Contribution of T-cell receptor gamma gene rearrangement by polymerase chain reaction and immunohistochemistry to the histological diagnosis of early mycosis fungoides

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ABSTRACT

الأهداف: دراسة دور الكيمياء الهيستولوجية المناعية ومستقبل الخلايا التائية (TCR) وتحليل إعادة ترتيب جينات غاما عند تشخيص الفطار الفطراني (MF).

الطريقة: أجريت دراسة استرجاعية وتم اختيار 73 حالة من أرشيف قسم علم الأمراض، كلية الطب، جامعة كوكوروفا، أدانا، تركيا، خلال الفترة من 2 يناير 2004م حتى 30 ديسمبر 2009م. ضمت الدراسة 39 حالة مصابة بالفطار الفطراني (MF) مع هيستولوجية تقليدية، 16 حالة مصابة بالفطار الفطراني (MF) مع هيستولوجيا مشكوك فيها كمجموعة غير حاسمة، 18 حالة مصابة بجلادات ملتهبة حميدة كمجموعة مراقبة مشاركة في الدراسة. تم تقييم الشرائح بوجود أو غياب المعايير الهيستوباثولوجية للفطار الفطراني. أجريت CD3K, CD4, and CD8 لجميع الحالات وتم تسجيل مجموعها ونسبة CD4/CD8. تم تحليل وجود مستقبل الخلايا التائية (TCR) وإعادة ترتيب جينات غاما عن طريق جهاز تقنية تفاعل البليمريز التتابعي.

النتائج: المؤشرات الهيستوباثولوجية الايحائية للفطر الفطراني هي التوجه البشروي (p=0.000)، وجود خراج بوتريبه المكروي (p=0.00) لمفاوية لا نموذجية (p=0.010) مؤشرات إحصائية هامة مع تحليل أحادي. وجود قابلية التنسيل في 76.9% في حالات الفطار الفطراني و37.5% في المجموعة الغير حاسمة. كانت قابلية التنسيل ايجابية لدى 4 من أصل 6 حالات في مجموعة غير حاسمة تم تشخيصهم بالفطار الفطراني عند إعادة الاختزاعات. لم تكن نسبة CD8 مختلفة احصائياً بين المجموعات الثلاثة.

خاتمة: أظهرت النتائج أن نسبة CD4/CD8 وTCR و إعادة ترتيب الجينات في الحالات المشكوك فيها يمكنها من مساندة الهيستوباثولوجية عند بداية الفطار الفطراني.

Objectives: To study the role of immuno-histochemistry and T-cell receptor (TCR) gamma gene rearrangement analysis in the diagnosis of mycosis fungoides (MF).

Methods: The study design was retrospective, and 73 cases were selected from the archive of the Pathology Department, School of Medicine, Cukurova University, Adana, Turkey, between January 2004 and December 2009. Thirty-nine MF cases with classical histomorphology, 16 cases with suspicious histomorphology for MF as the inconclusive group, and 18 cases with benign inflammatory dermatoses as the control group were involved in the study. The slides were evaluated for the presence or absence of the histopathological criteria for MF. Immunohistochemically, CD3, CD4, and CD8 were performed in all cases, and their counts and CD4/CD8 ratio were noted. Presence clonal TCR gamma gene rearrangement was evaluated by polymerase chain reaction (PCR).

Results: The histopathological parameters suggestive of MF were epidermotropism (p=0.000), presence of Pautrier microabscess (p=0.004), and atypical lymphocyte (p=0.000). Immunohistochemically, CD4 percentage (p=0.006) and CD4/CD8 ratio (p=0.010) were statistically significant parameters with univariate analysis. Clonality was present in 76.9% of MF cases and 37.5% in the inconclusive group. Four of 6 clonality positive cases in the inconclusive group were diagnosed as MF in rebiopsies. The CD8 percentage was not statistically different between the 3 groups.

Conclusion: In suspicious cases, CD4/CD8 ratio and TCR gamma gene rearrangement can support histopathology in early MF.

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Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma; characterized by the neoplastic proliferation of post-thymic T-lymphocytes.[1] Histologic diagnosis is generally easy in fully developed MF, because of the typical morphologic findings of the disease. However, it may be so difficult in early MF cases because these lesions show minimal changes mimicking inflammatory dermatoses. Many studies have attempted to establish certain criteria to diagnose MF.[2-7] Immunophenotyping and T-cell receptor gene rearrangement analysis have been researched as an adjunct to the histopathologic diagnosis.[8-11] There is no agreement on immunophenotyping results, because immunophenotypic changes are generally similar to inflammatory dermatoses in early stages. Besides, in the literature, molecular biology techniques can detect MF in a wide range (42-90%).[7-11] Our aim is to study the contribution of polymerase chain reaction (PCR) and immunophenotyping methods to the histopathologic diagnosis of MF.

Methods. In this retrospective study, 73 biopsy specimens belonging to 69 cases with 3 patient groups were selected from the archive of Pathology Department, School of Medicine, Cukurova University, Adana, Turkey, from January 2004 to December 2009. Mycosis fungoides group consisted of 39 biopsies with classical histomorphology. Histopathologic criteria for MF diagnosis were epidermotropism (infiltration of lymphocytes in the epidermis without spongiosis) (**Figure 1A**), Pautrier micro abscess, and intraepidermal atypical lymphocyte (lymphocyte with medium-large sized, hiperchromathic and convulated nucleus) (**Figure 1B**). Inconclusive group contained 16 cases suspicious for early MF clinically, but lacking major histopathological criteria like epidermotropism, Pautrier microabscess or atypical lymphocyte. Control group contained 18 cases with benign inflammatory dermatoses (12 lichen planus, 6 contact dermatitis) both clinically and histopathologically. Mycosis fungoides and inconclusive group patients were questioned for MF by the dermatologist. Mycosis fungoides group included biopsies showing at least one of the diagnostic criteria for MF. The cases without any of the diagnostic criteria were excluded from MF group and taken to the inconclusive group.

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Histopathological evaluation. Five-micron thick sections were sampled from the formalin fixed, paraffin embedded skin biopsy tissues and were stained hematoxylin and eosin (H&E). Slides were evaluated under the light microscope (Nikon E600, Tokyo, Japan). We reviewed all the slides in terms of those histopathological criteria; epidermotropism, Pautrier microabscess, atypical lymphocyte, spongiosis and fibrosis.

Immunohistochemical staining. Standard avidin-biotin-immunoperoxidase technique was performed to the polysin slides with tissue sections. Antibodies to CD3 (T-cell polyclonal rabbit anti-human, ref. no N1580, Dako, Denmark), CD4 (mouse, ref. no 08-1282, Zymed, San Francisco, US), and CD8 (T-cell mouse anti-human, ref. no N1592, Dako, Denmark) were performed according to the manufacturer's instructions. Two hundred mononuclear cells were counted on at least 4 randomly selected high power (x400) fields of CD3 stained slides for each case. CD4 and CD8 positive stained cells were counted and percentages of results were recorded (**Table 1**).

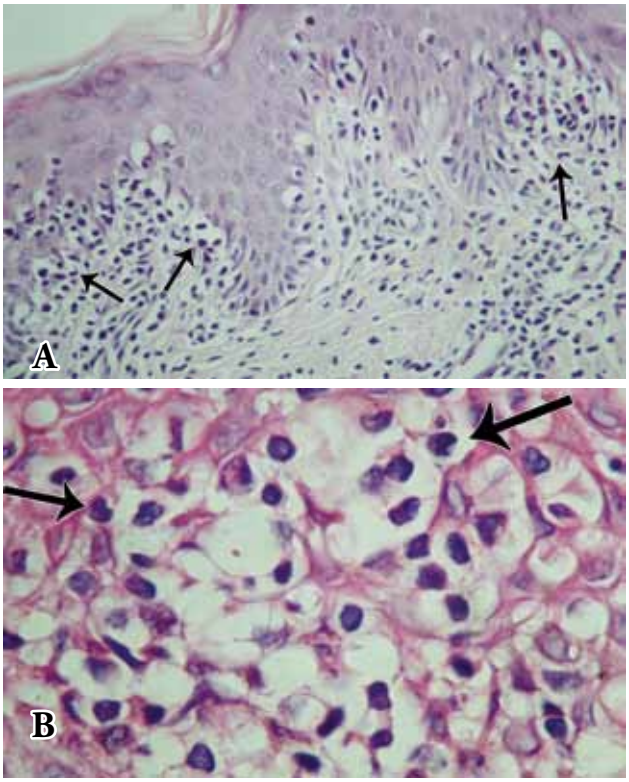

Figure 1 - Histopathology of mycosis fungoides **A)** epidermotropism (H&E x200) and **B)** atypical lymphocytes (H&E x1000).

Table 1 - The histopathological, immunohistochemical, and T-cell receptor gamma gene rearrangement results of all patient groups and the statistical significance between the 3 groups for each criterion.

Parameter	MF group (n=39)	Inconclusive group (n=16)	Control group (n=18)	*P*-value*
Epidermotropism (%)	39 (100.0)	12 (75.0)	1 (5.6)	0.000
Pautrier microabscess (%)	11 (28.2)	0	0	0.004
Atypical lymphocyte (%)	18 (46.2)	0	0	0.000
Spongiosis (%)	6 (15.4)	16 (100.0)	13 (72.2)	0.010
Fibrosis (%)	19 (48.7)	0	7 (38.9)	0.003
CD4 (%)	43.3	24.6	32.4	0.006
CD8 (%)	33.7	38.8	40.3	0.393
CD4/CD8 (%)	3.9	1.99	2.1	0.010
Clonality (%)	30 (76.9)	6 (37.5)	2 (11.1)	0.000

*p<0.05 significant, MF - mycosis fungoides

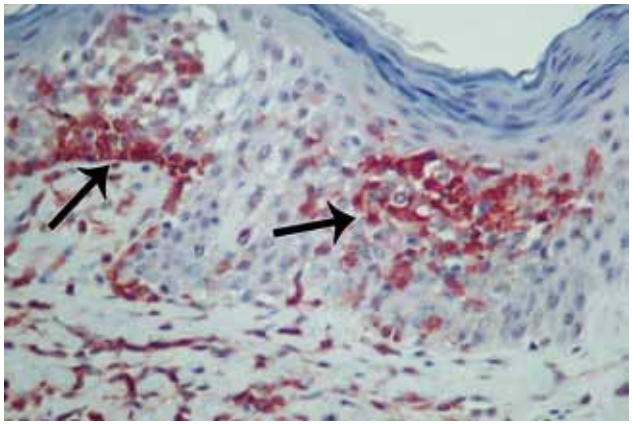

Figure 2 - CD4 positive lymphocytes showing epidermotropism (IHC, x400).

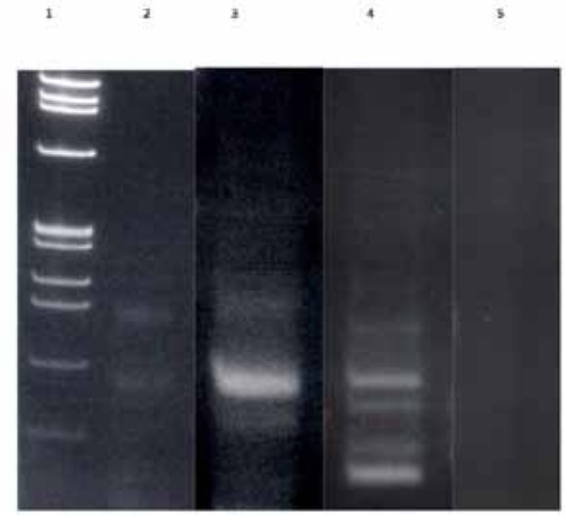

Figure 3 - T-cell receptor gamma gene rearrangement with polymerase chain reaction. Lane 1: marker, Lane 2 and 3: monoclonal, Lane 4: policlonality, Lane 5: negativity

T-cell receptor (TCR) gamma gene rearrangement analysis by PCR was performed to all cases. Genomic DNA was extracted from paraffin tissue by following manufacturer's protocol (High Pure PCR Template Preparation Kit-Cat.no: 11796828001, Roche). Three multiplex PCR reactions were developed that, when combined, were expected to amplify greater than 95% of rearrange TCR-γ chain genes.[12] The DNA amplification was performed with PCR core kit (Promega, M7660) and primaries of the 4 main groups (Family I-IV) for variable-region (V) genes and joining-region (J) genes as described by Lamberson et al.[12] The DNA was visualized by ethidium bromide stained polyacrylamide gel at 250 V for 2,5 hours.

Statistical analysis. A univariate analysis was performed for each criterion by Chi-square test. Mann Whitney test was used for comparison of couple of group. Roc curve was used to search for cut-off value of the parameters. *P*-value less than 0.05 was accepted statistically significant and 95% confidence interval was used for the mean.

Results. The study included 69 patients (58% females, 42% males) with a mean age of 45.6 years (3-82). The TNM stages of the MF were: 1A (51.4%), 1B (40%), stage 3A (5.7%) and, stage 2B (2.9%). The results of the histopathological evaluation, percentages of CD3, CD4, CD8 and PCR results with statistical *p*-values are summarized in **Table 1**. These parameters were also compared between couple groups, in which **Table 2** shows the statistical *p*-values for each parameter. With the ROC curve, the best cutoff value for the CD4 count as a discriminator between the MF and control groups was 26% (sensitivity 82%, specificity 69%). The cutoff value for CD4/CD8 ratio was 0.65 (sensitivity 74% and specificity 69%).

Table 2 - The statistical significance between couple groups for each parameter.

Parameters	MF versus inconclusive group	MF versus control group	Inconclusive versus control
Epidermotropism	0.001	0.000	0.000
Pautrier microabscess	0.018	0.012	*
Atypical lymphocyte	0.001	0.000	*
Spongiosis	0.080	0.003	0.324
Fibrosis	0.001	0.489	0.005
CD4 (%)	0.004	0.055	0.135
CD8 (%)	0.328	0.230	0.878
CD4/CD8	0.006	0.069	0.224
Clonality	0.005	0.000	0.070

Data express as p-value. *No statistics were computed because parameter was constant. Epidermotropism, Pautrier microabscess, atypical lymphocyte, fibrosis, CD4 count and clonality are statistically significant between mycosis fungoides (MF) and inconclusive groups.

The histopathological, immunohistochemical, and T-cell receptor gamma gene rearrangement results of all patient groups and the statistical significance between the 3 groups for each criterion are summarized in **Table 1**.

Epidermotropism, Pautrier microabscess, atypical lymphocyte, fibrosis, CD4 count, and clonality are statistically significant between MF and inconclusive groups are summarized in **Table 2**.

Discussion. The diagnosis of MF is a dilemma in early stages, both clinically and histologically. It can easily be confused with inflammatory dermatoses histologically. In our study, epidermotropism, Pautrier microabscess and intraepidermal atypical lymphocyte were significantly different between the 3 groups (**Table 1**). Inchara et al[3] have found that disproportionate epidermotropism/epitheliotropism, tagging of lymphocytes, haloed lymphocytes, larger epidermal lymphocytes, convoluted lymphocytes, eccrine infiltration, follicular infiltration, absence of dermal edema, papillary dermal fibrosis and monomorphous lymphoid infiltrate were the histological features useful in discriminating MF from inflammatory skin diseases. In present study, epidermotropism was present in 100% of the MF cases. However, it may also be present in inflammatory dermatitis (5.6%). Therefore, it is not a specific marker for MF. Naraghi et al[4] indicated that pagetoid pattern was not detected in any of the inflammatory disorders, so it was found to be a specific, but not sensitive feature. Pautrier microabscess is reported in 28.2% of MF cases in our study. This feature is reported to be an absolutely specific (96%) low sensitive (41%) parameter for early MF, which is an agreement in the literature.[4,6,8] The presence of atypical lymphocytes in the epidermis was another diagnostic clue for MF which was not detected in an inconclusive or control groups. However, 46,2% of early MF cases showed this parameter. So, its absence does not exclude MF. Mild spongiosis was found in all 3 groups in our study, similar to other studies.[3-5] Mild degrees of spongiosis does not exclude MF, however, presence of microvesiculation is rather rare,[4] although MF with bullae formation have been reported.[13] Dermal fibrosis was observed in both MF (48.7%) and control (38.9%) groups. It was not a discriminator (p=0.489). It may be associated with the chronicity of the lesion.

None of these histopathological features solve the problem in diagnosis of early stage MF. We need additional techniques to diagnose early MF. Immunohistochemistry and molecular techniques are searched for this purpose. Although many patterns of aberrant antigen expression have been described, the most mentioned ones include absence of CD7, Leu8; less commonly CD2 and CD5, and expression of CD4, CD8.[5] We investigated the expression of CD3, CD4, CD8, and CD4/CD8 ratio (**Figure 2**). CD4 and CD4/CD8 results were statistically significant between the 3 groups (p=0.006, p=0.010). Different CD4/CD8 ratios to be diagnostic value are described in the literature from 2-10 and there is not an agreeable cutoff value.[14] Mean CD4/CD8 ratio in MF group was 3.9 in our study similar with literature. However, when MF group was compared with control group, this parameter was not statistically significant (p=0.069). We found the cutoff value for CD4/CD8 as 0.65. No correlation found with the values from the literature. We found that this controversial finding may be associated with the hypothesis that defends for early MF lesions and may not show the immunophenotypic aberrancies, which are seen in advanced lesions.[5] Additionally, mean CD8 counts were 33.7% in control group and 32.5% in MF group, which was not statistically significant in agreement with the literature (p=0.393). This result is not surprising due to the presence of CD8 positive lymphocytes as a component of immune response to neoplastic CD4 positive T lymphocytes and partially as a rare subtype of MF with CD8 positive cell profile.

Recently, several PCR methods with different techniques have been performed to determine T-cell receptor beta or gamma gene rearrangement. Clonality has been found in 41-90% of the MF cases in different studies,[8,10,11] while in the present study it was 76.9% (statistically significant between the 3 groups [p=0.000])

(**Figure 3**). It is important to state that monoclonality may also be found in benign dermatoses. The range was 3.7-24% in the literature,[8] while in the present study it was 11.1% (2 of 18 cases). The results show that monoclonality is not always malignant. In spite of this, there is an agreement on that, demonstration of monoclonality of TCR beta or gamma gene rearrangement in suspicious lesions is a helpful data to the histopathological findings.

In the literature, various PCR studies on MF have shown 100% clonality in tumor stage, 73% clonality in plaque stage, 52-75% clonality in patch-plaque stage, and 83% clonality in eritrodermic stage.[8,10,11,14] In our study, clonality was found in 15/19 (78.9%) patch stage, 11/12 (91.7%) plaque stage, 1/3 (33.3%) patch-plaque stage, 3/4 (75%) eritrodermic stage, and no clonality was found in one tumor stage case. Six of the 9 cases with no clonality have shown CD4/CD8 ratio under 3.9. This clonal negativity may be associated with the low density of lymphocyte infiltration. The other 2 cases with negative clonality showing CD4/CD8 ratio higher than 2 have had PUVA therapy. Therapy may be an important factor especially in recurrent lesions. The last case was at tumor stage and showed high CD4/CD8 ratio with 6. In this case we think there may be a technical reason for negativity.

The positive clonality of the inconclusive group (6/16 cases, 37.5%) was between MF (76.9%) and control (11.1%) group. Clinical information on the 2 positive cases have not received. Besides, 4/6 positive cases underwent re-biopsies after 7 months to one year after the first biopsy, and they were all reported as MF. The result demonstrates that some of the cases in the inconclusive group could be reported as early MF by the help of PCR.

There were some limitations of the study. The amplification method and kind of material (fresh or paraffin) seem to affect the PCR results. Our cases were paraffin embedded and amplified by PAGE. We investigated only 3 T-cell antigens. There have been other aberrations reported in the literature like loss of CD2, CD5, CD7, which were not significant alone. We could not get the clinical information about 2 clonality positive cases in inconclusive group.

In conclusion, neither CD4/CD8 ratio, nor clonality of TCR gamma gene rearrangement is diagnostic for early MF alone. The gold standard for diagnosis of early MF cases is to correlate clinical and histopathological features with CD4/CD8 ratio and PCR results. Further studies with larger series are needed to assess the role of PCR.

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References

1. Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, et al. WHO-EORTC classification for cutaneous lymphomas. ***Blood*** 2005; 105: 3768-3785.
2. Latkowski JA, Heald P. Cutaneous T-cell lymphomas. In: Freebag IM. Eisen AZ. Wolff K. Austen KF. Goldsmith LA. Katz SI. Fitzpatrick's Dermatology in General Medicine. 6th ed. New York (NY): McGraw Hill; 2003. p. 1537-1558.
3. Inchara YK, Rajalakshmi T. Early mycosis fungoides vs. inflammatory mimics: how reliable is histology? ***Indian J Dermatol Venereol Leprol*** 2008; 74: 462-466.
4. Naraghi ZS, Seirafi H, Valikhani M, Farnaghi F, Kavusi S, Dowlati Y. Assessment of histologic criteria in the diagnosis of mycosis fungoides. ***Int J Dermatol*** 2003; 42: 45-52.
5. Furmanczyk PS, Wolgamot GM, Kussick SJ, Sabath DE, Olerud JE, Argenyi ZB. Diagnosis of mycosis fungoides with different algorithmic approaches. ***J Cutan Pathol*** 2010: 37: 8-14.
6. 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: 134-139.
7. Pimpinelli N, Olsen EA, Santucci M, Vonderheid E, Haeffner AC, Stevens S et al. Defining early mycosis fungoides. ***J Am Acad Dermatol*** 2005; 53: 1053-1063.
8. Ponti R, Quaglino P, Novelli M, Fierro MT, Comessatti A, Peroni A, et al. T-cell receptor gamma gene rearrangement by multiplex polymerase chain reaction/heteroduplex analysis in patients with cutaneous T-cell lymphoma (mycosis fungoides/Sézary syndrome) and benign inflammatory disease: correlation with clinical, histological and immunophenotypical findings. ***Br J Dermatol*** 2005; 153: 565-573.
9. Cho-Vega JH, Tschen JA, Duvic M, Vega F. Early-stage mycosis fungoides variants: case-based review. ***Ann Diagn Pathol*** 2010; 14: 369-385.
10. Xu C, Wan C, Wang L, Yang HJ, Tang Y, Liu WP. Diagnostic significance of TCR gene clonal rearrangement analysis in early mycosis fungoides. ***Chin J Cancer*** 2011; 30: 264-272.
11. Alessi E, Coggi A, Venegoni L, Merlo V, Gianotti R. The usefulness of clonality for the detection of cases clinically and/or histopathologically not recognized as cutaneous T-cell lymphoma. ***Br J Dermatol*** 2005; 153: 368-371.
12. Lamberson C, Hutchinson RE, Shrimpton AE. A PCR assay for detecting clonal rearrangement of the TCR-gamma gene. ***Mol Diagn*** 2001; 6: 117-124.
13. Abeldaño A, Arias M, Benedetti A, Ochoa K, Maskin M, Pellerano G et al. Unusual variants of mycosis fungoides. ***Skinmed*** 2011; 9: 218-222.
14. Florell SR, Cessna M, Lundell RB, Boucher KM, Bowen GM, Harris RM et al. Usefulness (or lack thereof) of immunophenotyping in atypical cutaneous T-cell infiltrates. ***Am J Clin Pathol*** 2006; 125: 727-736.