

DIRECT IMMUNOFLUORESCENCE IN LESIONAL AND UNINVOLVED SKIN IN DLE

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Direct immunofluorescence was evaluated in 16 patients with DLE including 1 case of lupus profundus and 4 with disseminated DLE. A BMZ band with multiple immunoreactants was demonstrated in lesional skin in 14 patients (87.5%), while 2 had only single immunoreactants. C3 and IgM were the commonest reactants followed by IgG, IgA and fibrinogen. A perifollicular prominence was seen in several patients. Four patients showed a band in uninvolved skin. This may indicate a potential to develop SLE. ANA was also positive in 5 patients but ds DNS was negative.

Key words: Direct immunofluorescence, Discoid lupus erythematosus, Lupus band

Introduction

In the lupus erythematosus spectrum of disease discoid lupus erythematosus (DLE) represents a relatively benign disorder of the skin, consisting of a localised form with lesions confined to above the neck, and a disseminated form in which lesions also occur on trunk and limbs.¹ The immunoreactants deposited at the dermo-epidermal junction (DEJ) can be demonstrated by direct immunofluorescence (DIF) as a characteristic lupus band, which is seen in lesional skin only in DLE and both in lesional and uninvolved skin in SLE.² A small percentage of DLE patients usually of the disseminated variety can progress to develop SLE.¹ This study was undertaken to define the DIF findings in lesional

and uninvolved (exposed and covered) skin in patients with DLE.

Patients and Methods

Sixteen patients with DLE including 1 with lupus profundus and 4 with disseminated lesions were studied. Lesional skin from all patients and uninvolved exposed skin (extensor forearm) and covered skin (buttocks) from 14 patients were biopsied for DIF studies.

DIF was performed as follows:-

The specimens were washed in phosphate buffered saline (PBS), snap frozen using liquid nitrogen and stored in the deep freezer at -70°C. Frozen sections (6-8) were obtained and incubated with FITC (fluorescein isothiocyanate) conjugated rabbit anti-human antibodies (DAKO) to IgG, IgM, IgA, C3 and fibrinogen respectively for about an hour. After washing in PBS (three 10 minute changes) and

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air drying, the sections were mounted using buffered glycerol and observed with a fluorescence microscope (Leitz Laborluk D). The class of immunoreactants, sites, pattern and intensity of staining were noted. An ANA assay by indirect enzyme immunoassay using wells coated with Hep-2 nuclear extract antigen (DIASAT Kit), LE cell test (by rotatory bead method) and anti-double stranded DNA (dsDNA) were also done in 14 patients. Histopathology (HPE) was performed in 12 cases.

Results

Sixteen patients (13 females, 3 males) ranging in age from 22 to 48 years with a mean age of 36.25 years with lesions clinically suggestive of DLE were studied. Scalp was the commonest site of involvement (11 patients) and in 5 of them this was the only site involved. Other sites involved were ears, nose, cheek and lips. Four patients had disseminated DLE with lesions below the neck. Duration of disease varied from 8 months to 10 years (mean duration 3-43 years). Histopathology was consistent with DLE in all. DIF results are shown in table I. In 5 patients the basement membrane zone (BMZ)

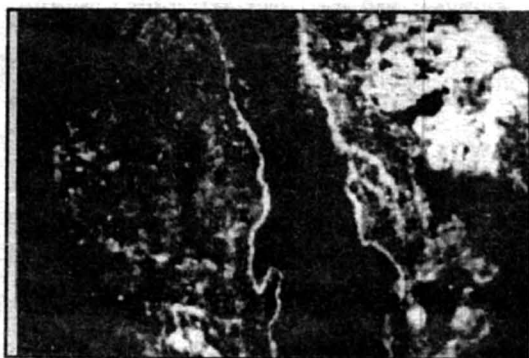


Fig 1. DIF perifollicular IgG deposits

band tended to be discontinuous with some immunoreactants but had a perifollicular

prominence (Fig1.) Intensity of staining also varied between immunoreactants and IgM usually showed the strongest deposits. Ten patients

Table I. DIF results

DIF BMZ	Lesion n=16	Exposed n=14	Covered n=14
IgG	11(69%)	2(14%)	1
IgM	12(75%)	4(29%)	3
IgA	9(56%)	0	0
C3	14(88%)	2(14%)	1
Fib	8(50%)	0	0

Table II. Correlation of patients with uninvolved skin and ANA positivity

No.	Type	ANA	DIF Exposed	DIF Covered
1	D	+ve	ENS	ENS
2	D	-ve	+ve	+ve
3	D	+ve	+ve	+ve
4	D	+ve	-ve	-ve
5	LP	+ve	+ve	-ve
6	L	+ve	+ve	+ve

D = disseminated, LP = lupus profundus, L = localised, ENS = epidermal nuclear staining.

had a predominantly granular pattern of deposits while it was more homogeneous in 5 with granules scattered within the band. Four patients showed a BMZ band in the exposed skin and 3 of them also had discontinuous bands in covered skin. 5 patients had a positive ANA test, but dsDNA and LE cell test were negative in all. The correlation of patients with uninvolved skin and ANA positivity are shown in table II.

The patient with lupus profundus had lesions over both cheeks, and lesional skin showed a thick strongly positive, homogenous to granular band of IgM, IgA, C3 and fibrin. Her exposed uninvolved skin showed a moderately positive IgM band with fibrillar extension into the upper dermis. H & E was consistent with lupus profundus. ANA was positive but dsDNA and LE cell tests were negative.

Table III. Frequency of BMZ bands

immunoreactants	No.	%
G,M,C	1	6.25
G,A,F	1	6.25
G,M,C,F	2	12.5
G,M,A,C	3	18.75
G,M,A,C,F	2	12.5
M,C,F	1	6.25
M,A,C,F	3	18.75
G	1	6.25
C	1	6.25
Total	16	100

Discussion

High diagnostic sensitivity of upto 90% has been reported with the DIF test in DLE.²⁻⁴ Others have reported it to be a less sensitive test.^{5,6} In this study a BMZ band was seen at least with one immunoreactant in all patients and, with multiple immunoreactants in 14 patients (87.5%) in lesional skin (Table III). The high percentage of positivity in this study could be because all the patients had lesions for a longer duration and most of the lesional biopsies were from scalp or retroauricular area, as early lesion (<2 months) and those on the trunk tend to be negative when compared to scalp lesions which are almost always positive.³ In the two Indian studies, Joshi et al⁷ reported a positive band in 4 of 6 patients studied while George et al⁶ found lesional lupus band in 15 of 26 patients (58%) only. Although uninvolved skin is expected to be negative in DLE, four of

our patients had uninvolved skin BMZ band and 1 had epidermal nuclear staining, all 5 patients had a positive ANA assay in addition. George et al⁶ also reported band in exposed skin in one of 3 patients studied. This could indicate a potential to progress to SLE and hence would call for a closer monitoring of these patients.

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