental school in southern India. *Indian J Dermatol*. 2012;**57(2)**:107-9.
2. Gupta S, Jawanda MK. Oral Lichen Planus: An Update on Etiology, Pathogenesis, Clinical Presentation, Diagnosis and Management. *Indian J Dermatol*. 2015;**60(3)**: 222-9.
3. Weston G, Payette M. Update on lichen planus and its clinical variants. *Int J Womens Dermatol*. 2015;**1(3)**:140-9.
4. Okpala IC, Akinboro AO, Ezejoifor IO, Onunu AN, Okwara BU. Metabolic Syndrome and Dyslipidemia among Nigerians with Lichen Planus: A Cross-Sectional Study. *Indian J Dermatol*. 2019;**64(4)**:303-10.
5. Nevile BW, Damm DD, Allen CM, Jerry EB. Oral and maxillofacial pathology. 2nd ed. Pensylvania wes philadephia, editor. W.B. Saundres Company; 2009. pp. 782–788.
6. Schwager Z, Stern M, Cohen J, Femia A. Clinical epidemiology and treatment of lichen planus: A retrospective review of 2 tertiary care centers. *J Am Acad Dermatol*. 2019;**81(6)**:1397-9.
7. Kiran MS, Vidya S, Aswal GS, Kumar V, Rai V. Systemic and Topical Steroids in the Management of Oral Mucosal Lesions. *J Pharm Bioallied Sci*. 2017;**9(Suppl 1)**:S1-S3.
8. Lozada-Nur F, Miranda C. Oral lichen planus: topical and systemic therapy. *Semin Cutan Med Surg*. 1997;**16(4)**:295-300.
9. Thongprasom K, Dhanuthai K. Steriods in the treatment of lichen planus: A review. *J Oral Sci*. 2008;**50(4)**:377-385.
10. Vaidya T, Badri T. Mycosis Fungoides. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020.
11. Campbell JJ, Clark RA, Watanabe R, Kupper TS. Sezary syndrome and mycosis fungoides arise from distinct T-cell subsets: a biologic rationale for their distinct clinical behaviors. *Blood*. 2010;**116(5)**:767-71.
12. Cerroni L, Fink-Puches R, Bäck B, Kerl H. Follicular Mucinosis: A Critical Reappraisal of Clinicopathologic Features and Association With Mycosis Fungoides and Sézary Syndrome. *Arch Dermatol*. 2002;**138(2)**:182-9.
13. Jawed SI, Myskowski PL, Horwitz S, Moskowitz A, Querfeld C. Primary cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome): Part I. Diagnosis: Clinical and histopathologic features and new molecular and biologic markers. *J Am Acad Dermatol*. 2014;**70(2)**:205.e1-16;quiz 221-2.
14. Whittaker SJ. Cutaneous lymphoma and lymphocytic infiltrates. 8th ed. In: Burns, T. Breathnach, S. Cox, N. Griffiths C (eds.)., editor. Wiley-Blackwell; 2010.
15. Pimpinelli N, Olsen EA, Santucci M, Vonderheid E, Haeffner AC, Stevens S, *et al*. Defining early mycosis fungoides. *J Am Acad Dermatol*. 2005;**53(6)**:1053-63.
16. Reddy K., Bhawan J. Histologic mimickers of mycosis fungoides: a review. *J Cutan Pathol*. 2007;**34(7)**:519-25.
17. Kelati A, Gallouj S, Tahiri L, Harmouche T, Mernissi FZ. Defining the mimics and clinico-histological diagnosis criteria for mycosis fungoides to minimize misdiagnosis. *Int J Womens Dermatol*. 2017;**3(2)**:100-6.
18. Arafah M, Zaidi SN, Kfoury HK, Al Rikabi A, Al Ghamdi K. The Histological Spectrum of Early Mycosis Fungoides: A Study of 58 Saudi Arab patients. *Oman Med J*. 2012;**27(2)**:134-9.
19. Hodak E, Amitay-Laish I. Mycosis fungoides: A great imitator. *Clin Dermatol*. 2019;**37(3)**:255-67.
20. Zackheim HS, McCalmont TH. Mycosis fungoides: the great imitator. *J Am Acad Dermatol*. 2002;**47(6)**:914-8.
21. Sajjan V, Chandela M, Pandit AM, Manjunathswamy B. Mycosis fungoides: The great imitator. *Our Dermatol Online*. 2015;**6(1)**:76-9.
22. Litvinov IV, Netchiporouk E, Cordeiro B, Doré MA, Moreau L, Pehr K, *et al*. The Use of Transcriptional Profiling to Improve Personalized Diagnosis and Management of Cutaneous T-cell Lymphoma (CTCL). *Clin Cancer Res*. 2015;**21(12)**:2820-9.
23. Trager MH, Geskin LJ. Current status of histone deacetylase inhibitors in cutaneous T-cell lymphoma. *G Ital Dermatol Venereol*. 2019;**154(6)**:681-95.
24. Jawed SI, Myskowski PL, Horwitz S, Moskowitz A, Querfeld C. Primary

cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome): part II. Prognosis, management, and future directions. *J Am Acad Dermatol*. 2014;**70(2)**:223.e1-17;quiz 240-2.
25. Fonatsch C, Duchrow M, Rieder H, Schlüter C, Gerdes J. Assignment of the human Ki-67 gene (MKI67)to 10q25-qter. *Genomics*. 1991;**11(2)**:476-7.
26. Apte SS. Ki-67 monoclonal antibody (MAb) reacts with a proliferation associated nuclear antigen in the rabbit Oryctolagus cuniculus. *Histochemistry*. 1990;**94(2)**:201-4.
27. Sobecki M, Mrouj K, Camasses A, Parisis N, Nicolas E, Llères D, *et al*. The cell proliferation antigen Ki-67 organises heterochromatin. *Elife*. 2016;**5**:e13722.
28. Bruno S, Darzynkiewicz Z. Cell cycle dependent expression and stability of the nuclear protein detected by Ki-67 antibody in HL-60 cells. *Cell Prolif*. 1992;**25(1)**:31-40.
29. Humayun S, Prasad VR. Expression of p53 protein and ki-67 antigen in oral premalignant lesions and oral squamous cell carcinomas: An immunohistochemical study. *Natl J Maxillofac Surg*. 2011;**2(1)**:38-46.
30. Scholzen T, Gerdes J. The Ki-67 protein: From the known and the unknown. *J Cell Physiol*. 2000;**182(3)**:311-22.
31. Taneja P, Maglic D, Kai F, Zhu S, Kendig RD, Fry EA, *et al*. Classical and novel prognostic markers for breast cancer and their clinical significance. *Clin Med Insights Oncol*. 2010;**4**:**15-34**.
32. Xu L, Liu YH, Ye JM, Zhao JX, Duan XN, Zhang LB, *et al*. Relationship between Ki67 expression and tumor response to neoadjuvant chemotherapy with anthracyclines plus taxanes in breast cancer. *Zhonghua Wai Ke Za Zhi*. 2010;**48(6)**:450-3.
33. Kanavaros P, Bai M, Stefanaki K, Poussias G, Rontogianni D, Zioga E, *et al*. Immunohistochemical expression of the p53, mdm2, p21/Waf-1, Rb, p16, Ki67, cyclin D1, cyclin A and cyclin B1 proteins and apoptotic index in T-cell lymphomas. *Histol Histopathol*. 2001;**16(2)**:377-86.
34. Jankowska-Konsur A, Kobierzycki C, Reich A, Grzegrzolka J, Bieniek A, Dziegiel P. Expression of SATB1, MTI/II and Ki-67 in Mycosis Fungoides. *Anticancer Res*. 2016;**36(1)**:189-97.
35. Edinger JT, Clark BZ, Pucevich BE, Geskin LJ, Swerdlow SH. CD30 Expression and proliferative fraction in nontransformed mycosis fungoides. *Am J Surg Pathol*. 2009;**33(12)**:1860-8.
36. Gambichler T, Bischoff S, Bechara FG, Altmeyer P, Kreuter A. Expression of proliferation markers and cell cycle regulators in T cell lymphoproliferative skin disorders. *J Dermatol Sci*. 2008;**49(2)**:125-32.
37. Soini Y, Kamel D, Pääkkö P, Lehto VP, Oikarinen A, Vähäkangas KV. Aberrant accumulation of p53 associates with Ki67 and mitotic count in benign skin lesions. *Br J Dermatol*. 1994;**131(4)**:514-20.
38. Nickoloff BJ, Griffiths CEM. Intraepidermal but not dermal T lymphocytes are positive for a cell-cycle - Associated antigen (Ki-67) in mycosis fungoides. *Am J Pathol*. 1990;**136(2)**:261-66.
39. Vinogradova IuE, Iliushkina EA, Kaplanskaia IB, Lutsenko IN, Zybunova EE, Kremenetskaia AM, *et al*. Diagnosis and treatment of skin T-cell lymphoma undergoing transformation in lymphosarcoma. *Ter Arkh*. 2009;**81(7)**:57-61.
40. Bittencourt AL, Barbosa HS, Vieira MDG, Farr L. Adult T-cell leukemia/lymphoma (ATL) presenting in the skin: Clinical, histological and immunohistochemical features of 52 cases. *Acta Oncol*. 2009;**48(4)**:598-604.
41. Bittencourt AL, Mota K, Oliveira RF, Farré L. A dyshidrosis-like variant of adult T-cell leukemia/lymphoma with clinicopathological aspects of mycosis fungoides. A case report. *Am J Dermatopathol*. 2009;**31(8)**: 834-7.