PATTERN ANALYSIS OF IMMUNOREACTANTS IN LICHEN PLANUS

By

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In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the Guidance of

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A line from Sanskrit Shlokha Says “Guru r brahma guru r vishnu guru r devo maheshwaraha, guru r sakshaath parabrahma tasmayshri gurave namaha” - meaning a teacher is next to god and without him knowledge is always incomplete.

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ABSTRACT

Introduction

Lichen planus (LP) is an inflammatory skin disease characterized by faintly erythematous to violaceous, flat topped, polygonal papules with adherent scales. The lesions usually involve flexural areas of the skin, the oral mucous membranes & genitalia. The etiology of lichen planus is unknown, although many studies have investigated and support an immunologic pathogenesis. Lymphocytes, particularly T-cells, play a major role. Other factors include antigen-presenting cells, adhesion molecules and inflammatory cytokines. While most cases of lichen planus are idiopathic, some are linked to medication use or hepatitis C virus (HCV) infection.

The diagnosis is based on the clinical characteristics of the lesions and histopathological examination. There are characteristic direct immunofluorescence findings of lichen planus such as the presence of subepidermal colloid bodies demonstrating IgM and less commonly IgA, IgG, C3 and fibrinogen. A linear broad band of fibrin at the dermo-epidermal junction has been suggested to be typical of lichen planus.

Objective of the study

To determine the pattern of immunoreactants in lichen planus by direct immunofluorescence

Materials and methods

The present study was carried out in the Department of Pathology in collaboration with Department of Dermatology, Venereology and Leprology BLDE University's Shri B.M.Patil Medical College, Hospital and Research Centre, Bijapur during the period; October 2011 to June 2013. Ten patients who were clinically diagnosed as lichen planus were included in the study. Skin biopsy was performed from the lesional skin and divided into 2 halves – one for H & E and the other for direct immunofluorescence (IMF)

Results

All 10 cases were proved by histopathology. The most common morphological type was classical lichen planus followed by hypertrophic, generalized and vesicular. Immunoglobulin deposition was seen at the basement membrane zone (BMZ) and the colloid bodies. The most common marker at thebasement membrane zone was fibrinogen(80%) followed by IgM (50%), C3(50%), Ig G(30%) and Ig A(30%). The most common marker present in colloid body was IgM, followed by fibrinogen and C3 in various combinations.

Conclusion

Histopathology remains gold standard for the diagnosis of lichen planus however diecrt IMF helps in diagnosing the diseases with ambiguous features e.g. lupus erythematosus.

LIST OF ABBREVIATIONS USED (In alphabetical order)

ANA	Anti-nuclear antibodies
BMZ	Basement membrane zone
CB	Colloid bodies
DEJ	Dermo-epidermal junction
DIF	Direct immunofluorescence
FITC	Fluorescein isothiocyanate
Ig	Immunoglobulins
HCV	Hepatitis C virus
H & E	Hematoxylin& eosin
IIF	Indirect immunofluorescence
IMF	Immunofluorescence
ICS	Intercellular space
LP	Lichen planus
LE	Lupus erythematosus
PBS	Phosphate buffered saline

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

INTRODUCTION

Lichen planus is an inflammatory skin disease characterized by faintly erythematous to violaceous, flat topped, polygonal papules with adherent scales. The lesions usually involve flexural areas of the skin, the oral mucous membranes & genitalia.[1]Rare cases of esophageal and ocular involvement are also reported.[2]Lichen planus frequently occurs between the ages of 30 & 60 years.[3] There is no predilection for sex or race.[1] The prevalence of the disease is approximately 1% in general population.[4]

Etiology

The etiology of lichen planus is unknown, although many studies have investigated and support an immunologic pathogenesis. Lymphocytes, particularly T-cells, play a major role.[4]Other factors include antigen-presenting cells, adhesion molecules and inflammatory cytokines. While most cases of lichen planus are idiopathic, some are linked to medication use or hepatitis C virus (HCV) infection.[5]

In the past few years, lichen planus has been linked to HCV infection, with studies demonstrating a higher prevalence of anti-HCV antibody titers in patients with cutaneous and oral lichen planus, compared with control subjects.[5]The reported rates of association have differed widely, probably because of varying study design, oral versus cutaneous lichen planus and geography.

Although the manner in which HCV infection predisposes patients to the development of lichen planus is unknown, some speculate that, long-term infection

may lead to an aberrant immunologic response.[6] Considering the current evidence, it is appropriate to screen all patients with lichen planus for HCV infection.

Lichenoid drug eruptions are reactions that may occur after exposure to various medications. These eruptions may exhibit a cutaneous and histologic appearance identical to that of idiopathic lichen planus and, thus, must be considered in every patient with lichen planus. While an exhaustive list of possible offending agents is quite long, the most common include gold, antimalarial agents, penicilamine, thiazide diuretics, beta blockers, non-steroidal anti-inflammatory drugs, quinidine and angiotensin-converting enzyme inhibitors.[7]

The interval between administration of the offending medication and the development of the lichenoid drug eruptions is usually a few months, although it may range from 10 days to several years. While the eruptions spontaneously clear anywhere from weeks to months after discontinuation of the medication in many patients, some patients require systemic therapy. Unfortunately, no test is available to confirm the causality of a particular medication. If the patient is taking a potentially offending medication, it should be discontinued whenever possible.[7]

Signs and symptoms

The typical rash of lichen planus is well-described by the "6 Ps": well-defined pruritic, planar, purple, polygonal, papules and plaques. The commonly affected sites are near the wrist and the ankle. The rash tends to heal with prominent blue-black or brownish discoloration that persists for a long time. Besides the typical lesions, many morphological varieties of the rash may occur. The presence of cutaneous lesions is not constant and may wax and wane over time. Oral lesions tend to last for longer than cutaneous lichen planus lesions.[8]

Cutaneous lichen planus:

Variants of cutaneous lichen planus are distinguished based upon the appearance of the lesions and/or their distribution.[9]

Annular lichen planus occurs in approximately 10 % of lichen planus cases. "Annular" means ring shaped, and this variant is so named because the papules develop in circular groups with clear, unaffected skin in the center.[9]

Linear lichen planus is so called because the papules are arranged in a line (the "Blaschko line").It may develop secondary to trauma (koebnerization) or uncommonly as a spontaneous, isolated eruption, usually on the extremities, and rarely on the face.[9]

Hypertrophic lichen planus (also known as "Lichen planusverrucosus") usually occurs on the extremities, especially the shin and interphalangeal joints, and tends to be the most pruritic variant of lichen planus.[9]

Atrophic lichen planus is a rare variant of lichen planus, and characterized by the presence of a few well-demarcated, white-bluish papules or plaques with central superficial atrophy.[9]

Bullous lichen planus (also known as "Vesiculobullous lichen planus") is a rare variant of lichen planus, characterized by the development of vesicles and bullae with the skin lesions.[9]

Ulcerative lichen planus is a rare variant of lichen planus presenting with chronic, painful bullae and ulceration of the feet, often with cicatricialsequelae evident.[9]

Lichen planuspigmentosus is an uncommon variant of lichen planus characterized by hyperpigmented, dark-brown macules in sun-exposed areas and flexural folds. [9]

Lichen planusactinicus (also known as "Actinic lichen niditus")is a variant of lichen planus that is more common in Middle Eastern countries in spring and summer, where sunlight appears to have a precipitating effect, such that exposed areas of the face, dorsal hands and arms, and nape of the neck are usually affected by papules that are hyperpigmented and violaceous-brown in color with a thready, rolled edge showing well-defined borders. [9]

Palmoplantar lichen planus -- lichen planus of the palms and soles.[9]

Inverse lichen planus is a variant of lichen planus characterized by violaceous papules and plaques appear in intertriginous areas of the skin.[9]

Lichen planus of nails

Lichen planus can involve the nails, termed nail lichen planus, and it is characterized by irregular longitudinal grooving and ridging of the nail plate, thinning of the nail plate, pterygium formation, shedding of the nail plate with atrophy of the nail bed, subungual keratosis, longitudinal erythronychia (red streaks), and subungual hyperpigmentation. Trachyonychia is present in around 10% of individuals with nail lichen planus.[9]

Scalp lichen planus

Lichen planus of the scalp (also termed lichen planopilaris,), can appear as violaceous, scaly, pruritic papules. Scalp lichen planus can cause scarring alopecia if it is untreated. This is a form of hair loss which involves scarring, and is considered to have an autoimmune cause. Lichen planopilaris is a distinct variant of cicatricial alopecia, a group of rare disorders which destroy the hair follicle, replace it with scar tissue, and cause permanent hair loss.[9]

Mucosal lichen planus

Oral lichen planus

Oral lichen planus can occur in the mouth alone, or in combination with cutaneous lichen planus and/or lichen planus of other mucosal surfaces. In contrast to cutaneous lichen planus, oral lichen planus is often more chronic and resistant to treatment.Oral lichen planus can present in different forms.[9]

Esophageal lichen planus

Mucosa lichen planus can affect the esophageal mucosa, but this is rare. The histologic appearance of the lesions more closely resembles oral lichen planus than cutaneous lichen planus. Esophageal lichen planus can cause dysphagia, caused by esophagitis and the development of an esophageal stricture.[9]

Genital lichen planus

Lichen planus may involve the genitals, and this is more common in males. Lesions may occur on the mucosa of the glans penis or the skin of the scrotum. In females, the vulva and the vagina can be involved, and may be associated with urethral stenosis, dyspareunia and pruritus. A severe variant of lichen planus is sometimes termed vulvovaginal-gingival syndrome, or gingivo-vulvar syndrome.The vulva, vagina, and gums are involved. It is associated with HLA-DQB1. The equivalent condition in males is termed peno-gingival syndrome. This is considered a distinct variant of classic lichen planus, and it may cause scarring, vaginal stricture formation, or vulva destruction. [9]

DIAGNOSIS

The diagnosis is based on the clinical characteristics of the lesions and histopathological examination. The histologic features consists of hyperkeratosis, wedge-shaped hypergranulosis, irregular acanthosis, vacuolar degeneration of basal layer with the presence of intra-epidermal or sub-epidermal colloid bodies(apoptotic keratinocytes), saw- toothed rete- ridges and papillary dermal band like lymphocytic infiltrate.[10]

However, direct immunofluorescence studies may be helpful in disease differentiation for cases with no specific clinical or histological characteristics, or with ambiguous features of other diseases, e.g. lupus erythematous which shows ragged fibrin band at basement membrane zone and clusters of colloid bodies with IgM and C3; to a lesser extent with other classes of immunoglobulin. Hence this study was undertaken.[11]

OBJECTIVE OF THE STUDY

To determine the pattern of immunoreactants in lichen planus by direct immunofluorescence.

REVIEW OF LITERATURE

The beginning of direct immunofluorescence dates back to 1942, when Albert Coons and Kaplan showed the labeling of anti-pneumococcal antibodies with fluorescein in the pulmonary tissue. This technique was later extensively worked upon by Mary Osborne.[12]

Immunofluorescence is an antigen-antibody reaction where the antibodies are tagged (labeled) with a fluorescent dye and the antigen-antibody complex is visualized using ultra-violet (fluorescent) microscope. Fluorochromes are dyes that absorb ultra-violet rays and emit visible light. This process is called fluorescence. One of the most used fluorochromes is fluorescein isothiocyanate (FITC), of green color, with absorption and emission peak wavelengths of 490nm and 520nm, respectively. Rhodamine, another agent used in DIF, of red color, has distinct absorption and emission peak wavelengths (520 and 610nm).[13]

The two main methods of immunofluorescence are **direct** and **indirect.**

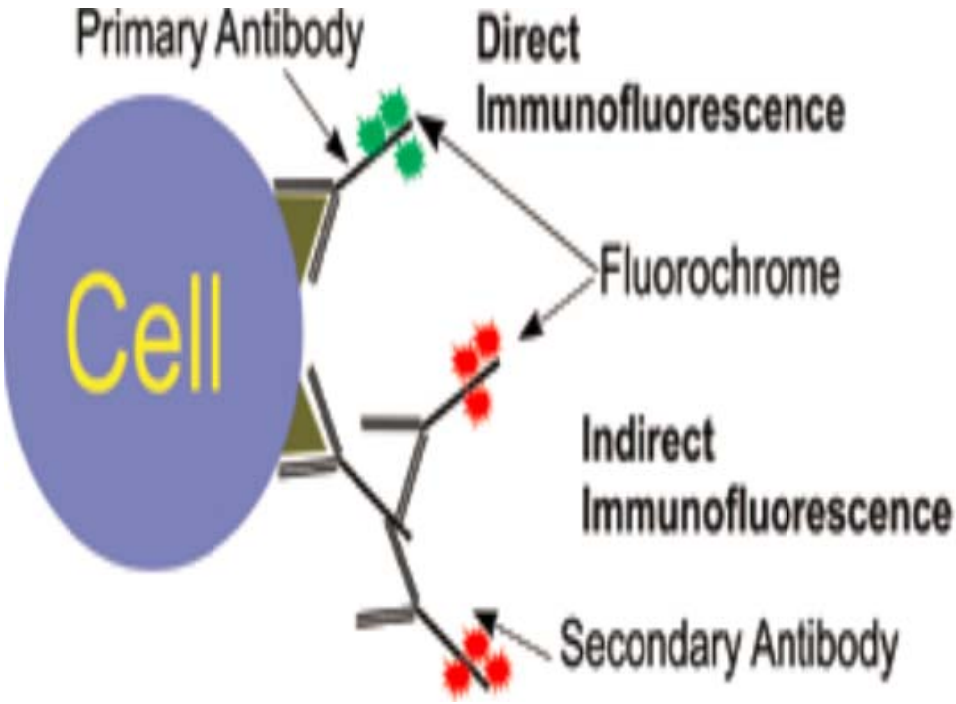
Direct immunofluorescence:

This technique is used to detect antigen in clinical specimens using specific fluorochrome labeled antibody. The steps involved are: Fixation of smear on the slide, treating with labeled antibody, incubation, washing to remove unbound excess labeled antibody and visualization under fluorescent microscope. When viewed under fluorescent microscope, the field is dark and areas with bound antibody fluoresce green. This technique can be used to detect viral, parasitic, tumor antigens from

patient specimens or monolayer of cells. Another application is identification of anatomic distribution of an antigen within a tissue or within compartments of a cell.[13]

Indirect immunofluorescence:

Indirect immunofluorescence is employed to detect antibodies in patient serum. The antigen on smear are made to react with specific unlabeled antibody and washed. The unbound antibody gets washed off. The presence of specific antibody bound to the antigen on smear is detected by adding another antibody. The second antibody is labeled anti-gamma globulin antibodies. This antibody binds to Fc portion of first antibody and persists despite washing. The presence of the second antibody is detected by observing under fluorescent microscope. It is often used to detect autoantibodies. Commonly used in the detection of anti-nuclear antibodies (ANA) found in the serum of patients with SLE.[13]



I. Direct Immunofluorescence in skin lesions

Direct immunofluorescence (DIF) is a one-step procedure used to detect and localize immunoreactants deposited in vivo in the patient's skin or mucosa. The immunoreactants include antibodies, complement components and fibrinogen.[14]

Frozen sections 5μm in thickness are cut with the cryotome and placed on slides. These are dried for ten minutes with an electric fan. Inadequate drying of sections between processing steps may lead to their detachment during washing. The sections are then washed in PBS at a pH of 7.4 for ten minutes to remove surrounding OCT compound. The sections are fan-dried once more and incubated with monospecific fluorescein isothiocyanate (FITC)-labeled antisera for thirty minutes at 37° C. Antisera to IgG, IgA, IgM, fibrinogen and the C3 component of complement should be routinely employed. Antisera to particular subclasses of immunoglobulins and other components of complement are also available but are less commonly used. Sensitivity and specificity of staining may be maximized by the use of the optimal dilution of the labeled antisera. This is determined by a chess-board titration procedure utilizing a known positive tissue specimen. The sections are washed in PBS to remove unbound antisera, fan-dried and mounted in a drop of buffered glycerol. They are then viewed with the fluorescence microscope.[14]

The distribution and type of immunoreactant deposition is recorded. The class and subclass of immunoglobulins and the presence or absence of complement is noted. Excessive fibrin depostion indicates that immunoreactants have been present more than 24-48 hours. Immunoreactants are deposited in two main patterns: in the

epidermal intercellular space (ICS) and along the basement membrane zone (BMZ). Intercellular space immunoreactants may be found throughout the epidermis or restricted to certain layers. Basement membrane zone deposits may be smooth and linear, granular and discontinuous or a combination of the two. [14]

II. Indirect Immunofluorescence in skin lesions

Indirect immunofluorescence (IIF) is a two-step procedure used to identify circulating autoantibodies to cutaneous or mucosal structures in a patient's serum. These antibodies are most commonly of lgG or lgA classes.[14]

In the first step, serial dilutions of the patient's serum in phosphate buffered saline (PBS) are incubated with frozen sections of the substrate. At least two 5μm thick sections are prepared by being alternately fan-dried, washed in PBS and fan-dried for ten minutes each. The initial serum dilution to 1:10 or 1:80 is incubated with the sections for thirty minutes at 37° C. If positive, subsequent incubations are used with increasingly higher dilutions of sera. Autoantibodies in the serum bind to components of the epidermis and basement membrane zone. Three washings of ten minutes each in PBS are carried out to remove unbound serum.[14]

In the second step, the bound autoantibodies are labeled with fluorescein isothiocyanate (FITC)-conjugated anti-human immunoglobulins. Class-specific antibodies are routinely used but subclass-specific antibodies are also available. Incubation with the antisera for thirty minutes at 37° C is followed by three washings in PBS of ten minutes each. The sections are then mounted in a drop of buffered glycerol and viewed with the immunofluorescent microscope.[14]

The class or subclass of immunoglobulins, the pattern and the site of deposition are noted. Circulating antibodies most commonly IgG, IgA are present in certain disorders whileimmunoglobulins of other classes are less common. Immunoglobulin may bind in a linear or discontinuous manner either along the BMZ or in the ICS. [14]

Advantages of direct immunofluorescence

Shorter sample staining time and simpler dual and triple labeling procedures.In cases where one has multiple antibodies raised in the same species, for example two mouse monoclonals, a direct labeling may be necessary.[13]

Disadvantages of direct immunofluorescence

Lower signal, generally higher cost, less flexibility and difficulties with the labeling procedure when commercially labeled direct conjugates are unavailable.[13]

Advantages of indirect immunofluorescence

Greater sensitivity than direct immunofluorescence. There is amplification of the signal in indirect immunofluorescence because more than one secondary antibody can attach to each primary.Commercially produced secondary antibodies are relatively inexpensive, available in an array of colors, and quality controlled.[13]

Disadvantages of indirect immunofluorescence

Has potential for cross-reactivity and the need to find primary antibodies that are not raised in the same species or of different isotypes when performing multiple-labeling experiments. Samples with endogenous immunoglobulin may exhibit a high background.[13]

Applications of immunofluorescence in dermatology

Immunofluorescence was introduced into Dermatology in the 1960s, when Beutner and Jordon revealed through this technique, tissue and circulating antibodies in autoimmune vesiculo-bullous dermatosis, especially in pemphigus vulgaris (PV), pemphigus foliaceus (PF) and bullous pemphigoid (BP).[15] Currently, immunofluorescence studies are vital for the laboratory diagnosis of autoimmune bullous dermatosis, but they are also important in the investigation of other diseases, such as inflammatory dermatosis -lupus erythematosus, lichen planus, porphyrias, vasculitis.

Direct immunofluorescence in lichen planus

Direct immunofluorescence studies in patients with lichen planus show the deposition of multiple immunoglobulins at the cytoid bodies & fibrin at the dermoepidermal junction.[16]

Colloid bodies (CB) or cytoid bodies or civattebodies areeosinophilic hyaline ovoid bodies which are often found in the subepidermal papillary regions or sometimes in the epidermis. They are usually seen in lichen planus (LP) and lupus erythematosus (LE). They can also be found in several dermatoses such as erythema multiforme (EM), bullous pemphigoid (BP) and diseases with suprabasal clefts. Colloid bodies, also known as civatte bodies or cytoidbodies, are generally believed to be derived from two origins. The first type originates from apoptosis of keratinocytes causes by epithelium damage created by circulating disorders. CB of this type are usually found locally both in the epidermis and papillary dermis. The other origin derives from the destruction of thickened basement membranes which are found only in the papillary dermis.[16]

Various studies were conducted to demonstrate the uses of immunofluorescence in lichen planus. These are as follows:

Kanokvalai K *et al*. studied 72 patients from 1996 to 2004. Of the 72 studied, 36 were females and 36 were males. Skin biopsy was taken from all the patients and studied under direct immunofluorescence. Distribution of immunoreactants in lichen planus was as follows - their study showed immunoreactants deposits at DEJ and CB in 53% and 60% cases respectively. Most common immune reactant at the DEJ was fibrin which was positive for 100% of cases. Other immunoreactants in the order of importance were C3,IgG, IgM and IgA. IgM was the most common immunoreactant at CB accounting for 93% ,followed by C3, fibrin,Ig and IgG.[17]

Sandra A *et al*. performed direct immunofluorescence in 18 patients who presented with clinical features of lichen planus. Lesional biopsy was taken for both histopathology and direct IF. Sections were stained with FITC labeled antisera to IgG, IgA, IgM, C3 and fibrinogen. The most common immunoreactant in this study was C3 accounting for 72%, followed by IgM in 66%, IgA in 50% and IgG 44%.[18]

E.H Baart De *et al*. performed direct IF in 40 patients of lichen planus. They reported the occurrence of fibrin or related substances and of IgM in the upper part of the dermis in all 40 lichen planus lesions examined. Different amounts of various complement components and fragments were located in the epidermal basement zone, and in the colloid bodies. [19]

Leena C *et al* in their study "Colloid Body deposition in direct Immunofluorescence" studied 502 patients with various skin lesions. Colloid bodies in direct immunofluorescence are usually found in interface dermatitis, various skin lesions and even in normal skin. Interface dermatitis includes lupus erthematosus,

discoid lupus erythematosus, lichen planus, erythema multiforme and dermatomyositis. The study concluded that immunoreactant deposits at CB alone can be found in various diseases but brighter intensity and higher quantity of CB was detected in interface dermatitis. CB alone is more common in lichen planus.[16]

Raghavendra R *et al*. studied twenty five patients with clinical diagnosis of lichen planus. Indirect IF using autologous lesional skin showed characteristic fluorescent IgG deposits in the upper dermis, at the level of the stratum granulosum and stratum spinosum in 88% patients. Direct IF also done in this study showed Ragged fibrin band 100%, IgM in 84%, C3 in 60% and IgA in 11% patients. [20]

Anita Nangia*et al.* conducted direct immunofluorescence in twenty-five patients with clinically diagnosed lichen planus. They observed that the male to female ratio was 1:1.77. The peak incidence was observed in 11-20 years age group. Most (96%) of the patients presented with moderate to severe itching within two months of onset of symptoms. Sixty percent of the patients had violaceous papules; 20% had both papules and plaques. Simultaneous oral involvement was seen in 4% of cases; 8% showed nail changes. Thirteen patients had classical LP, 9 had lichen planushypertrophicus, 2 had lichen planusactinicus, and one had lichen planopilaris. Direct immunofluorescence revealed fibrin deposition in 64% of cases as a linear pattern at dermo-epidermal junction, as coarse granular deposits of IgM sub-epidermally in 24 of cases and at the dermo-epidermal junction as C3 in20% of cases. Civatte bodies were seen in 5 cases with H&E staining, but direct immunofluorescence for IgM, fibrin and C3 was observed only in two cases. This suggests activation of complement and fibrinogen cascade. [21]

METHODOLOGY

The present study was carried out in the Department of Pathology in collaboration with Department of Dermatology, Venereology and Leprology BLDE University's Shri B.M.Patil Medical College, Hospital and Research Centre, Bijapur during the period; October 2011 to June 2013.

Ten patients who were clinically diagnosed as lichen planus were included in the study. Patients who were already on treatment for lichen planus were excluded from this study. Detailed clinical history, informed consent and examination findings of the patients was done as per the proforma.

The patients were subjected to the following procedure which was carried out in department of dermatology –

Skin biopsies were performed by using a 3mm disposable punch. The lesions to be biopsied were cleaned with an antiseptic & under aseptic precautions skin biopsy was obtained after infiltrating the desired area by a local anaesthetic (2% xylocaine). The biopsied skin was tightly packed and dressed. The biopsy was divided into 2 halves. One half of the biopsy (for DIF) was fixed in normal saline and the other half (for H & E) was fixed in formalin.

The skin biopsy specimens for DIF were embedded in cryomatrix embedding medium and snap frozen at -70^0C until sectioned. Frozen sections were cut on a cryostat, air dried,washed twice with phosphate buffered saline for 10 minutes and overlain for 30 minutes with fluorescein isothiocyanate-conjugated rabbit antihuman

antibodiesIgG, IgA,IgM,C_3 and fibrinogen. Thereafter section slides were mounted with the mounting medium and viewed with a fluorescent microscope.

The skin biopsy for H & E were fixed in 10% formalin for 12 hours. Paraffin blocks were made and sections of 4-5 microns thickness were cut. The slides were stained by routine H & E stain and viewed under light microscopy.

RESULTS

A total of 10 patients, clinically diagnosed as lichen planus were included in this study. The diagnosis was confirmed by histopathology in all ten cases. Among 10 patients, 5 were males and 5 females showing equal sex incidence. The youngest was 10 years old and the oldest was 60 years old. Maximum number of patients were between third and fourth decade.

Table 1.Age & sex incidence in lichen planus

Age in years	No. of cases
10-20 years	2 cases
21- 30 years	2 cases
31- 40 years	2 cases
41- 50 years	3 cases
51- 60 years	1 case

5 = males , 5 = females, Total = 10

Graph No 1 : Bar diagram showing age incidence

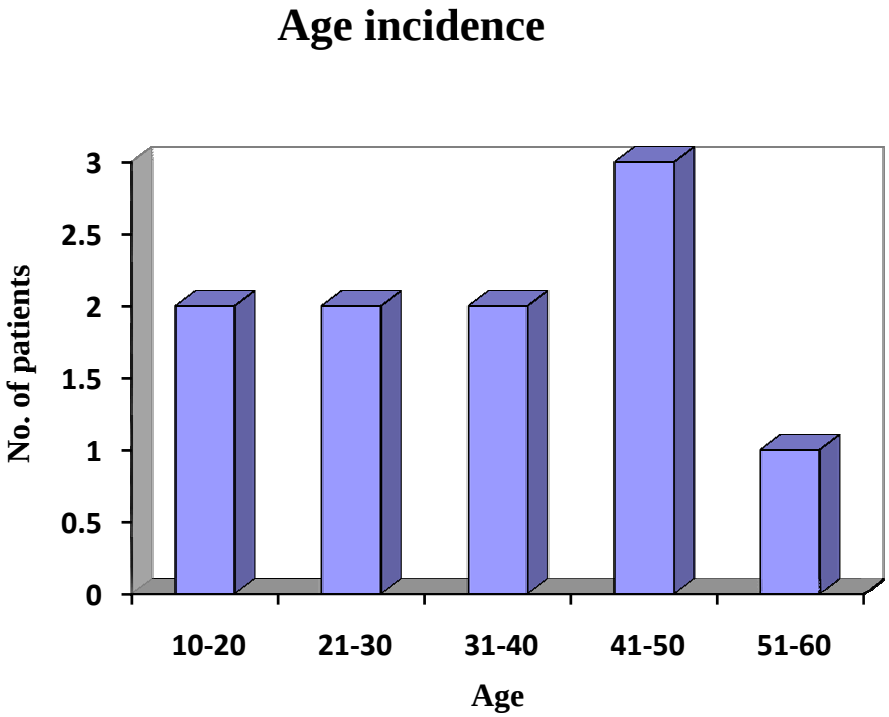


Table 2.The various morphological types encountered in the study

Clinical types	No. of cases
Classical	6
Hypertrophic	2
Generalized	1
Vesicular	1

The commonest type of lichen planus was classical type followed by hypertrophic, generalized and vesicular.

Graph No 2: The various morphological types encountered in the study

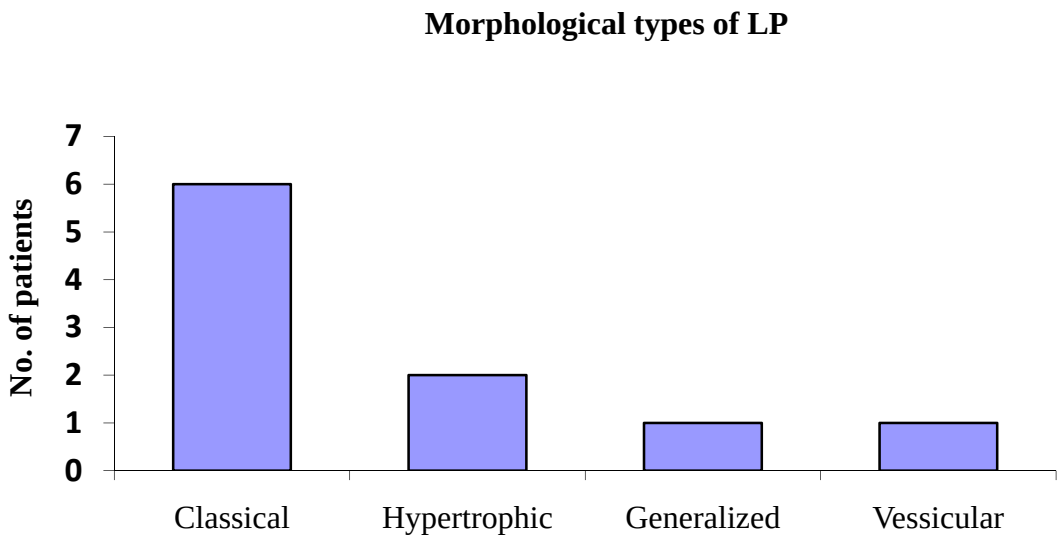


Table 3. Presence ofimmunoreactants in decreasing order of frequency

Immunoreactants	No. of cases
Fibrinogen	8
Ig M	5
C3	4
Ig A	4
Ig G	3

The most common immunoreactant was fibrinogen followed by IgM, C_3,IgA and Ig G.

Graph No 3 : Bar diagram showing deposition of various immunoreactants in various cases.

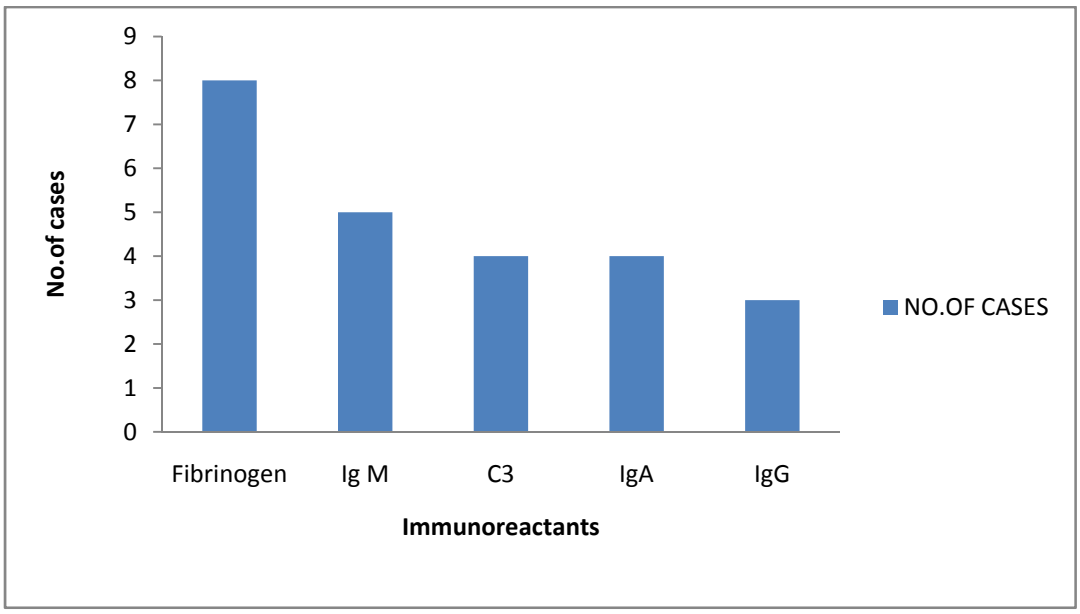


Table 4.Colloid body deposition

Sl no.	Case no.	Fibrinogen	Ig M	Ig G	Ig A	C3
1	2	+	+	-	-	+
2	4	+	+	-	-	-
3	5	-	+	-	-	+
4	7	+	+	-	-	-

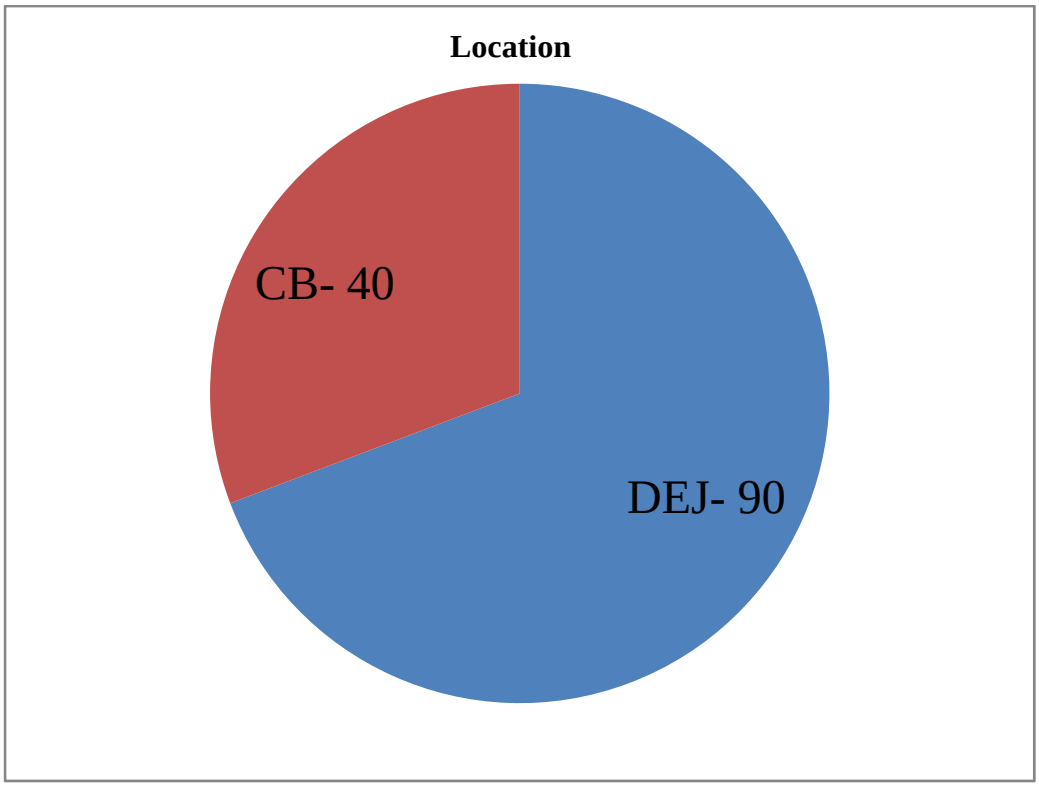
Colloid bodies were seen only in 4 cases (40%) , with 4 cases positive for IgM, 3 cases positive for fibrinogen and 2 cases positive for C_3 in various combinations.

Table 5. Distribution of immunoreactants in lichen planus

Location	Percentage
Dermoepidermal junction	90%
Cytoid bodies	40%

DEJ showed presence of immunoreactants in 90% of cases and CB in 40% of cases.

Graph No 4 : Distribution of immunoreactants in lichen planus

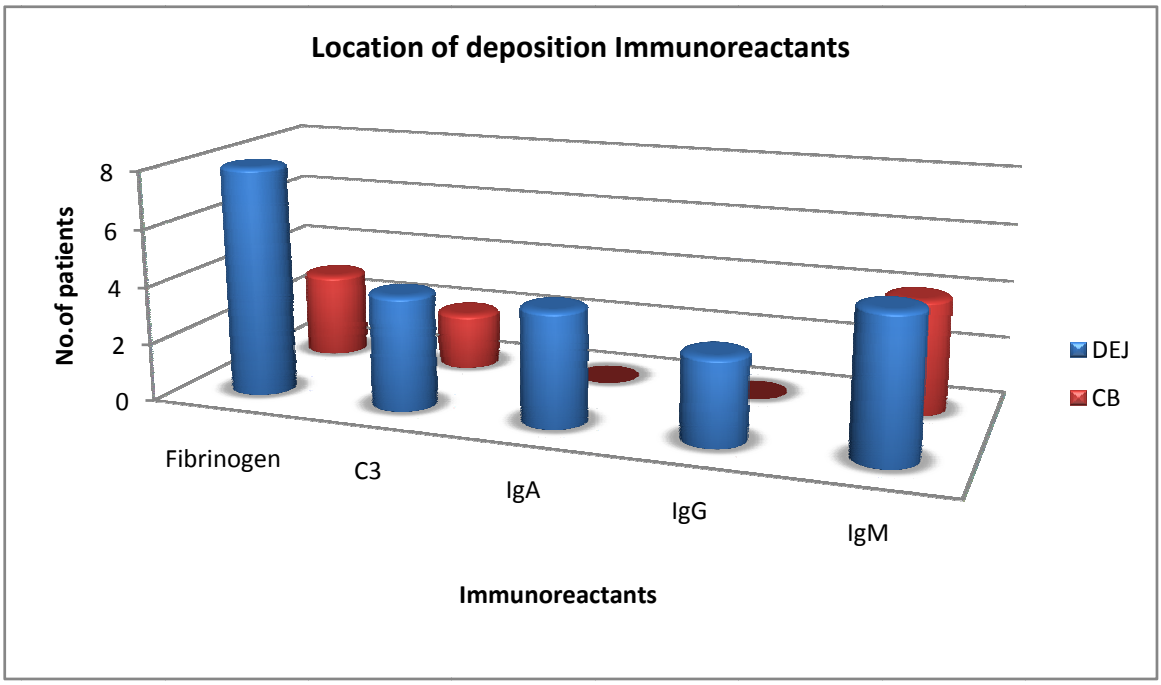


Pie chart showing presence of immunoreactants in DEJ (90%) and CB (40%).

Table 6.Details of immunoreactant deposits at various location

	No. of cases	
Immunoreactant	**DEJ**	**CB**
Fibrinogen	8	3
C_3	4	2
IgA	4	0
IgG	3	0
IgM	5	4

Graph No 5 : Bar diagram showing deposition of various immunoreactants at DEJ and CB



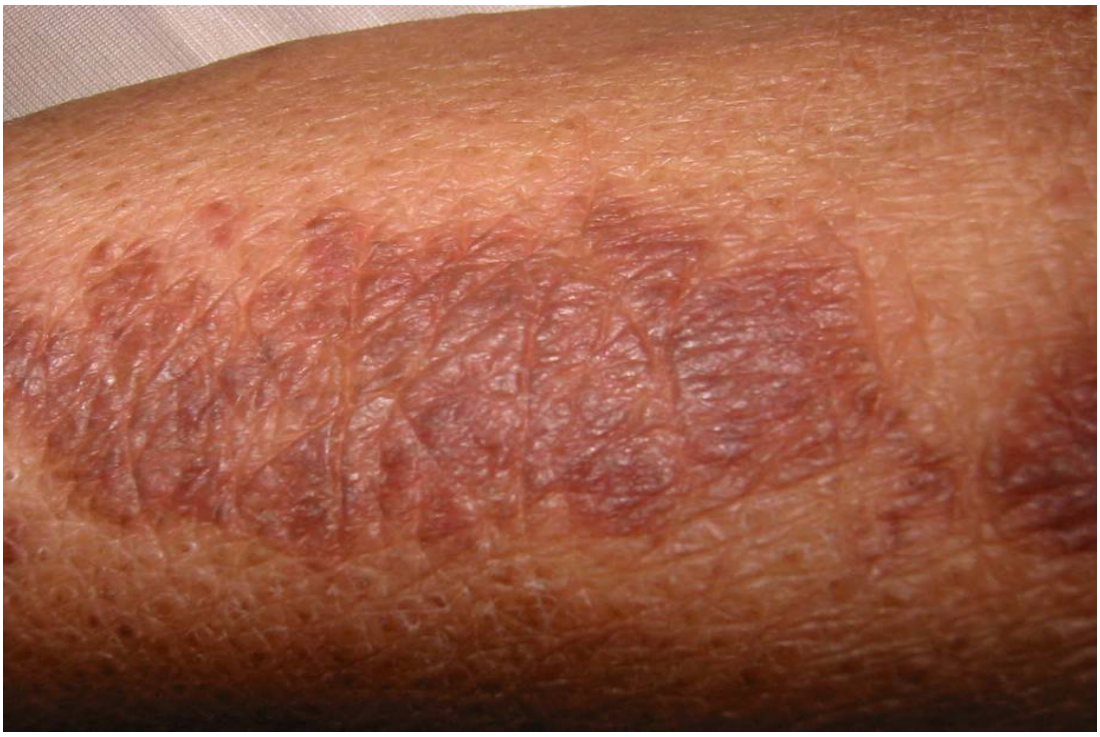


Fig 1. Erythematous to violaceous papules on the forearm suggestive of lichen planus

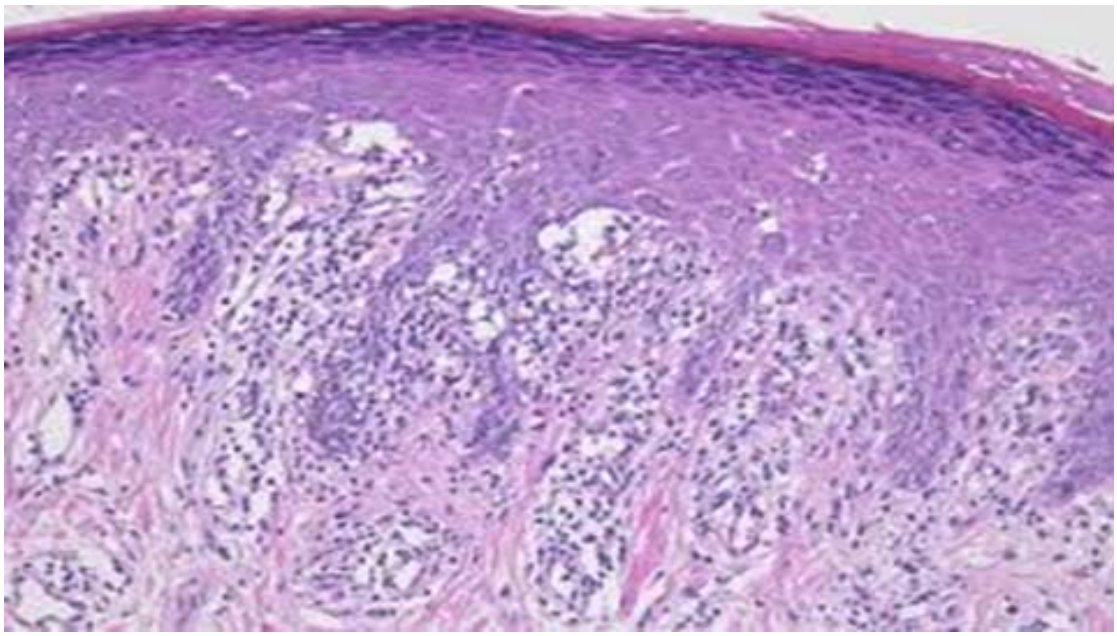


Fig 2. Microphotograph showing hyperkeratosis, hypergranulosis, saw toothed rete ridges and band like lymphocytic infiltration along the dermoepidermal junction suggestive of lichenplanus.

(H& E stain 10 X)

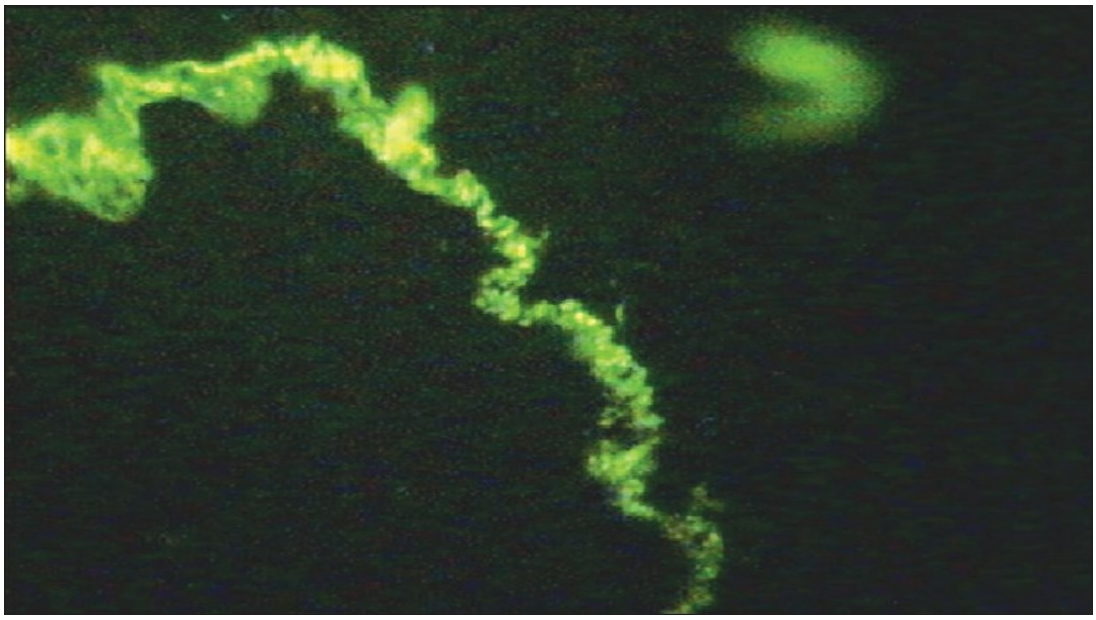


Fig 3.IMF microphotograph showing deposition of fibrinogen in the form of ragged band at the basement membrane zone

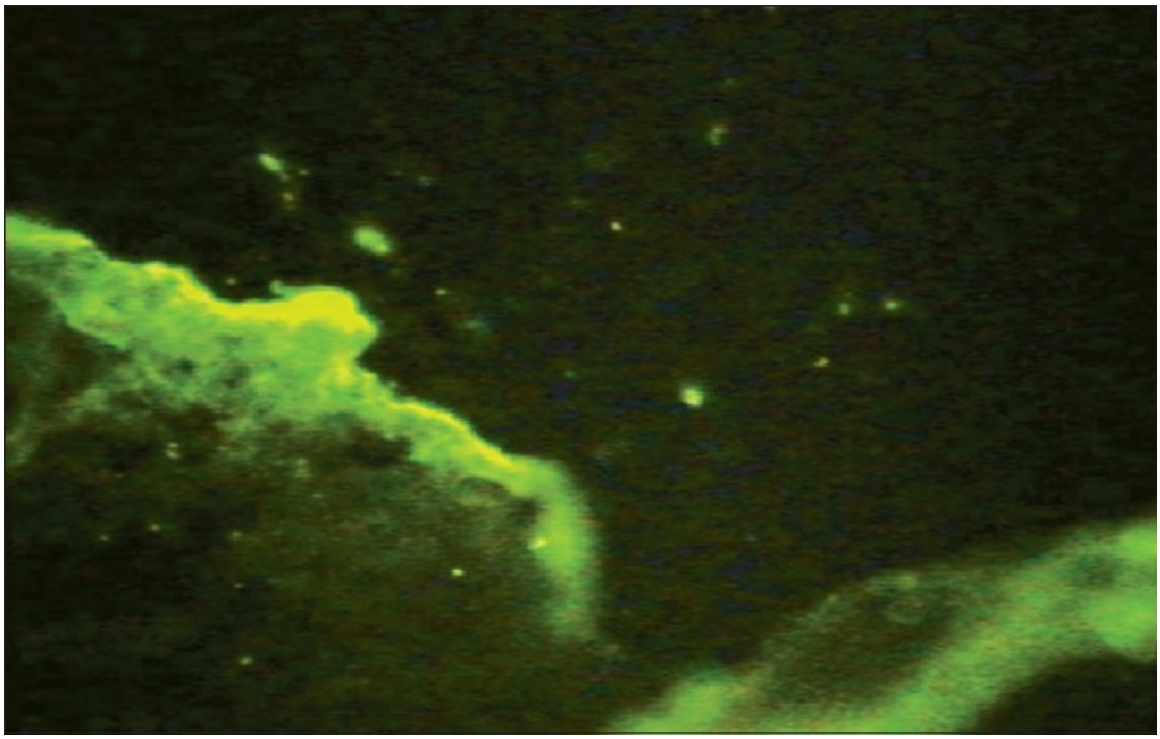


Fig 4.IMF microphotograph showing deposition of Ig M at the basement membrane zone

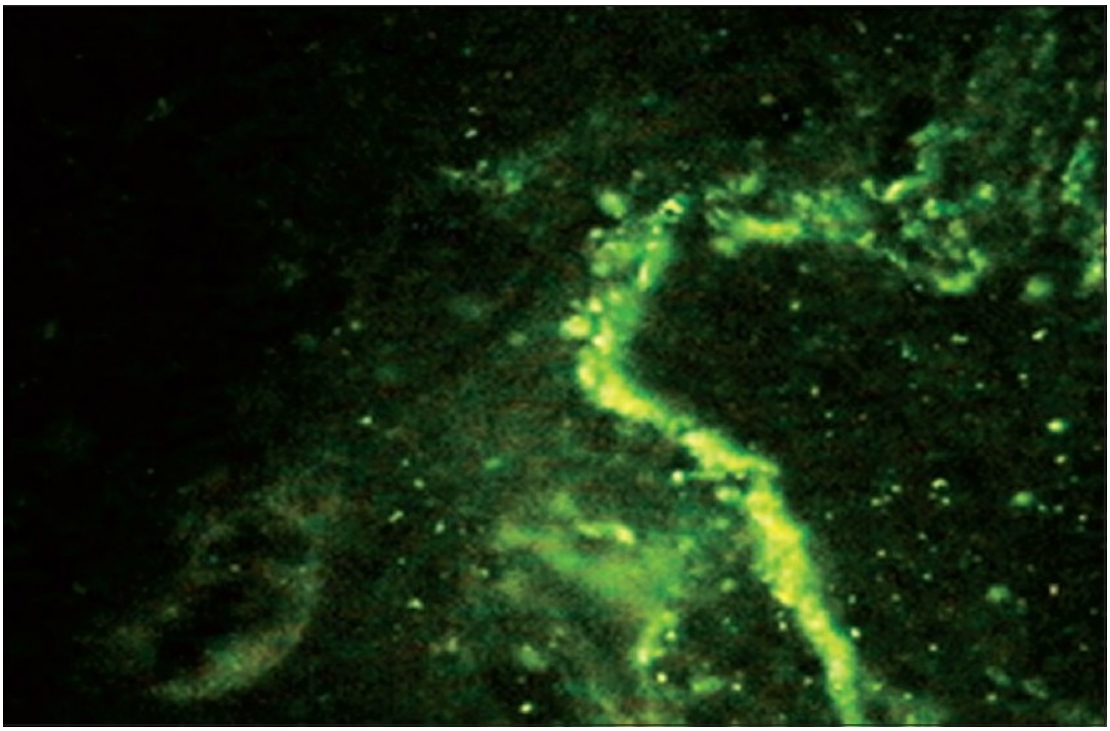


Fig 5.IMF microphotograph showingdeposition of C_3at the basement membrane zone

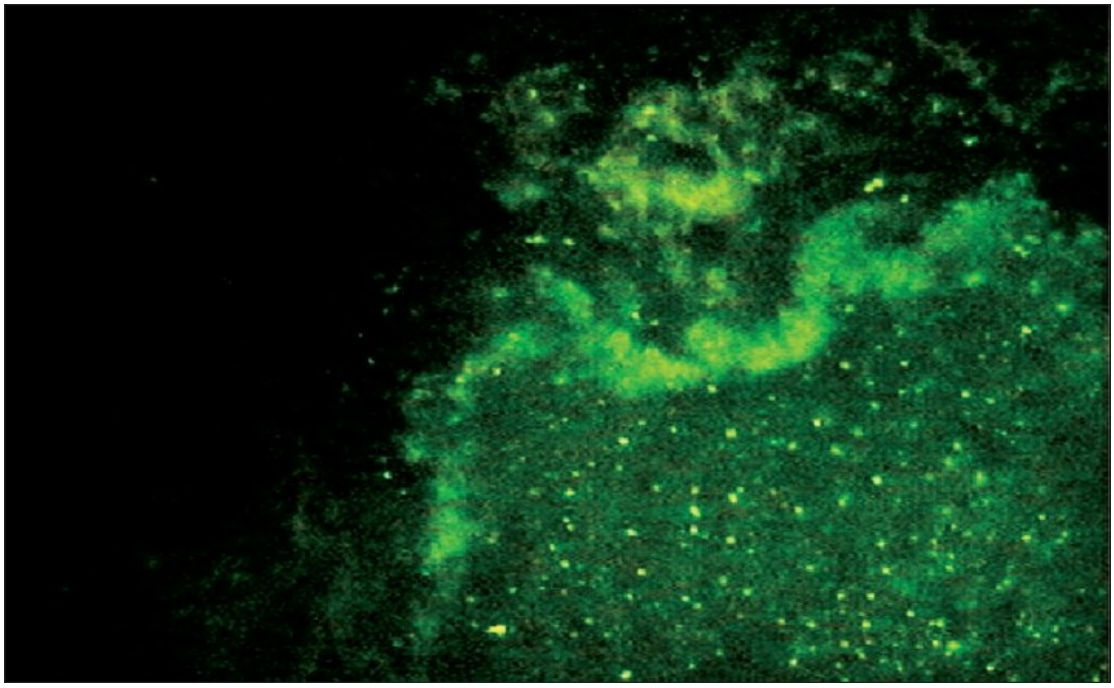


Fig 6. IMF microphotograph showing deposition of IgA at the basement membrane zone

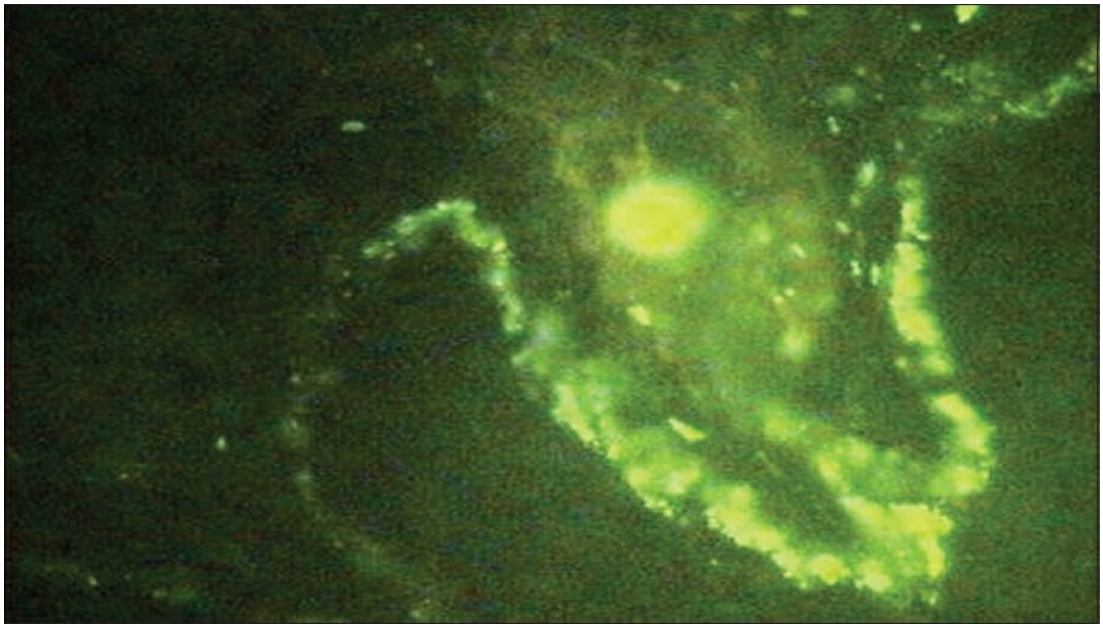


Fig 7.IMF microphotograph showing deposition of IgG at thebasement membrane zone

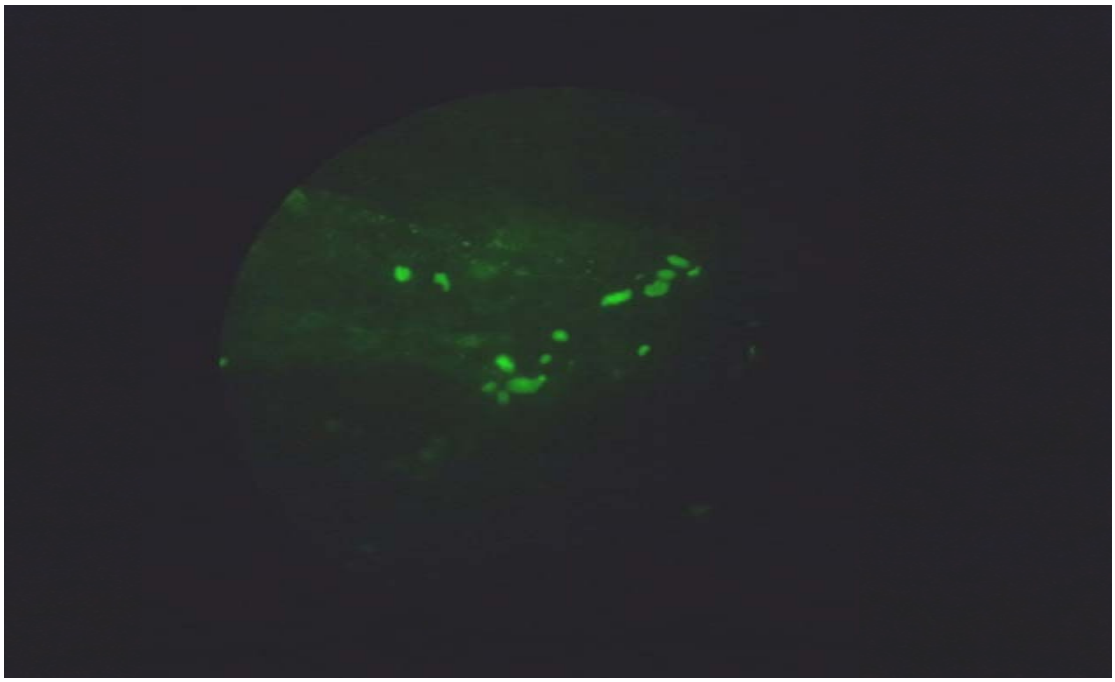


Fig 8. IMF microphotograph showingIgM deposition at the Colloid bodies.

DISCUSSION

Lichen planus is a papulo-squamous disease of the skin and mucous membrane of worldwide distribution. The exact etiology of lichen planus remains unknown. Recent studies have shown that lichen planus represents cell mediated immune response to an induced antigenic change in the epidermal cells in a genetically predisposed individual.[22]

There are characteristic direct immunofluorescence findings of lichen planus such as the presence of subepidermal colloid bodies demonstrating IgM and less commonly IgA, IgG, C_3 and fibrinogen. A linear broad band of fibrin at the dermo-epidermal junction has been suggested to be typical of lichen planus.

These characteristic direct immunofluorescence findings suggest that IMF may be a useful adjunct to clinical and histopathological findings in confirming diagnosis of confusing cases such as lupus eryhtematosus.

The present study was done to know the pattern of imunoreactants in lichen planus using direct immunofluorescence.

Age and Sex distribution

The youngest patient was 10 years old male and the oldest was 60 years old male. Most of the patients were between 30 to 40 years of age. Lichen planus is commonly seen in the age group of 30 to 70 years.[4] The presence of lichen planus in a younger age group in present study is concordant with the observation of Anita *et al*.,[21] whose study is also from India which is a tropical country.

Most studies have reported a female predominance but in the present study there is equal sex incidence with 5 male and 5 female patients.Similar findings were observed in the study done by Kanokvalai K *et al.*[17]

Morphologic variants

The most common morphological type was classical lichen planus followed by hypertrophic, generalized and vesicular. Similar findings were observed by Raghavendra R *et al.*[20]

Pattern of immunoreactants

The most common immunoreactant was fibrinogen (80%) followed by IgM (50%), C3(50%), Ig G(30%) and Ig A(30%). These findings were concordant with the studies done by Anita *et al.*[21] and Raghavendra R *et al.*[20]while this finding was discordant with the observation done by Kanokvalai K *etal.*[17] where fibrinogen was followed by C_3, IgG,IgM and IgA.

The presence of fibrinogen was seen in 80% in the present study. Study done by Sandra *et al.*[18]and Kanokvalai K *et al.*[17] showed fibrinogen positive in 100% cases.

The present study observed presence of cytoid bodies in 40% of cases. Study done by Kanokvalai K *et al.*[17]showed presence of cytoid bodies in 60% of cases and Anita *et al.*[21] in 20%. The most common marker present in colloid body was IgM, followed by fibrinogen and C3 in various combinations which was similar to the findings observed by other authors.[21,17]

CONCLUSION

Lichen planus is an inflammatory skin disease characterized by faintly erythematous to violaceous, flat topped, polygonal papules with adherent scales. The lesions usually involve flexural areas of the skin, the oral mucous membranes & genitalia.

The diagnosis is based on the clinical characteristics of the lesions and histopathological examination. The histologic features consists of hyperkeratosis, wedge-shaped hypergranulosis, irregular acanthosis, vacuolar degeneration of basal layer with the presence of intra-epidermal or sub-epidermal colloid bodies, saw-toothed rete- ridges and papillary dermal band like lymphocytic infiltrate.

However, direct immunofluorescence studies may be helpful in disease differentiation for cases with no specific clinical or histological characteristics, or with ambiguous features of other diseases, e.g. lupus erythematous which shows ragged fibrin band at basement membrane zone and clusters of colloid bodies with IgM and C3; to a lesser extent with other classes of immunoglobulin.

The most common morphological type was classical lichen planus followed by hypertrophic, generalized and vesicular. The most common immunoreactant was fibrinogen(80%) followed by IgM (50%), C3(50%), Ig G(30%) and Ig A(30%). The present study observed presence of cytoid bodies in 40% of cases.

SUMMARY

The present study was done to determine the pattern of immunoreactants in lichen planus by direct immunofluresence. The salient features of the study are as follows;

- Total of 10 cases clinically diagnosed as lichen planus were included in the study.
- All 10 cases were proved by histopathology.
- The youngest patient was 10 years old male and the oldest was 60 years old male.
- Most of the patients were between 30 to 40 years of age.
- There was equal sex incidence in the present study.
- Deposition of the immunoreactants were seen at the DEJ and CB.
- The most common immunoreactant at the DEJ was fibrinogen followed by presence of IgM , C3, Ig G and Ig A.
- CB was seen in 40% of cases.
- The most common marker present in colloid body was IgM, followed by fibrinogen and C3 in various combinations.

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ANNEXURES

SCHEME OF CASE TAKING

1) Name : CASE NO :

2) Age : IP NO :

3) Sex : DOA :

4) Religion : DOD :

5) Occupation :

6) Residence :

7) Presenting Complaints :

9) Past History :

10) Personal History :

11) Family History :

12) Treatment History :

13) General Physical Examination

Pallor present/absent

Icterus present/absent

Clubbing present/absent

Generalized Lymphadenopathy	present/absent
Anasarca	present/absent
Built	Poor/Average /Well
Nourishment	Poor / Average /Well

Vitals:-

PR	:	BP	:
RR	:	Temp	:
Weight	:		

Systemic Examination:

i. Respiratory System

ii. Cardiovascular System

iii. Central Nervous System

iv. Per abdomen examination

v. Dermatological examination:

Provisional Clinical Diagnosis:

Investigations:

Hematological examination:

Other investigations:

Histopathological examination:

Macroscopy:

Microscopy:

Special stains:

Final Diagnosis:

B.L.D.E.A'S SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER ,BIJAPUR-586103

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT : PATTERN ANALYSIS OF IMMUNOREACTANTS IN LICHEN PLANUS

PRINCIPAL INVESTIGATOR : Dr. YESHASWINI. JAYAKUMAR

P.G.GUIDE : Dr. B.R.YELIKAR$_{M.D.}$

PURPOSE OF RESEARCH:

I have been informed that the present study will include the skin biopsy for further evaluation.

PROCEDURE:

I understand that after having obtained a detailed clinical history a thorough clinical examination will be done. Skin biopsy will be performed in all the patients with clical diagnosis of lichen planus.

RISK AND DISCOMFORTS:

I understand that I may experience some pain and discomforts during the skin biopsy. This is mainly the result of my condition and the procedures of this study are

not expected to exaggerate these feelings which are associated with the usual course of treatment.

BENEFITS:

I understand that my participation in the study will have no direct benefit to me other than the potential benefit of the treatment.

CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of hospital records and will be subject to the confidentiality. Information of sensitive personal nature will not be part of the medical record, but will be stored in the investigations research file.

If the data are used for publication in the medical literature or for teaching purpose, no name will be used and other identifications such as photographs will be used only with special written permission. I understand that I may see the photograph before giving the permission.

REQUEST FOR MORE INFORMATION :

I understand that I may ask more questions about the study at anytime.

Dr. Yeshaswini Jayakumar will be available at the Department of Pathology to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation. A copy of this consent form will be given to me to keep for careful reading.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice. I also understand that Dr. Yeshaswini Jayakumar may terminate my participation in the study after she has explained the reasons for doing so.

INJURY STATEMENT :

I understand that in the unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, the appropriate treatment would be available to me. But, no further compensation would be provided by the hospital. I understand that by my agreements to participate in this study and not waiving any of my legal rights.

I have explained to _____the

Purpose of the research, the procedures required and the possible risks to the best of my ability.

_____ _____

Dr. Yeshaswini. Jayakumar. Date

STUDY SUBJECT CONSENT STATEMENT:

I confirm that Dr. Yeshaswini Jayakumar has explained to me the purpose of research, the study procedure, that I will undergo and the possible discomforts as well as benefits that I may experience in my own language. I have been explained all the above in detail in my own language and I understand the same. Therefore I agree to give consent to participate as a subject in this research project.

_____ _____

(Participant) Date

_____ _____

(Witness to signature) Date

ETHICAL CLEARANCE CERTIFICATE

Ethical Committee BIJAPUR-586103 OUT WARD No 27/2011 Date 21-10-11 B.L.D.E.'s S.B.M. PATIL Medical College

B.L.D.E. UNIVERSITY'S

SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 20-10-2011 *at* 10-30 am *to scrutinize the Synopsis/Research projects of postgraduate/undergraduate student/Faculty members of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis/Research project has been accorded Ethical Clearance.*

Title "Pattern analysis of immunoreactants in Lichen planus"

—x—x—x—

Name of P.G./U.G. student/Faculty member Dr. Yashaswini Jayakumar Dept of Pathology

Name of Guide/Co-investigator Dr. B.R. Yelikar Prof & HOD. Pathology

DR.M.S.BIRADAR,
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Chairman
Ethical Committee
BLDEA'S Shri. B.M. Patil
Medical College
Bijapur-586103

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

MASTER CHART

Sl No	Name	Age	Sex	Duration Of Disease	Family History	Variant of LP	Immunoreactants					Pattern	
							Fibrinogen	IgM	IgG	IgA	C3	DEJ	CB
1	Shridevi	30	F	15 DAYS	NO	Classical LP	+	+	+	-	+	+	-
2	Kasturi	25	F	1 year	NO	Classical LP	+	+	-	+	-	+	+
3	Kenchappa	50	M	30 Days	NO	Generalised	+	-	-	-	+	+	-
4	Manjunath	60	M	3 Months	NO	Hypertrophic	+	-	+	+	-	+	+
5	Rathikant	44	M	4 Months	NO	Classical LP	-	+	-	-	+	+	+
6	Laxmi	47	F	2 Months	NO	Hypertrophic	+	-	-	-	-	+	-
7	Siddu	37	M	15 days	NO	Classical LP	+	+	+	-	-	+	+
8	Seema	17	F	1 year	NO	Classical LP	+	-	-	+	+	+	-
9	Vijaykumar	31	M	2 years	NO	Vesicular	+	+	+	-	+	+	-
10	Akshata	10	F	45 Days	NO	Classical LP	-	-	-	-	-	-	-