"ASSOCIATION OF MUCOCUTANEOUS LICHEN PLANUS WITH HEPATITIS C VIRUS INFECTION IN A ONE YEAR CROSS SECTIONAL STUDY AT K.L.E.S DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM"

By

DR. DEEPTI RANA

Dissertation

Submitted to the

KLE University

Belgaum, Karnataka

In partial fulfilment

of the requirements for the degree of

DOCTOR OF MEDICINE (M.D)

in

DERMATOLOGY, VENEREOLOGY AND LEPROSY

Under the Guidance of

DR. B. S. MANJUNATHSWAMY

DEPARTMENT OF DERMATOLOGY, VENEREOLOGY AND LEPROSY
J.N.MEDICAL COLLEGE, NEHRU NAGAR,
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I hereby declare that this dissertation entitled **"ASSOCIATION OF MUCOCUTANEOUS LICHEN PLANUS WITH HEPATITIS C VIRUS INFECTION IN A ONE YEAR CROSS SECTIONAL STUDY AT K.L.E.S DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM"** is a bonafide and genuine research work carried out by me, under the guidance of **Dr. B. S. MANJUNATHSWAMY**$_{\text{M.D, DVD}}$ Professor, Department of Dermatology, Venereology and Leprosy, J. N. Medical College, Belgaum.

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Place :

Date :

Dr. Deepti Rana

LIST OF ABBREVIATIONS USED

CD	=	Cluster of differentiation
HCV	=	Hepatitis C Virus
HLA	=	Human leukocyte antigen
KLES	=	Karnataka Lingayat Education Society
LP	=	Lichen planus
MRC	=	Medical Research Centre
NANB	=	Non A Non B
NS	=	Non-structural
OPD	=	Out Patient Department
OLP	=	Oral Lichen Planus
PUVA	=	Psoralen and Ultraviolet A light therapy
RNA	=	Ribonucleic acid

ABSTRACT

Background and Objectives: To find out the association of Hepatitis C Virus infection with mucocutaneous Lichen Planus (LP) and to study various clinical and histopathological manifestations of Lichen Planus.

Materials and Methods: The present study is a one-year, cross-sectional study from November 2007 to October 2008. The patient's demographic data, location of lesions, risk factors were noted in a pre-tested and pre-designed proforma after taking informed and written consent. All clinically diagnosed cases of Lichen Planus were subjected to Punch biopsy for histopathology and Second Generation ELISA test for HCV antibodies (Anti-HCV) in human serum.

Results: A total number of 50 cases were studied, none of the patients were positive for antibodies to HCV. Maximum cases were in the age group of 21-40 years (46%). Male to female ratio was 1.6:1. In the study 96% of patients had only cutaneous involvement, 8% had cutaneous and mucosal lesion and 4% patients had only mucosal involvement. 86% cases were of Classical LP.

Conclusion: No association was established between mucocutaneous LP and HCV infection.

Key words : Lichen planus, Hepatitis C Virus

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INTRODUCTION

Lichen Planus (LP) is a common inflammatory mucocutaneous disorder that exhibits distinct morphology and microscopic features, which might be associated with Hepatitis C Virus (HCV) infection.[1]

Lichen Planus was first described in 1869 by Erasmus Wilson. The term Lichen means 'to lick', which is derived from the Greek verb (Leichen). It is a self limiting condition that most commonly affects middle-aged adults. It can involve glabrous skin, mucous membrane, hair and nails.[1]

The classic cutaneous lesion of Lichen planus is variable and most commonly one finds pruritic, faintly erythematous to violaceous, flat-topped, polygonal papules with characteristic flexor distribution. Less common variants include hypertrophic, atrophic, vesicular and bullous lesions. Isomorphic phenomenon is characteristic of Lichen Planus.[1,13,18]

Many agents have been implicated in its etiology. It is associated with autoimmune and liver disorders. A more consistent association exists between Lichen Planus and HCV infection. The occurrence of Lichen Planus in a patient with HCV infection was first reported in 1991. Since then many studies have suggested an association between these two disorders and stress the importance of liver examination in mucocutaneous Lichen Planus. So Lichen Planus can be used as a marker of HCV infection in asymptomatic patients leading to diagnosis and early treatment, and possibly better prognosis. However, if this is not true association the routine testing of patients with Lichen Planus for HCV may result in unnecessary use of medical resources, with increase in monetary cost and increase in anxiety among

those tested. Therefore it is important to determine whether there is an association between Lichen Planus and HCV infection so that guidelines regarding the routine HCV testing of patients with Lichen Planus may be developed for clinicians.[13,18]

Histopathology and Immunofluorescence findings will confirm the diagnosis of Lichen Planus.

The duration of Lichen Planus is related to the extent and site of involvement. Follow up is essential to monitor possible malignant transformation, especially in mucosal lesions.

Multiple therapeutic options exist including corticosteroids, retinoids, immunosuppressive agents, PUVA, antibiotics and various newer drugs.

OBJECTIVES

Primary objective : To find out the association of Hepatitis C Virus infection with mucocutaneous Lichen Planus.

Secondary objective : To study various clinical and histopathological manifestations of Lichen Planus. The classical lesions of LP are Violaceous, flat-topped, polygonal papules. Histopathological features : orthokeratosis, hypergranulosis, irregular acanthosis and band like dermal lymphocytic infiltration.

REVIEW OF LITERATURE

HISTORICAL ASPECTS

Erasmus Wilson initially coined the term Lichen Planus in 1869. The dermatosis was described earlier by Hebra as "Leichen Ruber".[1] Hence it is also called as Lichen Ruber Planus.

Lichen Planus (Greek Leichen, tree moss"; Latin planus, "flat"). The term Lichen was applied to the disease because of the common presentation of the flat-topped lesions that suggested the similarity to a group of plants known as Lichens.[1,2]

Hallopeau (1887) and Darier (1892) considered Lichen Sclerosus et Atrophicus (LSEA) as being a modification of Lichen Planus due to the close similarity between clinical and morphological findings in both conditions.[3]

The first variant of lichen planus was described by Kaposi in 1892 and he termed Lichen Ruber Pemphigoides for widespread bullous eruption complicating typical Lichen Planus.[4]

Louis Frederick Wickham in 1895 described the characteristic appearance of whitish striae and punctuations on the top of flat surfaced papules.[5]

Lichen planopilaris, a term coined by Pringle in 1895, and follicular LP, a name proposed by Silver et al are clear terms that describe the clinical syndrome of Lichen Planus associated with cicatricial alopecia.[6]

Darier described the histopathological findings in 1909 and attributed the appearance of Wickham's striae to an increase in the granular cell layer.[1,3]

Graham Little reported scalp involvement in 1915. In 1930 Schreiner divided bullous Lichen planus into two groups, the first, Lichen Planus vesiculosus, and the second Lichen planus pemphigoides.

In 1941, Niles described Actinic Lichen Planus.[4] Thyresson and Meberger demonstrated colloid bodies at epidermo-dermal junction and suggested that they were formed from degenerating epithelial cells and attributed this change to viral infection.[3,5]

Glickman in 1964 attributed the pallor which produces the appearance of Wickham striae, to the absence of papillary capillaries in the centre of the lesion. Ryan (1966) on the other hand attributed the violaceous hue which surrounds the areas of pallor to radially arranged horizontally oriented capillaries of proliferative type.[3,5]

In 1969 Pinkus and Mehregan suggested that the basic histopathologic features of Lichen Planus are damage to basal layer of epidermis.[5] In 1970 Zaias described the histopathologic features of Lichen planus of the nail.[4]

An overlap between Lichen planus and Lupus Erythematosus (LE) was proposed in 1970. Triad of Lichen planus, myasthenia gravis and thymoma was first reported by Aronson and Soltani in 1978.[4]

A unique type of genital and oral mucosal Lichen planus was described by Pelisse et al. in 1982 as Vulvo-vaginal gingival (VVG) syndrome.[7]

Hanav and Sengel described perforating variant of Lichen planus in 1989. In the same year, the triad of hypertension, diabetes mellitus and Lichen planus was described as Grinspan's syndrome.[4,11]

In 1993 Cribier et al, described the male equivalent of the vulvo-vaginal gingival syndrome of erosive oral Lichen planus called peno-genital syndrome of erosive oral Lichen planus.[7]

With discovery of HCV, a single stranded RNA virus in 1989 and availability of test for anti HCV antibodies in 1991, first case of Lichen planus with HCV infection was reported.[7,8]

In 1954 Hard and Humberg used penicillin for treating Lichen planus. Sehgal et al. reported effectiveness of Griseofulvin (1971) and Levamisole (1978) for recalcitrant Lichen planus. Other treatment modalities like PUVA (1978), Isotretinoin (1985), Dapsone (1986), Antimalarials (1989), Phenytoin and Cyclosporine (1990) and many newer drugs have been tried in the treatment of Lichen planus.[4]

EPIDEMIOLOGY

Incidence:

The exact incidence and prevalence of Lichen planus are not known, but the overall prevalence is believed to be somewhat less than 1% of general population. Lichen planus has a worldwide distribution with no racial predisposition. Estimates between 0.14% and 0.80% have been reported worldwide.[1,4]

Age : Females are usually affected in their fifties and sixties, whereas males develop Lichen planus at an earlier age. The disease is less common in very young and the elderly.[1,4]

Sex : No sexual predisposition is evident.[1,4]

Seasonal variation:

The development of Lichen planus may be affected by seasonal or environmental factors. An increased incidence in December and January or from January to July has been reported.[1,4]

Triggering factors : Sunlight, trauma, friction

Genetic factors:

An increase in frequency of HLA-B7, -AW19, -B18 and CW8 haplotypes were noted in familial LP. HLA-A3, -A5, -A28, -B8, -B16 and –BW35 were noted in nonfamilial LP. [1]

HLA-B8 was common in patients of oral LP and HLA-BW35 with cutaneous LP. [1,18]

Aetiopathogenesis :

It is evident that immunologic mechanisms almost certainly mediate the development of lichen planus. Humoral immunity most likely is a secondary response in the immunopathogenesis. Cell-mediated immunity plays a major role in triggering the clinical expression of the disease. Both CD4+ and CD8+ T cells are found in lesional skin of Lichen Planus. Progression of disease may lead to preferential

accumulation of CD8+ cells. The majority of the lymphocytes in the infiltrate of lichen planus are CD8+ and CD45RO (memory) positive cells. This latter cell subtype is not normally found in healthy skin.[1,4]

These cells are considered responsible for the development of the most characteristic change observed in the lichenoid reaction, namely, apoptosis.[1,4]

The epithelial lymphocyte interaction can be divided into three major stages: antigen recognition, lymphocyte activation, and keratinocyte apoptosis.[1]

Lichen Planus-Specific Antigen Recognition:

Majority of the T cells in the infiltrate of LP are activated CD8+ cytotoxic lymphocytes. Evidence suggests that CD8+ lesional Tcells (within epithelium & adjacent to basal keratinocyte) recognise LP specific antigen associated with major histocompatibility complex (MHC) class I on lesional keratinocyte. The antigen may be autoreactive peptide suggesting that LP may be an autoimmune disease. Alternatively it may represent an exogenous antigen such as altered protein, drug, contact allergen, dental amalgam, viral infectious agents or unidentified immunogenic target.[1]

The role of T helper (CD4) cells in the etiopathogenesis of LP is not fully understood. These cells may become activated via antigen-presenting cells such as Langerhans cells in association with major histocompatibility complex (MHC) class II and specific cytokines.[1]

T helper (CD4) cells may propagate CD8+ cytotoxic lymphocytes through cellular cooperation & release of cytokines.

The exact nature of antigenic stimulation is not known. Contact sensitizers such as metals which may act as haptens eliciting an immunogenic response. Low grade chronic exposure to mercury and other metals stimulating a lymphocytic reaction manifesting as LP. The role of infections or microorganisms: syphilis, herpes simplex virus 2, HIV, amebiasis, chronic bladder infections, hepatitis C virus, Helicobacter pylori, and human papillomavirus in development of LP is still not clear.[1]

Cytotoxic lymphocyte activation

Activated T lymphocytes by TH1, TH2 and cytotoxic- suppresor cells, release soluble mediators: interlukin (IL) -2, IL-4, IL-10, interferon (INF)- gamma, tumor necrosis factor (TNF)-alpha, that attract lymphocytes & regulate their activities in epithelium. Both pro & anti inflammatory cytokines are generated simultaneously. The balance between lymphocytic activation & down regulation determines clinical behaviour of the disease. INF-gamma produced by T helper cells during the antigen recognition stage, induces keratinocytes to produce lymphotoxin-alpha &TNF-alpha & to upregulate MHC-II thus increasing interactions with helper T cells.[1,4]

INF-gamma also upregulates the expression of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 by basal keratinocytes, Langerhans cells, and other macrophage-dendritic cells.[1]

Laminin-5 and collagen types IV and VII, are increased in lesional LP and serve as ligands for β_1-integrin on surface of lymphocytes, thus allowing for enhanced association of lymphocytes with the basement membrane.[1]

This close interaction between lymphocytes & basement membrane, targets metalloproteins to alter extracellular matrix protein & integrins, thus causing apoptosis, BM disruption, reduplication and subepidermal cleft formation.

Keratinocyte apoptosis[1,4]

The exact mechanisms used to trigger apoptosis are not known. Possible mechanisms are :

1) T cell secreted TNF-alpha binding to TNF- alpha R1 receptor on keratinocyte surface.
2) T cell surface CD95L binding CD95 on keratinocyte.
3) T cell secreted granzyme B entering keratinocyte via perforin –induced membrane pores.

Basement membrane disruption may trigger apoptosis through the loss of basement membrane-derived cell survival signal that normally prevents the onset of apoptosis mediated by recruited lymphocytes.

Clinical Features:

LP affects the skin, mucous membrane, hair and nails.[1]

The classical cutaneous lesions of LP are flat-topped, faintly erythematous to violaceous polygonal pruritic papules of varying sizes, may be asymptomatic in some. A thin, transparent and adherent scale may be present over the papules. Fine, whitish puncta or reticulated networks referred to as “Wickham’s striae”, are present over the surface of many papules, which are easily observed after placing mineral or immersion oil over the lesions.[1,4,18]

Lesions are classically distributed symmetrically and bilaterally over flexural areas of wrists, arms, legs and lower back. The face, palms and soles are less commonly involved.[1,4,13]

In active disease, scratching, injury or trauma may induce an isomorphic response (KOEBNER PHENOMENON). The lesions usually heal with hyperpigmentation.[1,14,18]

Classification of Lichen Planus variants:[1]

Configuration

Annular

Linear

Morphology of lesion

Hypertrophic

Atrophic

Vesiculobullous

Erosive/Ulcerative

Follicular

Actinic

Lichen planus pigmentosus

Other forms: perforating or guttate

Site of involvement

Palms & Soles

Mucous membrane

Nails

Scalp

Special forms

Drug induced (lichenoid drug eruption)

Lichen planus- Lupus Erythematosus overlap

Lichen planus pemphigoides

Keratosis lichenoides chronica

Lichen planus and malignant transformation

Lichenoid reaction of graft versus host disease

Lichenoid keratosis

Lichenoid dermatitis

CONFIGURATION:

ANNULAR LICHEN PLANUS

They occur approximately in 10% of cases and commonly develop as arcuate grouping of individual papules that develop rings or peripheral extension of clustered papules with central clearing. Characteristically seen over penis and scrotum and more commonly seen in blacks.[1,14]

LINEAR LICHEN PLANUS

Linear lesions as a koebner's phenomenon are frequently found in LP but isolated linear lesions, usually made up of small papules in close apposition extending the whole length of a limb may occur. This type is usually seen in childhood. This variant must be distinguished from linear naevi and other dermatoses with linear variants. A zosteriform pattern of LP has also been described and LP can develop in the site of healed herpes zoster. Multiple linear LP lesions following the lines of Blaschko have been reported and multiple linear LP was documented in a human immunodeficiency virus (HIV) patient have been reported.[1,15,18]

MORPHOLOGY OF LESION:

HYPERTROPHIC LICHEN PLANUS (LP verrucosus)

These lesions are usually confined to the extremities, especially shins and interphalangeal joints and tend to be most pruritic variant. Lesions are thickened and elevated, purplish or reddish-brown in color, hyperkeratotic with occasional verucous plaques. The lesions heal with atrophic scar or hyper- or hypopigmentation. Chronic venous insufficiency is commonly present.[4,18]

ATROPHIC LICHEN PLANUS

This variant is rare and is characterized by few well-demarcated, white-bluish papules and plaques with central superficial atrophy. Commonly seen on lower extremities and trunk.[1,18]

VESICULOBULLOUS LICHEN PLANUS

Two types are seen :

a) Bullous lichen planus
b) Lichen planus pemphigoides

Bullous Lichen Planus : Rare variant, characterized by development of vesicles and bullae on pre-existing lesion of lichen planus.[1,4]

Lichen Planus Pemphigoides: This is a controversial entity with co-existence of lichen planus and bullous pemphigoid. Clinically, it consists of bullae on lesional and non-lesional skin, often on the extremities. These lesions can appear during flare of disease and may be associated with mild constitutional symptoms.[1,4]

Histologically, the typical changes of LP are seen along with sub-epidermal separation.[1]

EROSIVE AND ULCERATIVE LICHEN PLANUS

This rare variant has been described in oral cavity and the soles. Characterized by painful bullae and ulcerations of the feet. Permanent loss of toe nails and cicatricial alopecia of scalp are common. Progression to squamous cell carcinoma has been reported in few cases of chronic ulcerative lesions of LP. The rare associations of erosive lichen planus are Castleman's lymph node hyperplasia and malignant lymphoma.[1,4]

FOLLICULAR LICHEN PLANUS (LICHEN PLANOPILARIS)

This form may occur alone or in association with other forms of lichen planus. Keratotic follicular papules are present commonly over trunk, proximal extremities, may affect scalp by producing cicatricial alopecia. The triad of follicular lichen planus of skin with cicatricial alopecia of scalp and non-scarring alopecia of axilla and pubic area is known as 'Graham Little-Piccardi-Lassuer syndrome'.[1,4]

Other variants of follicular LP include the pseudopelade of Brocq, the lichen planus follicularis tumidus form, postmenopausal frontal fibrosing alopecia and lichen planoporitis.[1,4,7]

ACTINIC LICHEN PLANUS

This variant is also known as Lichen planus subtropicus, summer time actinic lichenoid eruption, lichen planus actinicus, and lichenoid melanodermatosis. It is more common in Middle-East countries in the spring and summers. Lesions are limited to sun-exposed areas and are hyerpigmented with violaceous-brown color and a thready, rolled edge showing well-defined borders with minimal pruritus and scaling.[1,4,16]

LICHEN PLANUS PIGMENTOSUS

This is a pigmentary disorder seen in India or in Middle East, which may not be associated with typical LP papules. It is an uncommon variant and is characterized by hyperpigmented, dark-brown macules in sun-exposed areas and flexural folds.[1,18]

The mucous membrane, palms and soles are never involved. Erythema dyschromicum perstans (ashy dermatosis) that occurs in sun-exposed areas bears similarity to this variant of LP.[1,4]

OTHER VARIANTS

Guttate LP: is characterized by widely scattered discrete lesions that seldom become chronic.[1]

Exfoliative and exanthematous forms: are very rare and may represent manifestations of lichenoid drug reactions.[1]

Invisible LP: lesions that are not perceptible with visible light illumination but become apparent with Wood's lamp examination. Pruritus is present and this entity may be a minimal variant of lichen planus “invisible de Gougerot."[1,4]

SITE OF INVOLVEMENT:

PALMOPLANTAR LICHEN PLANUS

This acral, localized variant is rare. Characteristic lesions are very prurigoginous, erythematous, scaly plaques with or without hyperkeratosis and are often seen on the internal plantar arch. Yellowish, compact keratotic papules or papulonodules are seen on the lateral margins of the fingers and hand surfaces. They appear like callosities with an inflammatory, erythematous halo.[1]

MUCOSAL LICHEN PLANUS

LP involves mucosal surfaces of mouth, vagina, esophagus, conjunctiva, urethra, anus, nose and larynx. Its prevalence is approximately 1% of adult population. It may be the only manifestation in 20-30 % of patients.[1,4]

Oral LP: Oral involvement occurs in 60%-70% of patients with cutaneous lichen planus. Oral lichen planus lesions have a characteristic bilaterally, symmetrical distribution and are usually asymptomatic unless erosions or ulcers develop.[1,4]

Oral LP may manifest as :

a) Reticular
b) Plaque-type
c) Atrophic
d) Erosive

Buccal, gingival and glossal mucosae most commonly affected.

a) **Reticular type** is the commonest presentation. These lesions appear as raised white, linear striations, bilaterally symmetrical, over buccal mucosa.[1,4]
b) **Plaque type** appears as a multiple diffuse, raised white plaques commonly over buccal mucosa and tongue.[1,4]
c) **Atrophic type** can be seen concomitantly with the erosive or reticular form. It is frequently seen on gingival mucosa.
d) **Erosive type** is the most painful form. These erosions are seen frequently in elderly with reticular form.

Genital LP : Involvement of the genitalia with cutaneous LP has been reported in 25% of men with typical lesions .They present with pruritis and burning sensation. Lesions consist of violaceous papules, commonly on glans penis. Annular lesions are frequently reported on the scrotum. Female genital involvement consists of patches of leukoplakia or erythroplakia, sometimes with erosions and generalized desquamative vaginitis. Anal lesions of mucosal lichen planus present with leukokeratosis, hyperkeratosis, fissuring, and erosions.[1,4]

A special form : involvement of vulvar and gingival tissue. The characteristic features are erythema and erosions of the gingivae and tongue and, occasionally, white reticulated plaques with pain and discomfort. Desquamation and erosions of vulva and vagina in association with burning pain, dyspareunia and vaginal discharge.[1]

Conjunctival lichen planus: may manifest as cicatricial conjunctivitis.[1]

LICHEN PLANUS OF NAIL

Nail involvement occurs in 10% to 15% of patients. Usually only a few finger nails or toe-nails are involved. Thinning, longitudinal ridging and dorsal splitting of nail plate (onychoschizia) are most common findings. Onycholysis, longitudinal striation (onychorrhexis), subungual hyperkeratosis or even absence (anonychia) of nail plate can also be seen. Twenty-nail dystrophy (trachyonychia) may be an isolated finding. Pterygium or forward growth of the eponychium with adherence to the proximal nail plate is a classic finding of lichen planus of the nail. The tenting or pup-tent sign is observed as a result of nail bed involvement that elevates the nail plate and may cause longitudinal splitting.[1,4,18]

LICHEN PLANUS OF THE SCALP

LP may affect the scalp. Typically individual keratotic follicular papules that coalesce and merge over the scalp to form patches are seen, affecting women more than men. Patients present with uni- or multifocal hair loss that may be extensive and involve the entire scalp. Perifollicular erythema and acuminate keratotic plugs are characteristic features. End-stage disease is characterized by scarring alopecia that has led to the use of several clinical terms describing the entity lichen planopilaris, folliculitis decalvans et atrophicus, lichen spinulosus et folliculitis decalvans and Graham-Little syndrome.[1]

INVERSE LICHEN PLANUS

Its a rare variety characterized by red-brownish, discrete papules and nodules seen mainly in the flexural areas such as axillae, groin and inframammary areas, and less likely, popliteal and antecubital areas.[1,4]

SPECIAL FORMS OF LP:

Lichenoid eruption or Drug-Induced Lichen Planus: a group of cutaneous reactions identical or simillar to LP. Lichenoid drug eruptions develop after ingestion, contact, or inhalation of certain chemicals. The eruptions usually appear symmetrically on the trunk and extremities, with photodistributed pattern. Mucous membrane involvement is less common.[1]

Lichen Planus-Lupus Erythematosus Overlap Syndrome: an overlap between LP and lupus erythematosus. Atrophic plaques and patches with hypopigmented and a livid red to blue-violet color with telangiectasia and minimal scaling are

characteristic. Lesions are most commonly seen on the extremities. Some patients may progress to SLE. In others, laboratory evaluation may reveal only a weak-positive antinuclear antibody. This disease variant is characterized by a prolonged course and lack of response to treatment.[1,4]

Histologically, features of LP and LE are usually present in the same biopsy sample. On DIF, features of LP with linear to granular deposits of IgM and C3 as seen in LE.[1]

Lichen Planus Pemphigoides: characterized by tense blisters on lesions of lichen planus or the development of vesicles on uninvolved skin.[1]

Keratosis Lichenoides Chronica (Nekam's Disease) :

A rare dermatosis characterized by violaceous popular and nodular lesions, often arranged in a linear and reticulate pattern on the dorsum of hands and feet, extremities and buttocks. The mucous membranes, genitalia, nails, palms and soles may be affected.[1,4]

Lichen Planus and Malignant Transformation: Malignant transformation of oral lichen planus is controversial. Risk factors for development of oral cancer are long-standing disease, erosive or atrophic types, and tobacco use. Only 0.5 - 5 % of patients develop SCC. The most common site for malignant transformation is the tongue, followed by buccal mucosa, gingiva, and, rarely, the lip. The lesions appear as indurated, non-healing ulcers or exophytic lesions with a keratotic surface.[1,4]

Lichenoid Reaction of Graft-Versus-Host disease (GVHD) : may present as lichenoid eruption over trunk, buttocks, hips, thighs, palms, and soles. In oral mucosa, xerostomia and oral ulcerations are occasionally seen. Oral mucosa shows ulcerations ocassionally with xerostomia.[1,4]

Hisologically, infiltrating CD3 + T lymphocytes are present in larger number in Oral LP than in lichenoid lesions of oral GVHD.[1]

Lichenoid Keratosis: commonly seen on sun-exposed skin of extremities as brown to red, scaling maculopapules. Showing histological features of LP with additional features of focal parakeratosis.

ASSOCIATED CONDITIONS

Idiopathic LP has been reported to be associated with diseases of altered immunity:[4]

- Alopecia areata
- Vitiligo
- Dermatomyositis
- Morphoea
- Lichen sclerosus et atrophicus
- Systemic lupus erythematosus
- Pemphigus vulgaris and paraneoplastic pemphigus

In association with thymoma:

- Myasthenia gravis

With gastrointestinal diseases:

- Primary biliary cirrhosis
- Primary sclerosing cholangitis
- Chronic hepatitis
- Ulcerative colitis

LP has also been associated with diabetes mellitus, hypertension.

LABORATORY DIAGNOSIS

HISTOPATHOLOGY OF LICHEN PLANUS

Typical papule of lichen planus shows [3,12]

- Compact orthokeratosis
- Wedge shaped hypergranulosis
- Irregular acanthosis
- Vacuolar alteration of the basal layer
- Band-like dermal lymphocytic infiltrate in close approximation to the epidermis.

The earliest finding is an increase in epidermal Langerhans cells, associated with a superficial perivascular infiltrate of lymphocytes and histiocytes, impinging on the dermal–epidermal junction (DEJ). Mild spongiosis is followed by vacuolar alteration and clefting along the dermal–epidermal junction, with accumulation of necrotic keratinocytes (colloid bodies).[12]

Hyperkeratosis: The cornified layer shows compact orthokeratosis and contains few parakeratotic cells, which are important for diagnosis.[12]

Focal hypergranulosis: the thickening of granular layer is uneven and wedge shaped. The granular cells appear increased in size and contain coarse and abundant keratohyaline granules.[12]

Acanthosis: is irregular and affects the spinous layer and suprapapillary plates. Keratinocytes of spinous layer appear larger and eosinophilic, possibly because of advanced keratinization.

The rete ridges show irregular lengthening and some are pointed at lower end giving saw-toothed appearance.

Alteration of basal cell layer: the cells are not clearly visible in early lesions, because the dense dermal infiltrate obscure demal-epidermal junction with vacuolar alteration and necrosis of these cells. In fully developed lesions, basal cells appear as flattened squamous cells.

Band-like dermal lymphocytic infiltrate: the infiltrate in the upper dermis is bandlike and sharply demarcated at its lower border, comprising entirely of lymphocytes with macrophages. Few eosinophils and or plasma cells may be seen. Melanophages are seen in upper dermis with subsequent pigment incontinence.

Colloid bodies: necrotic keratinocyte also referred to as colloid, hyaline, cytoid, civatte bodies are present in the lower epidermis and especially in papillary dermis. These are 20µ in diameter, homogenous, eosinophilic, they may occur in any interface dermatitis.

Max-joseph space: are small areas of artefactual separation between the epidermis and dermis

<u>Variations of LP - Histopathology:</u>

In hypertrophic LP: Epidermis shows irregular acanthosis, papillomatosis and hyperkeratosis. In the centre of the lesion the follicles may be expanded and at times have a 'cyst-like' appearance. Long-standing cases there will be dermal fibrosis adjacent to the inflammatory changes.[3,12]

In atrophic LP: Epidermis may be greatly thinned almost to the level of the granular layer, although relative compact hyperkeratosis remains. The rete ridges are usually completely effaced with relatively few colloid bodies. The papillary dermis shows fibrosis.

In Lichen planopilaris: Infiltrate extending around, and may permeate, the base of the hair follicle epithelium, with follicular keratin plugging.[3,12]

In actinic LP: Epidermis shows orthokeratosis and hypergranulosis. The prickle cell layer at the centre of lesion is thin and atrophic with loss of retepegs, at borders it is acanthotic with saw tooth appearance. The upper dermis shows classical band lymphocytic infiltrate hugging epidermis.[3,12]

Lichen planus pigmentosus: similar to classical LP, except for the stratum granulosum which looks normal and there is pigment incontinence that extends deep into reticular dermis.[3,12]

In Oral LP: features diagnostic of oral lichen planus include parakeratosis, alternating with both types of keratinization with presence of a granular layer. Epithelium is often atrophic.[3,12]

ELECTRON MICROSCOPY [3,12]

1) The basal keratinocytes with their desmosomes and hemidesmosomes, show degenerative changes.
2) Tonofilaments are decreased in early lesion and increased in late lesion.
3) The dermal infiltrate, on invading the epidermis, causes damage to the lamina densa such as fragmentation. This may be followed by duplication and irregular folding of the lamina densa. The dermal infiltrate contain mainly lymphocytes and macrophages.
4) Necrotic keratinocytes or colloid bodies are located in papillary dermis.

IMMUNOFLUORESCENCE [3]

In LP fibrinogen deposition can be demonstrated by Direct immunofluorescence (DIF) as shaggy deposits at dermal-epidermal junction. Occasionally there are granular deposits of IgM or linear deposits of C3 or both IgG and C3 in basement membrane zone. Necrotic keratinocytes are seen by DIF in 87% cases. They stain mainly for IgM but also for IgG , IgA, C3 and fibrin.[3,21]

In lichen planopilaris, DIF shows deposition of IgM and or IgA, IgG and rarely C3 at level of infundibulum and isthmus. The shaggy pattern of fibrinogen seen around affected follicles.[12]

In lichen planus pemphigoides, DIF of perilesional skin shows IgG, C3 arranged linearly along basement membrane zone.[12]

IMMUNOCYTOCHEMICAL STUDIES

The infiltrating cells in LP are predominantly T lymphocytes, with very few B lymphocytes. More than 90% are activated T lymphocytes expressing HLA-DR antigen and some interleukin-2 receptor. It is likely that both subsets participate in immunologic reaction.in the epidermis adjacent to the infiltrate, basal keratinocyte express HLA-DR surface antigen and ICAM-1, both of which are implicated in the enhancement of the interaction between lymphocytes and their epidermal targets resulting in keratinocyte destruction. Probably these surface antigens are induced by cytokines released by lymphocytes from the infiltrate.[12]

COMPLICATIONS

Hair fall : Patches of atrophic cicatricial alopecia develop over the scalp. It results from follicular destruction by the inflammatory infiltrate, with scarring.[1,18]

Pterygium unguis: Adhesion between the epidermis of the dorsal nail fold and the nail bed may cause partial destruction of the nail, rarely nail may be permanently lost.[1,18]

Malignant transformation: Risk of malignant transformation is fairly low. Risk factors for development of oral cancer are long standing disease, erosive or atrophic LP and use of tobacco. There's possibility of 0.5-5% patient of oral LP developing SCC.[1,4]

Risk of skin malignancy in cutaneous LP is extremely low.

DIFFERENTIAL DIAGNOSIS:

Lichenoid eruption:

The only differential diagnosis to be considered in a classical LP is that of lichenoid eruption. Lichenoid drug eruptions have been reported after ingestion, contact or inhalation of certain chemicals or drugs.[1,4]

Lichenoid reactions have also been described as a reflection of chronic graft-versus-host disease in patients after bone-marrow transplantation.

Lichenoid eruptions may be typical or atypical for classic LP, with localized or generalized eczematous papules and plaques.

The salient features of differentiation between classic LP and lichenoid eruptions are summarised as follows:[1]

Clinical and histopathological differentiation between classic LP and lichenoid eruption :[1]

	Classic LP	**Lichenoid eruption**
Lesion	Smaller	Larger and scaly
Wickham's striae	Usually present	Usually absent
Residual hyperpigmentation	Possible	Common
Alopecia	Uncommon	Common
Predilection	Flexural/ Extremities	Sun-exposed areas
Mucous membrane involvement	Very common	Less common
Colloids in granular layer	Very uncommon	Common
Parakeratosis	Not seen	Common

Papular lesions of LP should be differentiated from other papulosquamous lesions, particularly Psoriasis.[1,13]

Annular lesions may resemble granuloma annulare.[1]

Linear LP should be differentiated form nevus unis lateris, lichen striatus and epidermal nevus.[1]

Hypertrophic LP may resemble lichen simplex chronicus, prurigo nodularis, lichen amyloidosis and kaposi's sarcoma.[1]

Atrophic LP may mimic lichen sclerosis et atrophicus. Follicular LP may resemble lichen nitidus and lichen spinulosus.[1]

Lichen plano-pilaris should be differentiated from other causes of cicatricial alopecia such as lupus erythematosus, inflammatory folliculitis and cicatricial pemphigoid.[1]

Nail involvement may resemble psoriasis, onychomycosis, and alopecia areata.

Oral lesions should be differentiated from candidiasis, lupus erythematosus, and mucous patches of secondary syphilis or traumatic bite line.[1]

TREATMENT

Lichen planus is essentially a benign and self-limiting condition. Current treatment modalities consist of topical and systemic therapy.[1,4]

Topical therapy:[1,4]

- Steroids, Intralesional steroids, Tacrolimus, Pimecrolimus

Systemic therapy:[1,4]

- Steroids, retinoids, antibiotics, Dapsone, anti-malarials, photochemotherapy, immunosuppressive agents, atihistaminics.

Antiprurtic drugs are useful in relieving pruritus, precipitating factors such as alcohol, smoking, sharp tooth, ill fitting dental appliances should be eliminated.

Patients with actinic lichen planus must be protected by sunscreens.

Treatment for Cutaneous Lichen Planus

Topical :

1st line:

Topical

- Topical steroids
- Intralesional steroids
- Tacrolimus
- Pimecrolimus
- Psoralen
- Photochemotherapy

Systemic

- Systemic steroids
- Etretinate
- Acitretin
- Isotretinoin

2nd line:

- Cyclosporine
- Dapsone
- Hydroxychloroquine
- Azathioprine
- Mycophenolate mofetil

Special forms :

- Lichen planus pemphigoides: Doxycycline, tetracycline, and nicotinamide
- Generalized LP : Interferon- alpha 2b
- Generalized LP : Metronidazole
- Refractory LP : Cyclophosphamide, Methotrexate

Treatment for Oral Lichen Planus[1,4]

1st line:

Topical:

- Topical steroids
- Lidocaine
- Inlralesional steroids
- Tretinoin gel
- lsotretinoin gel
- Tacrolimus
- Pimecrolimus

Systemic:

- Anti-candidal.
- Systemic steroids
- Etretinate
- Acitretin
- Isotretinoin

2nd line:

Topical:

- Cyclosporine mouthwash
- Extracorporeal photochemotherapy
- PhotodynamicTherapy

Systemic:

- Cyclosporine
- Griseofulvin
- Hydroxychloroquine
- Thalidomide
- Azathioprine, cyclophosphamide
- Mycophenolate mofetil

CORTICOSTEROIDS:

Corticosteroids are the first line of drugs used in treatment of LP. Depending on the extent of involvement and type of lesion, steroids can be used as topical, systemic, intralesional forms.[1]

Topical: topical glucocorticoids are typically used for mucosal and limited cutaneous disease. Potent topical preparations like fluocinonide 0.05% and clobetasol propionate 0.05% under occlusive dressings may cause regression of limited cutaneous and hypertrophic lesions in most instances. Cortisone vaginal and rectal suppositories are helpful for mucosal involvement. Oral lesions can be treated with triamcinolone acetonide long lasting lozenges, betamethasone valerate aerosols and pellets.[18]

Systemic : Oral steroids are indicated for-[18]

- Extensive LP lesions interfering with patients normal life
- Nail atrophy and pterygium formation
- LP with extensive ulcerative lesions on oral/vaginal mucosa
- Follicular LP of the scalp
- Bullous LP

Prednisolone, Methyl prednisolone, Triamcinolone have been used. The minimum effective dose of prednisolone is 5-20mg/day, for 4-6weeks, and then gradually tapered for another 6weeks.[18]

In patients requiring long standing systemic steroids, oral minipulse therapy consisting of betamethasone 5mg given orally on two consecutive days in a week can be tried.[18]

Intralesional : Triamcinolone acetonide 5-10mg/ml is effective in treating hypertrophic, oral and nail LP.

RETINOIDS:

Topical retinoids: 0.1% isotretinoin gel has been shown to be effective in improving the lesions of oral LP, but relapses are common with discontinuation of the drug.[1,4]

Systemic retinoids :

- Acetretin in a dose of 30mg/day for 8weeks in severe cutaneous LP and 30-35mg daily for 2 weeks in patients with LP and LE overlap syndrome.[20]
- Etretinate or isotretinoin 0.6-1mg/kg/day three times daily for 2months.

PHOTOCHEMOTHERAPY:

Psoralen and ultraviolet A (UVA) light photochemotherapy is usually successful in generalized cutaneous lichen planus and recalcitriant erosive oral LP.

Initial dose is0.5-2J/cm^2. The maximum dose administered at the end of treatment should not exceed 7J/cm^2 in a single session, treated 3 times a week with an interval of 48hours between each session.[1,21]

IMMUNOSUPPRESSIVE DRUGS:

Cyclosporine A is used in recalcitrant LP, given in a dose of 3-10 mg/kg/day. Pruritus usually disappears after 1- 2 weeks. Rash disappears in 4-6 weeks.[1,22]

Tacrolimus: 0.1% ointment improves refractory erosive oral LP. It is effective in controlling symptoms but flare-ups do not occur in 1-2weeks of stopping the treatment.[1,23]

Pimecrolimus: 1% cream in oral LP cleared lesions in 4 weeks.

Azathioprine is useful in recalcitrant, generalized cutaneous lichen planus and in lichen planus pemphigoides.[1]

Mycophenolate mofetil at a dose of 1500 mg twice daily, also used in oral , hypertrophic and bullous LP.[24]

MISCELLANEOUS:

Antimalarials: Hydroxychloroquine at 200 to 400 mg/day has been used successfully in actinic lichen planus and erosive LP.[1]

Thalidomide can be used in erosive lichen planus, unresponsive to other therapies.

Dapsone: 200mg daily is used in the treatment of bullous and erosive LP for 4-6weeks.

Phenytoin: 100-200mg daily, orally for 2-8weeks in Cutaneous and oral LP.

Metronidazole - 500 mg orally twice daily for 1 to 2 months also reportedly clears the majority of cases of generalized lichen planus.[25]

Low molecular weight heparin in low doses has lymphoid antiproliferative and immunomodulatory properties.At a dose of 3 mg weekly. Four to six injections of heparin induced complete regression of lesions within 4 to 10 weeks.[26]

IFN-a2b has been administered for treatment of generalized lichen planus with improvement, but this biologic response modifier also has been implicated in development or exacerbation of LP.

SURGERY:

Split thickness skin grafting has been used to cover ulcerative lichen planus of the feet that is recalcitrant to other treatments.[1,18]

COURSE AND PROGNOSIS

Lichen planus is an unpredictable disease that typically persists for 1-2 years, but may follow a chronic relapsing course over many years. The duration varies according to the extent and site of involvement and morphology of the lesions. Generalized eruptions tend to have a rapid course and heal spontaneously faster than limited cutaneous disease. Spontaneous regression is also an uncommon feature of oral lichen planus. The mean duration for oral lichen planus is 5 years. Generally, the duration of disease has the following order, from shortest to longest duration - generalized, cutaneous, cutaneous and mucous membrane, mucous membrane, and hypertrophic and lichen planopilaris have the same course.[1,4]

Relapse of disease occurs in 15-20 percent of cases and tends to occur in the same area as the initial episode. Recurrences are more common in generalized lichen planus and are usually of shorter duration.[1]

Hair loss is usually permanent.[1]

HEPATITIS C VIRUS

- HCV was discovered in 1989, since then it has gained importance not only because it is the principal cause of post transfusion chronic hepatitis, but also because of its association with innumerable number of extrahepatic disorders like: Cryoglobulinemia, Porphyria cutanea tarda, Leucocytoclastic vasculitis, Livedo reticularis, Lichen planus.[28,34]

Virology:

The hepatitis C virus (HCV) is a single stranded RNA flavivirus that replicates in hepatocytes and peripheral blood mononuclear cells.[27]

Structure:

It consists of positive-sense, single-stranded RNA genome within a nucleocapsid. The nucleocapsid and RNA are packed in an envelope, derived from host membranes into which viral-encoded glycoproteins are inserted. It is 50-60nm size and contains 3011 aminoacids and 9033 nucleotides.[28,34]

Types and subtypes of HCV:

There are six main genotypes, each with a different worldwide prevalence.[27]

Type1a: United States and Western countries

Type1b: United States, Japan and Europe

Type 2 : Not very common

Type 3 : India, Pakistan and Australia

Type 4: Middle East and North Africa

Type 5: South Africa

Type 6: Asia

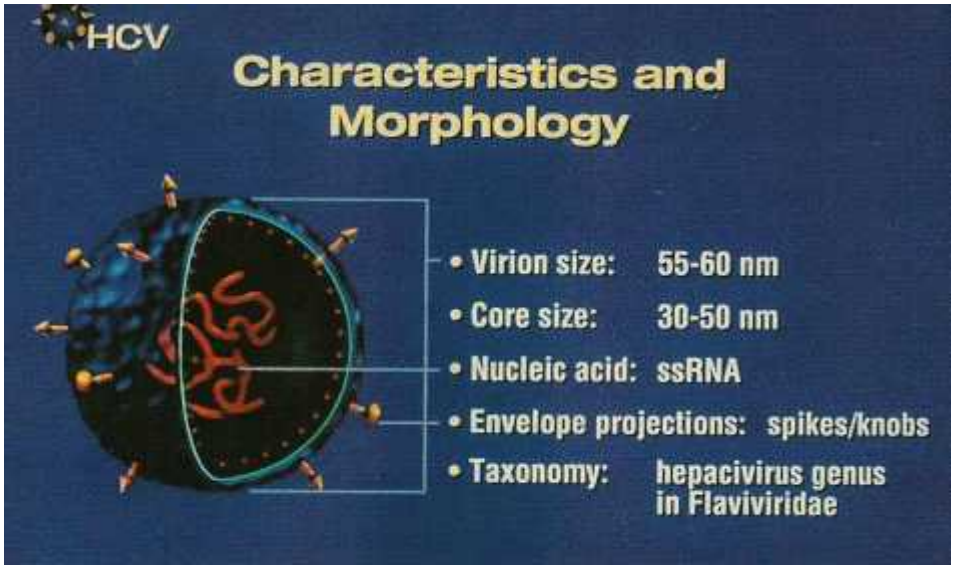


FIG. 1 : STRUCTURE OF HEPATITIS C VIRUS [34]

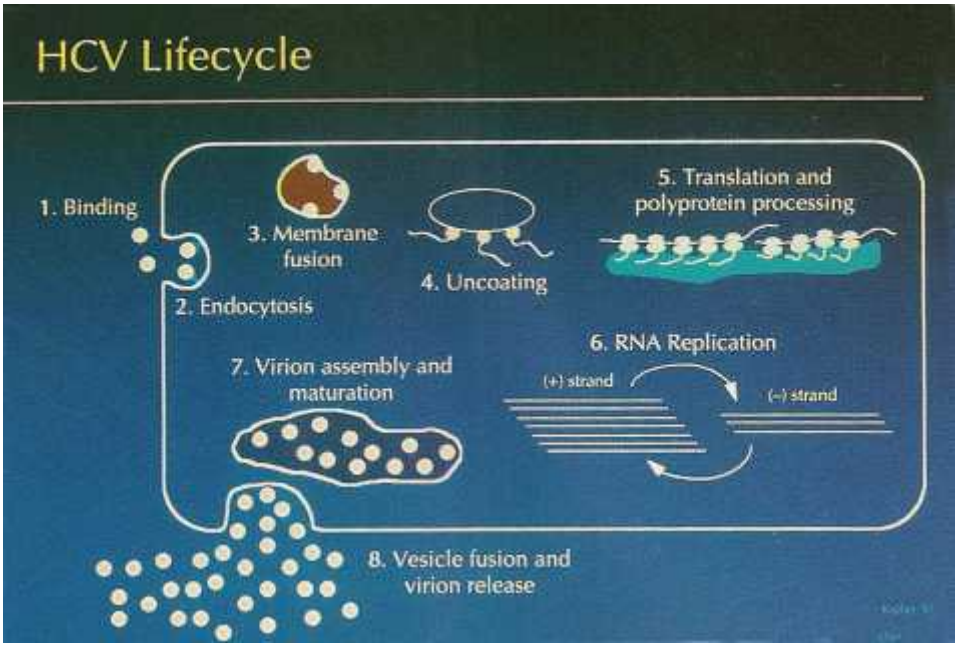


FIG. 2 : LIFE CYCLE OF HEPATITIS C VIRUS [34]

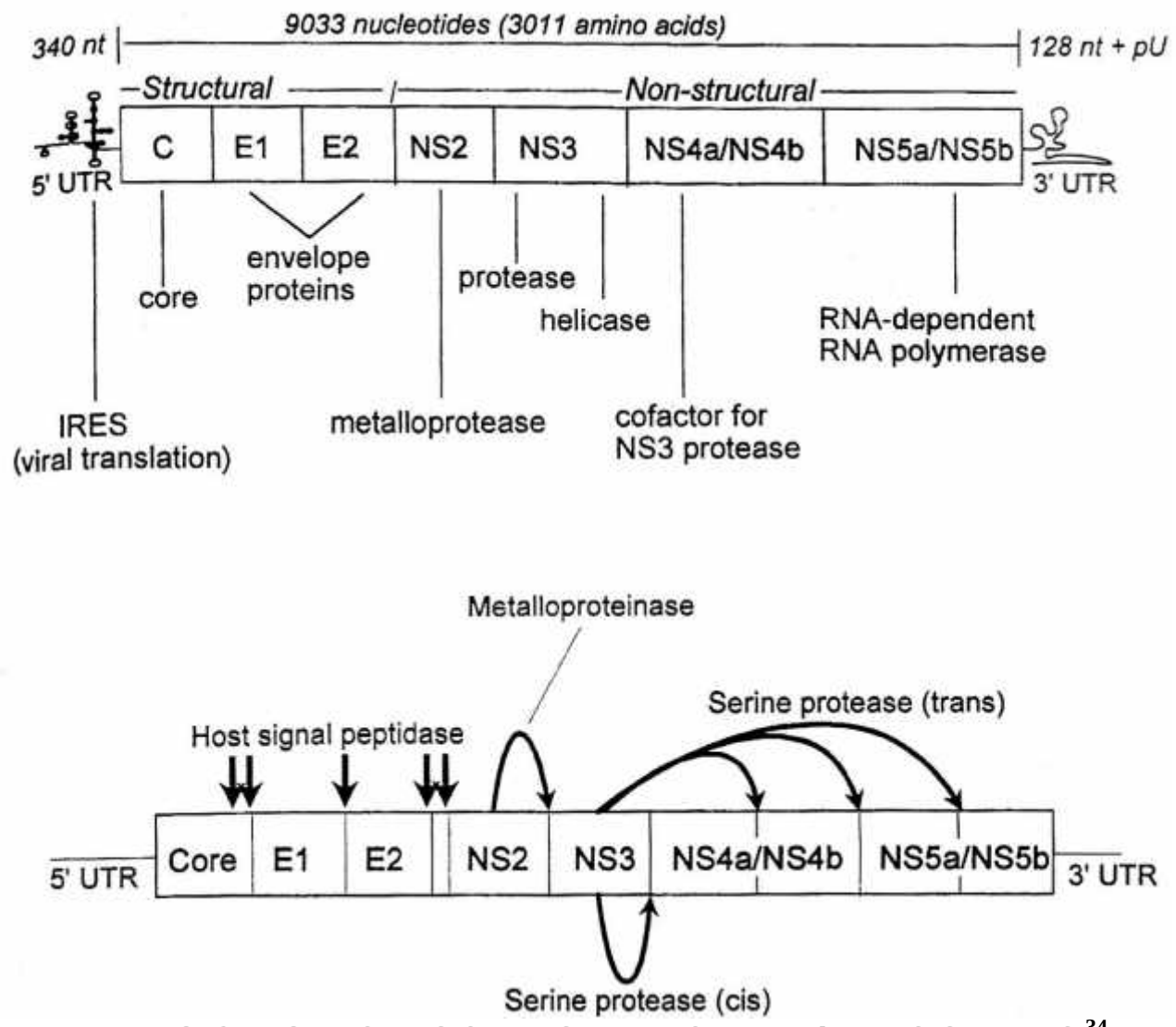


FIG. 3 : GENOMIC STRUCTURE OF HEPATITIS C VIRUS [34]

Modes of transmission:[27,29]

1) Transfusion of blood or blood products
2) Intravenous drug use
3) Nosocomial transmission
4) Sexual transmission

Pathogenesis:

The hepatic damage from HCV occurs by viral replication within liver hepatocytes. The mechanism of extrahepatic effects is uncertain and thought to be caused by viral antigens or viral-laden lymphocytes depositing in the skin.[28]

Immunology:

In most patients infected with hepatitis C virus, a wide array of humoral and cell-mediated immune responses are generated by HCV polypeptides. The antibodies that develop in response to hepatitis C structural and non-structural proteins form the basis for the detection assay of the host's exposure to HCV. Cellular immune response is also activated in these patients with the development of T-cells (CD4 and CD8) that recognize and respond to processed HCV antigens. However, despite these host immune responses HCV usually persists and develops into a chronic infection. Potential mechanisms for viral persistence include its replication with attenuated antigenicity, restricted expression of viral or class-I MHC proteins in infected hepatocytes or emergence of virus quasispecies.[34]

Clinical Manifestations:

Hepatitis C has a broad clinical spectrum of disease presentation and outcome. They can be divided into: [34]

1) **Hepatic manifestations :**
   - Acute and chronic hepatitis
2) **Extra Hepatic manifestations:**

   Cutaneous manifestations:
   - Cryoglobulinemia
   - Porphyria cutanea tarda
   - Leucocytoclastic vasculitis
   - Livedo reticularis
   - Lichen planus
   - Sjogren's syndrome

LICHEN PLANUS IN HCV INFECTION

Pathogenesis:

HCV related LP is associated mainly with classII HLA-DR6 allele.[29]

The proposed mechanisms for the causation of LP are : [8]

- Selective presentation of certain HCV-encoded peptides by HLA-DR6 molecules on the surface of monocytes to CD4+ cells that provokes lichenoid reaction.[29]
- HCV is capable of cytopathic replication in cell types outside the liver. Thus the presence of HCV in lesional tissue has been the object of several investigators.
- It may trigger an auto-immune process that is directed against antigens expressed on extra-hepatic cells.[10]

- As HCV infection can give rise to tissue-specific and non- tissue specific response, whose presence induces keratinocyte antigenic changes and hence generation of cell-mediated reaction manifesting as LP

This suggests that, HCV-specific T-cells may play a role in this immunological process, resulting in activated CD8 Tcells, cytokines and expansion of certain B cell clones which trigger and maintain the lichenoid reactions.

LP associated with HCV infection occurred mainly in 7^{th} to 8^{th} decade and women were twice as often affected as men. Clinically the lesions of HCV –related LP are similar to those of classic LP. Mucosal involvement is frequent in HCV-related LP, especially erosive type, but others found reticular variant to be more common. The lesion show more symmetrical distribution mainly on gingival, tongue and lips. The histological features do not show substantial differences, though few reported a high inflammatory infiltrate.[30,34]

Diagnosis:

Diagnostic tests for HCV infection is divided into two categories:[47]

1) Serological assays which detect anti-HCV antibodies.
    - Screening test e.g: ELISA
    - Supplemental antibody tests eg: (RIBA)

2) Molecular assays which detect, quantify and characterize HCV RNA genome.
    - PCR
    - HCV genotype tests

Screening and Supplemental antibody tests:

Enzyme linked immunosorbent assay (ELISA):

Antibodies to several different HCV antigens are simultaneously detected by ELISA. The first generation ELISA test used recombinant antigen C100-3 derived from NS4 region of HCV gene. The second generation (ELISA-II) assays incorporate recombinant proteins from the nucleocapsid core region, C22-3 and NS3 region C33-C. These assays are more sensitive and detect antibodies earlier than first generation. Anti C-100 antibody to C33 appears earlier between 11-20 weeks. A third generation (ELISA-III) immunoassay, which incorporates proteins from NS5 region and replaces some recombinant proteins with synthetic peptides earlier. This assay has 97% specificity.

Radio Immuno Blot Assay (RIBA):

It is used as supplementary test. Reactivity in an immunoassay is confirmed by incubation with a nitrocellulose strip that contains individual bands of recombinant or synthetic HCV proteins.

Polymerase chain reaction (PCR):

There are two types of PCR, qualitative and quantitative. The qualitative PCR detects fewer viral particles less than 50mRNA/ ml versus quantitative PCR, which detects more than 500mRNA/ml.

The qualitative PCR is used for confirmation and the quantitative test is used to monitor disease activity and response to therapy. PCR is a very sensitive, but too complicated, time consuming, costly technique.[27]

Treatment:

There is no benefit with rest, diet or vitamin supplements. Antiviral agents are given with variable response.[20,27]

a) Interferon- (INF-)
b) INF- and Ribavarin combination
c) Ursodeoxy Cholic Acid
d) Hepatic iron removal
e) Hepatic transplantation
f) Herbal remedies

METHODOLOGY

STUDY POPULATION

The study consisted of 50 cases of clinically diagnosed Lichen Planus, attending the outpatient Department of Dermatology, Venereology and Leprosy at K.L.E.S Dr. Prabhakar Kore Hospital and Medical Research Centre attached to Jawaharlal Nehru Medical College, Belgaum, from November 2007 to October 2008 for a period of 12 months.

Inclusion criteria

All cases (old and new), which are diagnosed clinically and confirmed histopathologically as Lichen Planus with involvement of skin, mucous membrane or both irrespective of age, sex, duration of illness and associated disease

Exclusion criteria

- Patients not willing to participate in the study.
- Clinically resembling LP which were initially included in the study but histopathologically not proved as Lichen Planus.

A sample size of 50 was selected and this was calculated by taking 80% of average cases of Lichen Planus in 3 years attending the Dermatology OPD at KLES Dr.Prabhakar Kore Hospital and MRC, Belgaum.

The patient's demographic data, location of lesions, risk factors were noted in a pre-tested and pre-designed proforma after taking informed and written consent. Diagnosis was made with history and clinical examination and confirmed with biopsy.

Following investigations are done in all patients with lichen planus:

- Urine examination and microscopy.
- Haematological such as Hb%, TLC, DLC, ESR
- Random Blood sugar
- Punch biopsy for histopathology
- The patients serum was tested for anti HCV antibodies by second generation ELISA (ERBA diagnostics).

RESULTS

THE PRESENT STUDY IS A ONE-YEAR CROSS-SECTIONAL STUDY OF 50 CASES OF LICHEN PLANUS WHO ATTENDED THE OPD AT K.L.E.S DR. PRABHAKAR KORE HOSPITAL AND RESEARCH CENTRE, BELGAUM from November 2007 to October 2008.

Table 1 : Age Distribution

Age	No. of cases	Percentage (%)
Below 10	01	2
11-20	04	8
21-30	11	22
31-40	12	24
41-50	09	18
51-60	09	18
Above 60	04	8
Total	**50**	**100**

In the present study, peak prevalence was seen in the age group of 21-40 years that is 46%. The youngest patient was 7years old and eldest was 70years old.

Graph 1 :

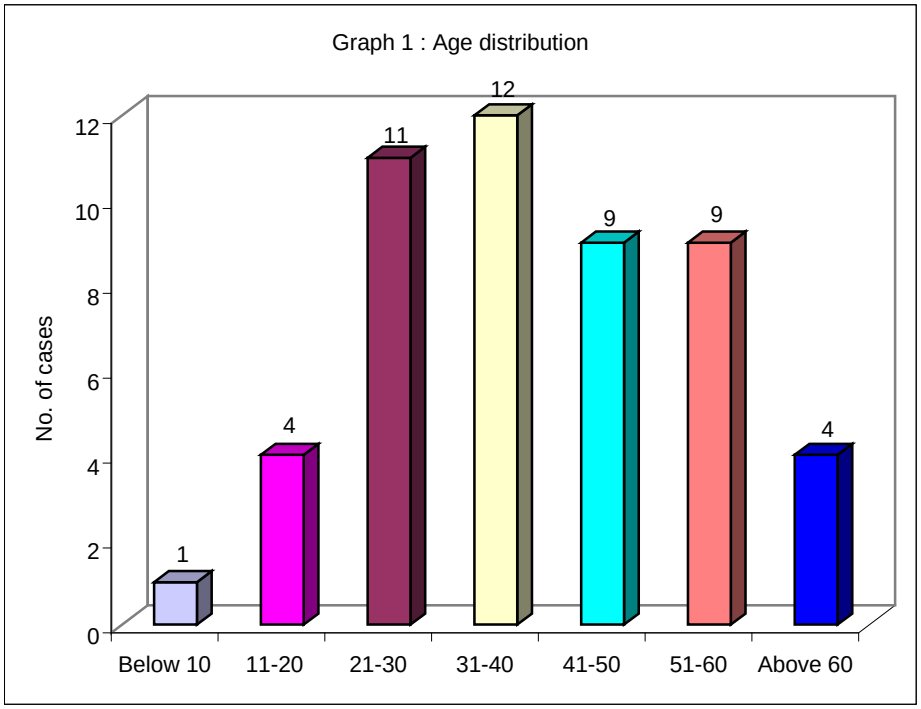


Table 2 : Sex distribution

Sex	No. of cases	Percentage (%)
Male	31	62
Female	19	38
Total	**50**	**100**

There was male preponderance in this study. Male to female ratio was 1.6:1.

Graph 2 :

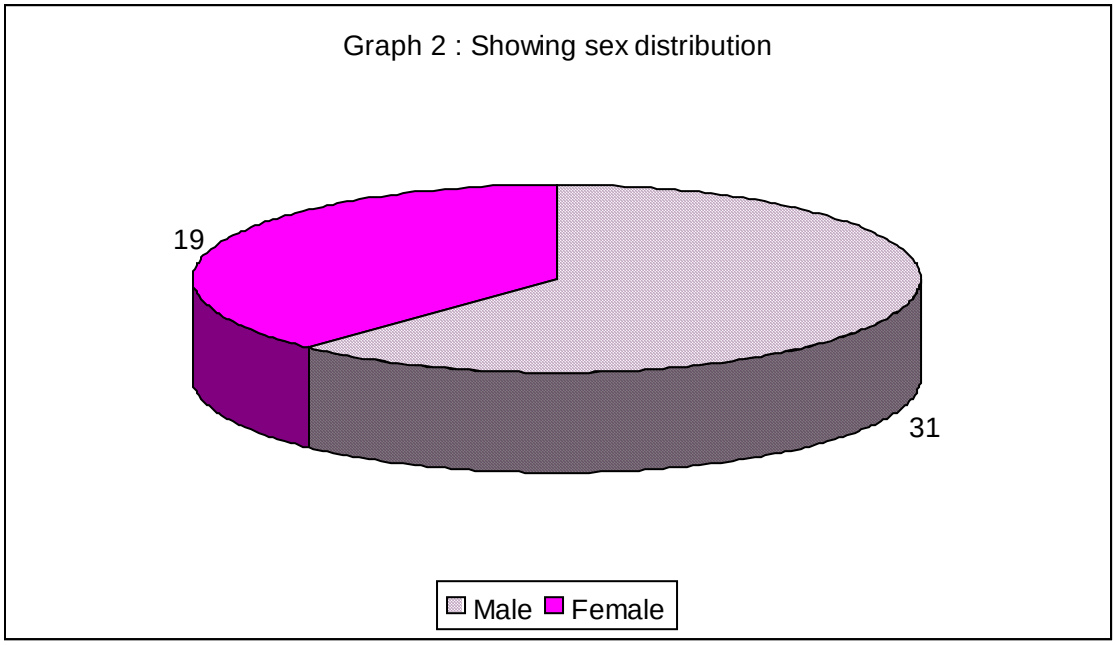


Table 3 : Occupation

Occupation	No. of cases	Percentage (%)
Housewife	14	28
Students	12	24
Agriculturists	13	26
Business	02	4
Officer	07	14
Nurse	01	2
Driver	01	2
Total	**50**	**100**

In the present study, Lichen planus was seen more commonly in housewives 28% followed by agriculturists26% and students 24%.

Graph 3 :

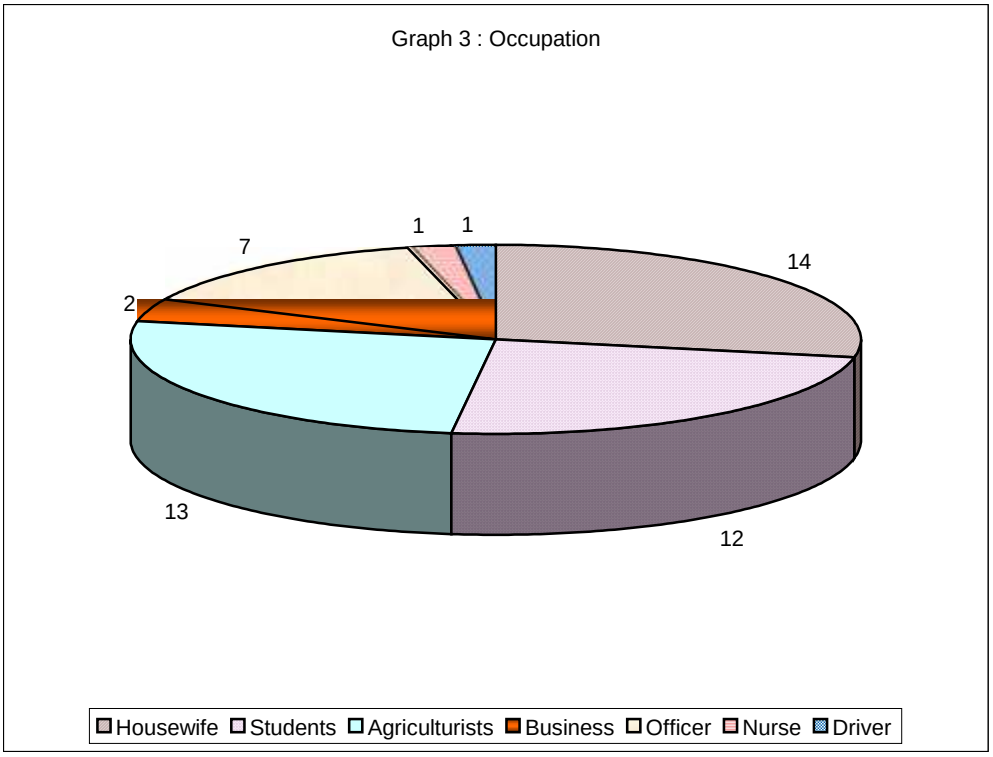


Table 4 : Duration of illness

Duration	No. of cases	Percentage (%)
0-1mths	08	16
2-3mths	18	36
4-5mths	05	10
6mths-1yr	10	20
>1year	09	18
Total	**50**	**100**

In the present study the maximum duration was 2-3 months, seen in 36% of the cases.

Graph 4 :

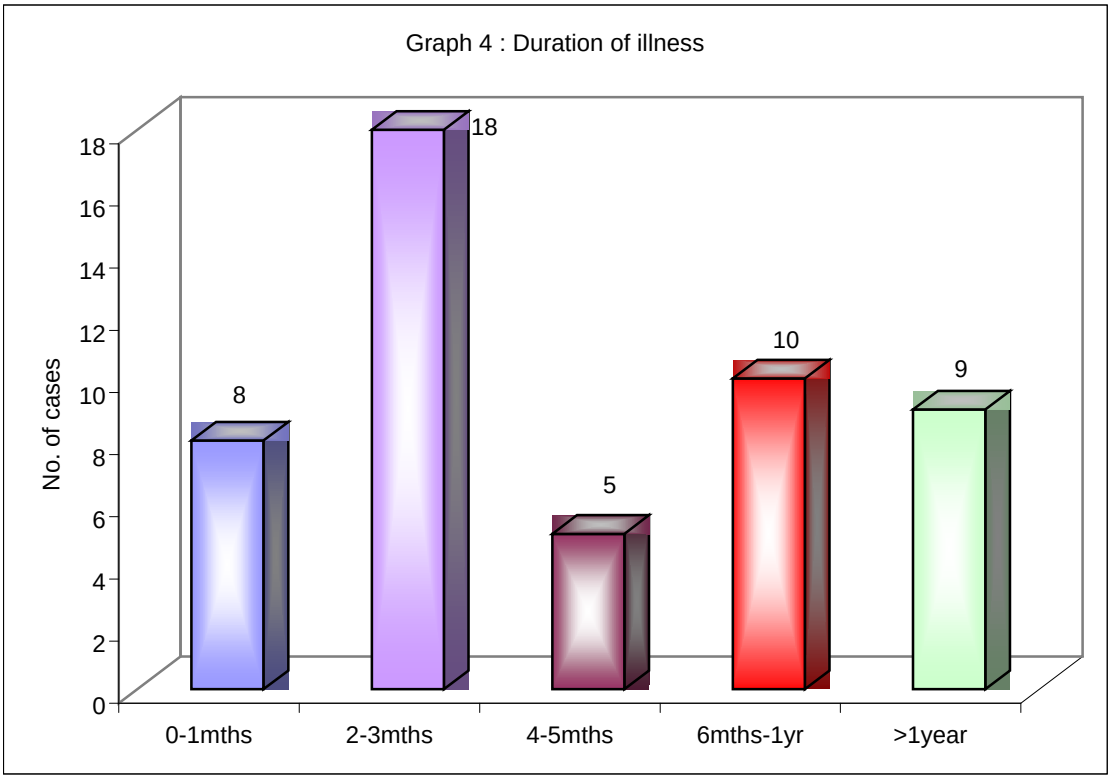


Table 5 : Symptoms

Symptoms	No. of cases	Percentage (%)
Asymptomatic	01	2
Mild itching	11	22
Moderate itching	21	42
Severe itching	17	34
Total	**50**	**100**

In the present study, itching was present in 98% of patients and only 2% of the patients were asymptomatic.

Graph 5 :

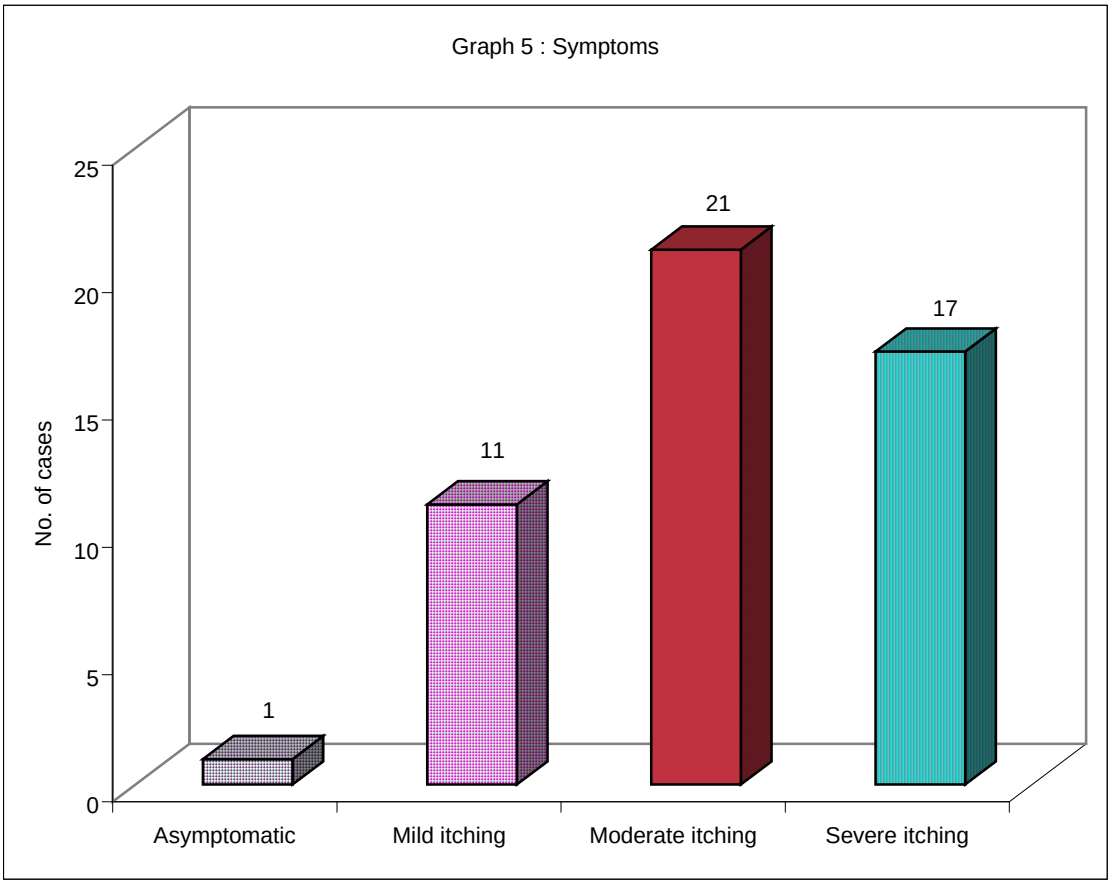


Table 6 : Risk factors

Risk factors	No. of cases	Percentage (%)
Surgeries/ interventions	6	12
Smoking	4	8
Drugs	9	18
Alcohol intake	8	16
Stress	5	10

In the present study, risk factors like drugs 18% followed by alcoholism 16%, previous surgery 12%, stress 10% and smoking 8%.

Graph 6 :

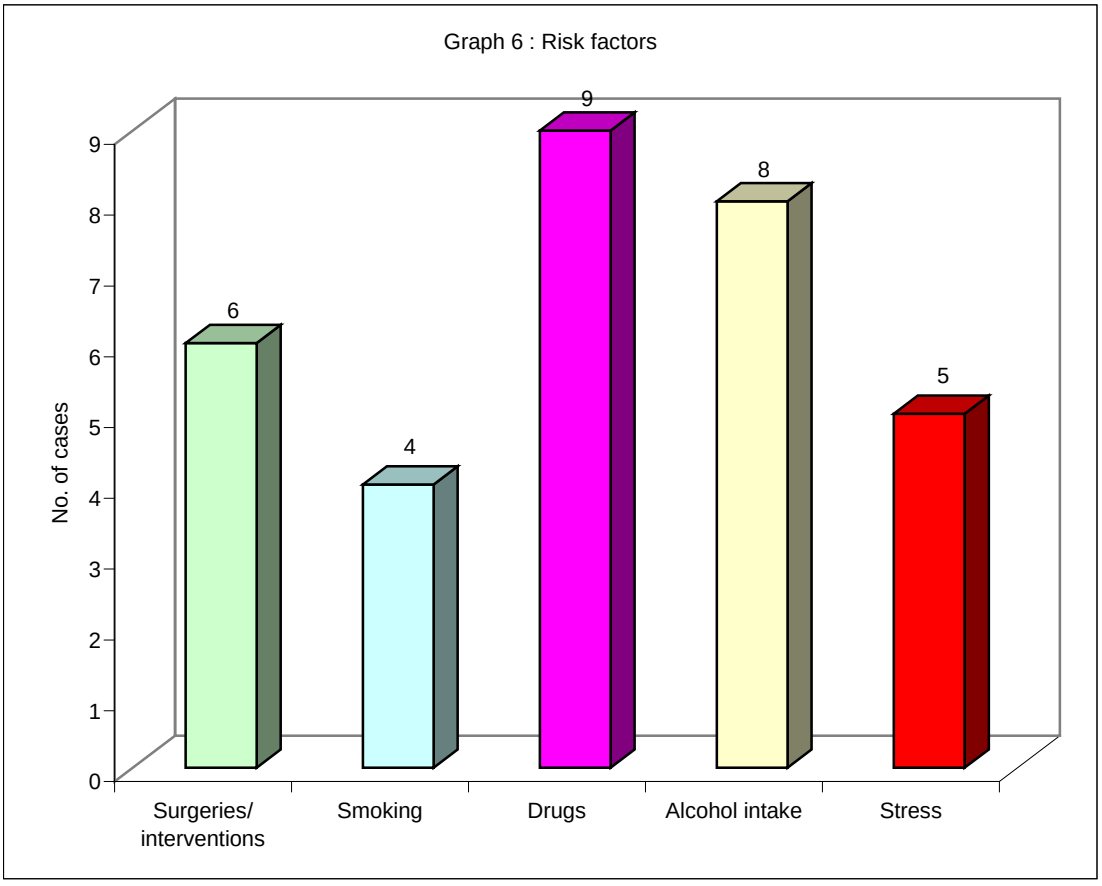


Table 7 : Previous medical history

Medical history	No. Of cases	Percentage (%)
Jaundice	5	10
Hypertension	6	12
Diabetes Mellitus	9	18

In the present study, lichen planus was associated with diabetes mellitus in 18% cases, hypertension in 12%, jaundice in 10%.

Graph 7 :

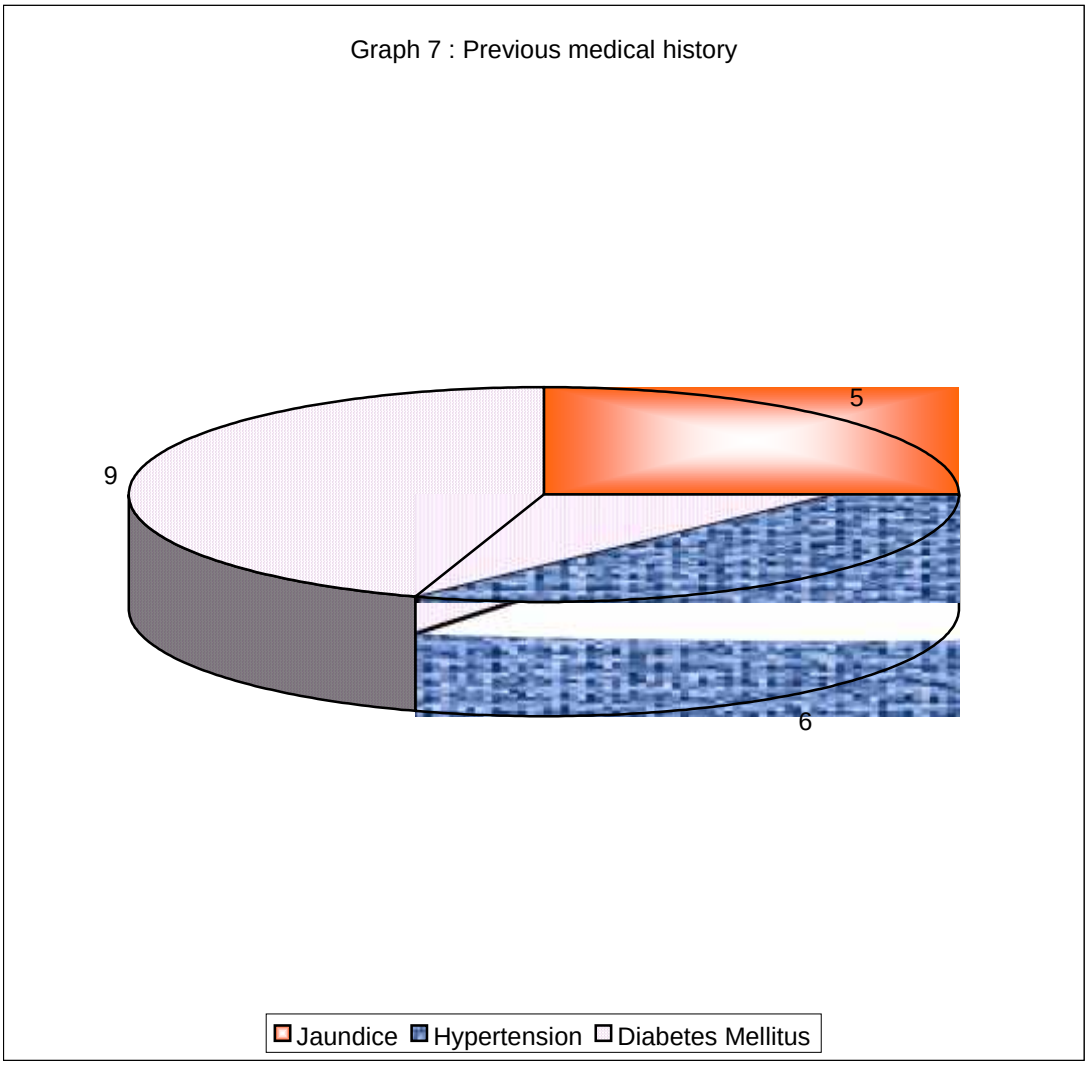


Table 8 : Clinical forms

Type	No. Of cases	Percentage (%)
Cutaneous	48	96
Cutaneous + Mucosal	04	8
Mucosal	02	4
Total	**50**	**100**

In the present study, 96% of cases had only cutaneous involvement. Cutaneous and mucosal involvement in 8% cases and only oral lesions in 4% of cases.

Graph 8

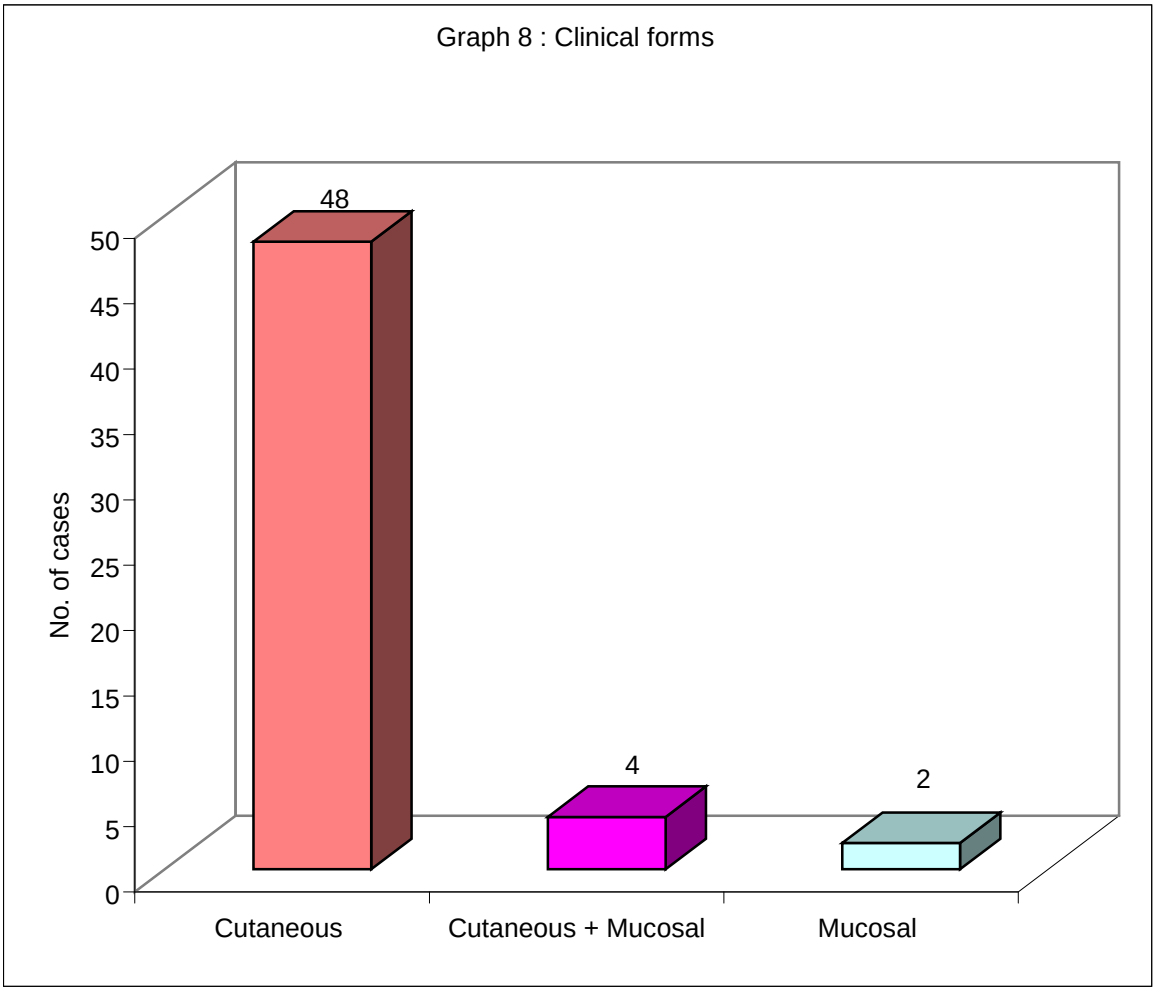


Table 9 : Clinical variants

Variants	No. of cases	Percentage (%)
Classical	43	86
Hypertrophic	03	6
Follicular	01	2
Actinic	01	2
Oral	02	4
Total	**50**	**100**

In the present study, commonest clinical type was Classical LP in 86% cases followed by Hypertrophic variant 6%, follicular type 2% and actinic type 2%.

Graph 9

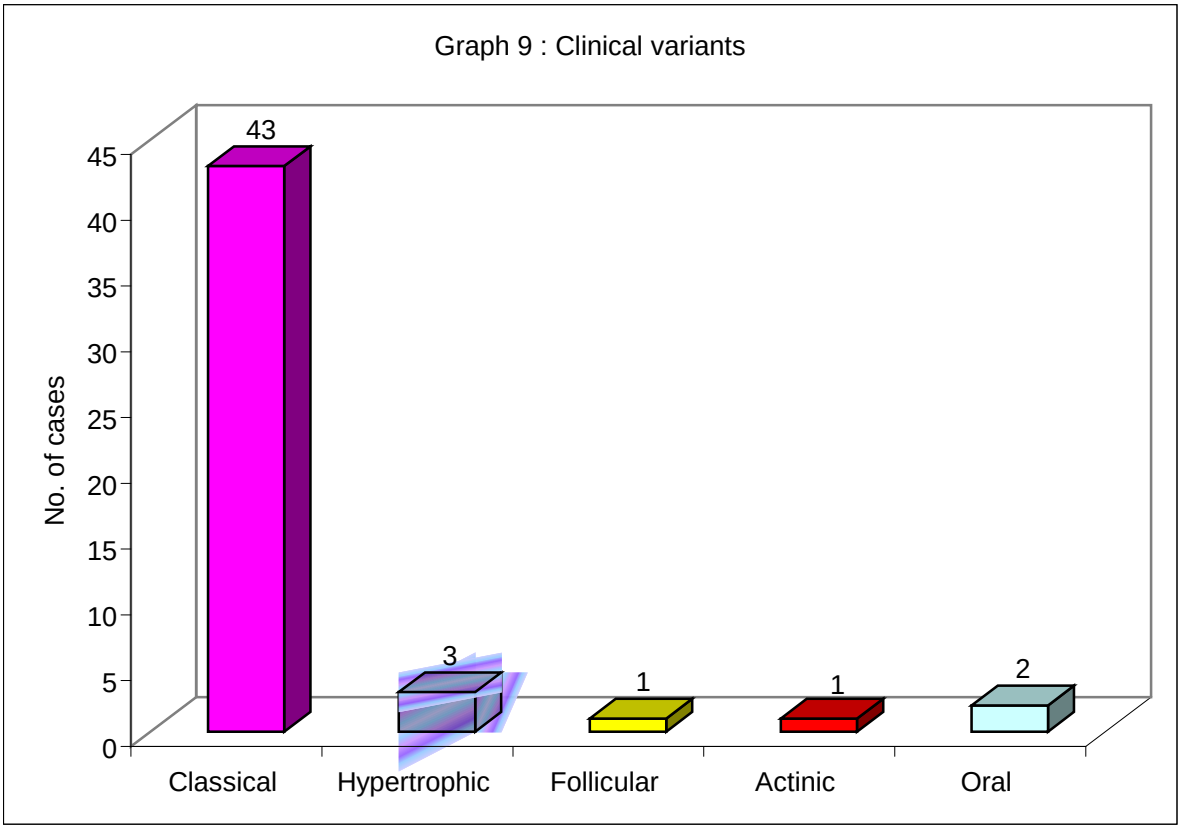


Table 10 : Distribution of lesions and Koebner's phenomenon

Distribution	No. of cases	Percentage (%)
Lower limbs	39	78
Upper limbs	32	64
Trunk	17	34
Face and neck	01	2
Oral cavity	06	12
Generalized	02	4
Nail	08	16
Koebner's phenomenon	03	6

In the present study, lower limbs were involved in 78% cases and upper limbs in 64%. Trunk was involved in 34%, face and neck in 2%. Nails in 16%, oral cavity in 12% cases.

Koebner's phenomenon was seen in 6% cases.

Graph 10

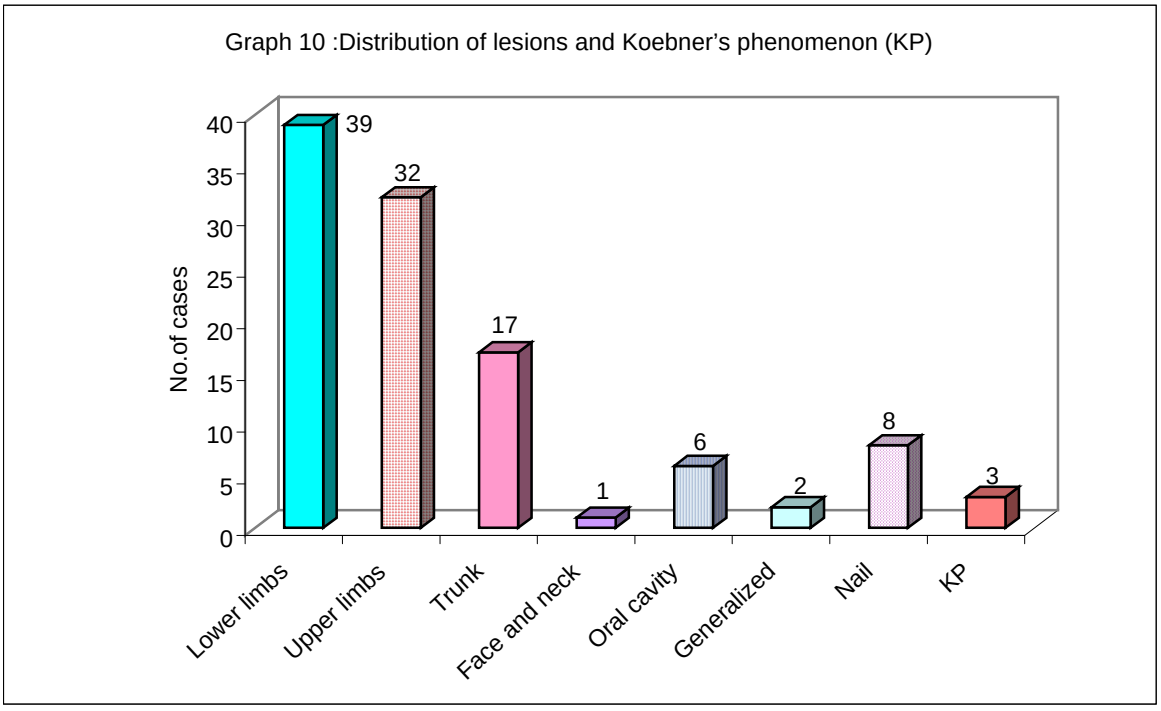


Table 11 : Types of oral lesions (n=6)

Types	No. of cases (%)			
	Lips	**Buccal**	**Tongue**	**Gums**
Reticular	-	05(83.3)	-	-
Papular	01(16.6)	-	-	-
Atrophic	-	-	-	-
Erosive	-	-	-	-
Annular	-	-	-	-
Plaque	-	01(16.6)	02(33.3)	

Reticular type of OLP on buccal mucosa was most common.

Table 12 : Nail changes (n=3)

Nail changes	Finger nail (%)
Longitudinal ridging	3(6)
Pitting	1(2)
Discoloration	1(2)

In the present study none of the patients had isolated nail involvement. 6% patients showed nail changes, out of which 3 had longitudinal ridges, 1 of them had pitting and discoloration.

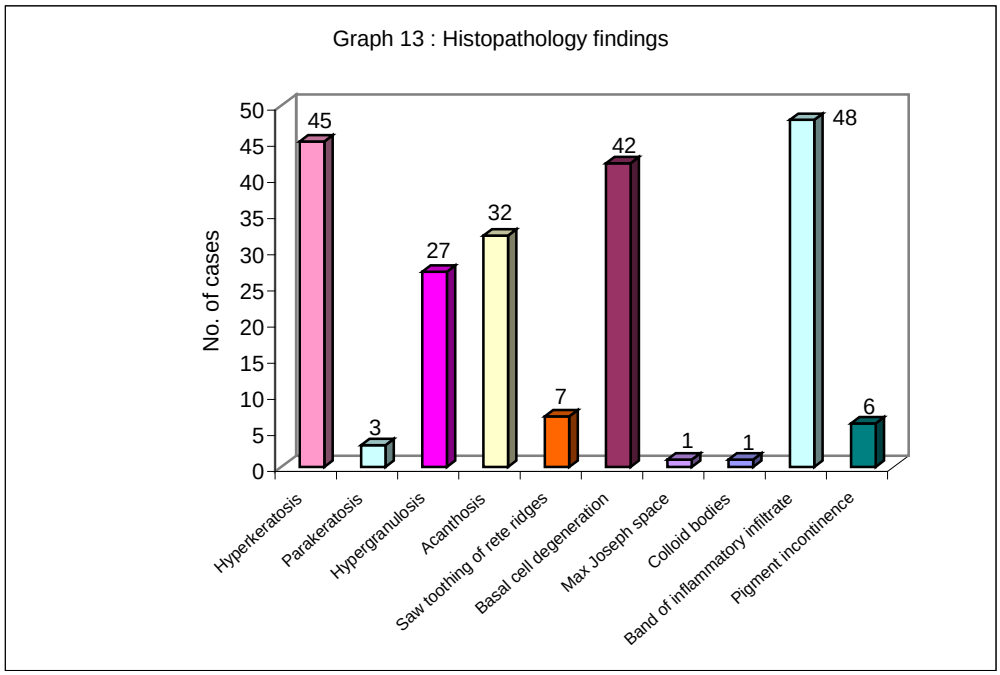
Table 13 : Histopathology of LP

Histopathology findings	No. of cases	Percentage (%)
Hyperkeratosis	45	90
Parakeratosis	03	6
Hypergranulosis	27	54
Acanthosis	32	64
Saw toothing of rete ridges	07	14
Basal cell degeneration	42	84
Max Joseph space	01	2
Colloid bodies	01	2
Band of inflammatory infiltrate	48	96
Pigment incontinence	06	12

In the present study, epidermis showed hyperkeratosis in 90% cases, acanthosis in 64%, hypergranulosis in 54%, basal cell degeneration 84%, parakeratosis 6%, saw toothing of rete ridges in 14%, colloid bodies in 2% and Max Joseph space in 2%.

96% cases showed band of chronic inflammatory infiltrate at dermo-epidermal junction.12% cases showed pigment incontinence.

Graph 11



Anti- HCV antibodies were negative in all the 50 patients of LP

"ASSOCIATION OF MUCOCUTANEOUS LICHEN PLANUS WITH HEPATITIS C INFECTION IN A ONE YEAR CROSS-SECTIONAL STUDY AT K.L.E.S. DR. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM"

By

Dr. DEEPTI RANA

Dissertation is submitted to the
KLE University
Belgaum, Karnataka
In partial fulfillment of the requirements for the degree of

Doctor of Medicine (MD)

In

(Dermatology, Venereology and Leprosy)

Done under the guidance of

Dr. B.S.MANJUNATHSWAMY, *M.D, DVD*

Professor

Department of Dermatology, Venereology and Leprosy
JawaharLal Nehru Medical College, Belgaum-590010

May 2010

CLINICAL PHOTOGRAPHS

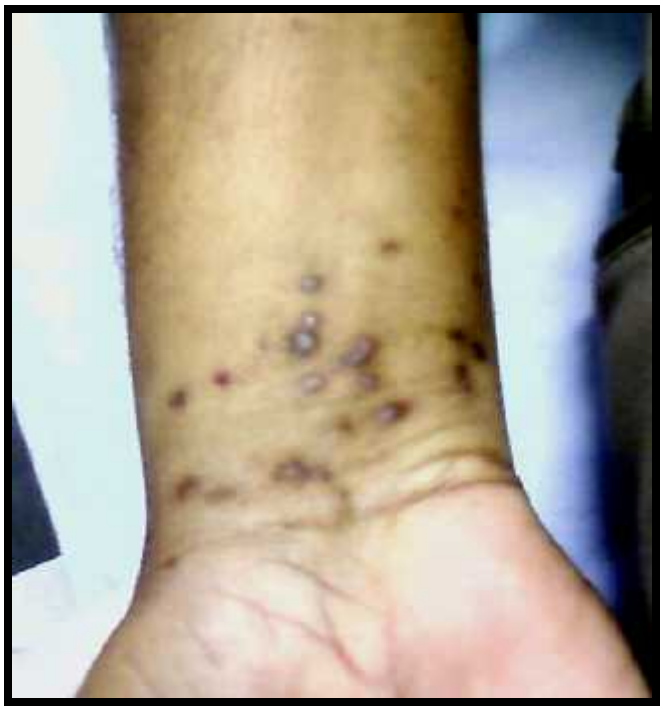


FIG. 4 : Classical lichen planus showing flat-topped, violaceous papules on the flexor aspect of wrist.

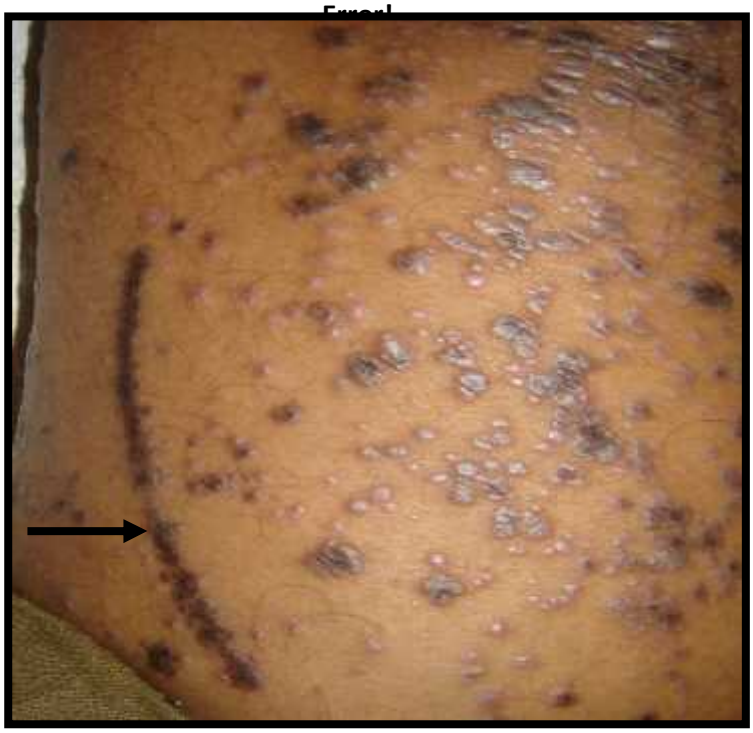


FIG. 5 : Classical Lichen Planus with Koebner's phenomenon (arrow)

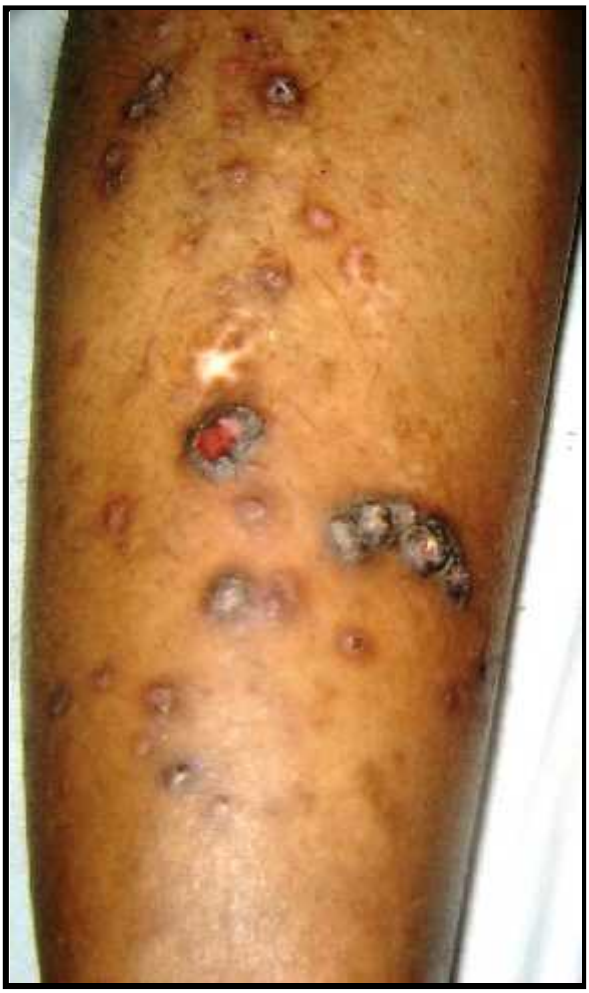


FIG. 6 : Hypertrophic Lichen Planus

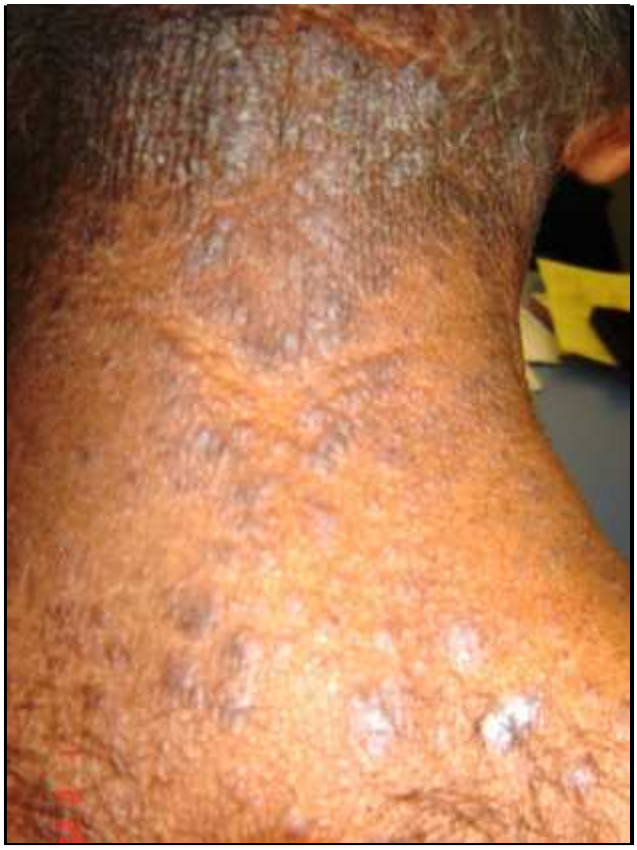


FIG. 7 : Actinic Lichen Planus

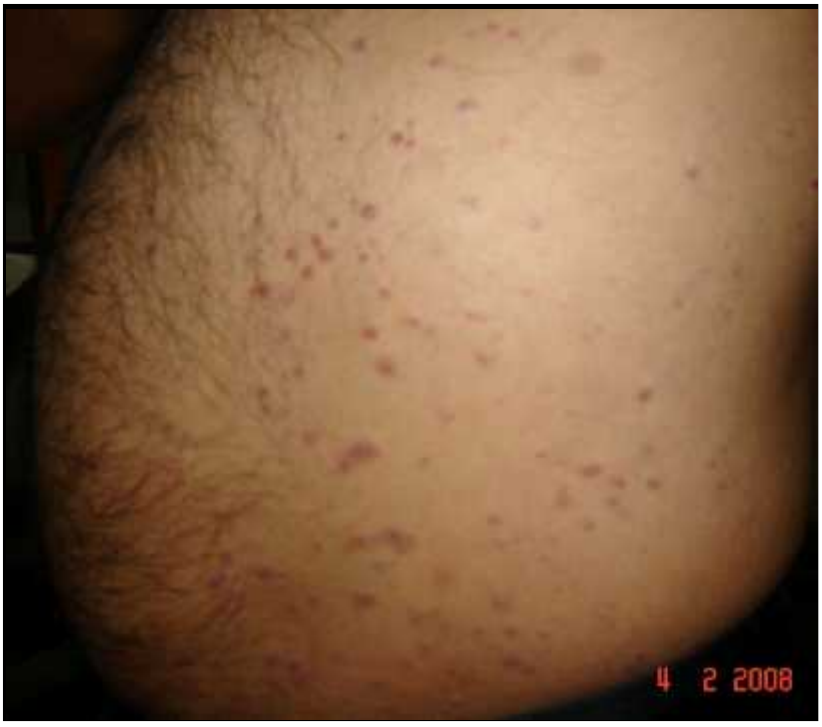


FIG. 8 : Generalized Lichen Planus

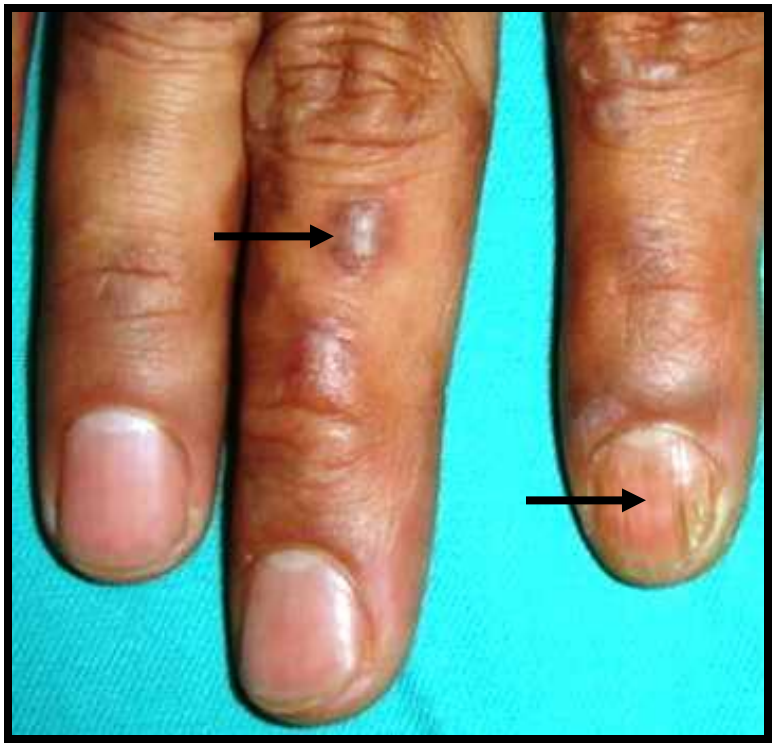


FIG. 9 : Actinic Lichen Planus with Longitudinal ridging

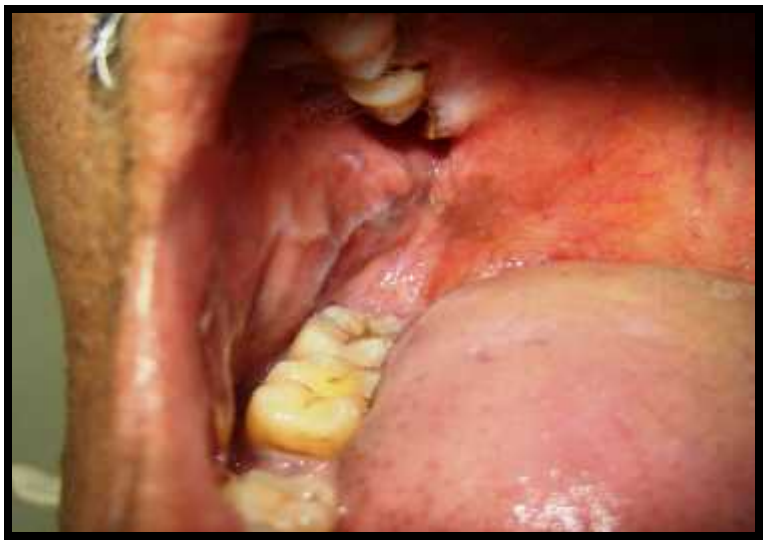


FIG. 10 : Reticular type of oral LP

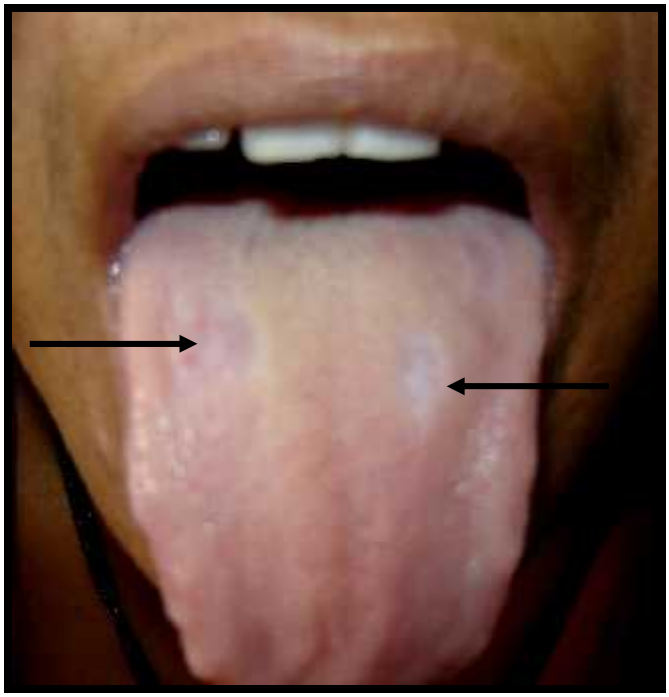


FIG. 11 : Plaque type lesions on tongue

HISTOPATHOLOGICAL PHOTOGRAPHS

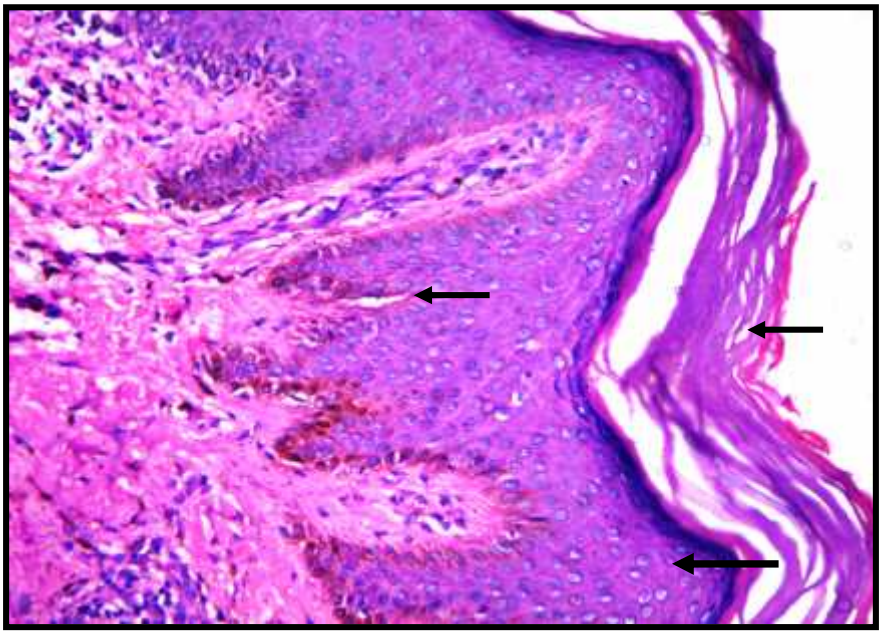


FIG. 12 : Microscopy showing hyperkeratosis, hypergranulosis, saw-toothed rete ridges, acanthosis. (200X, H&E)

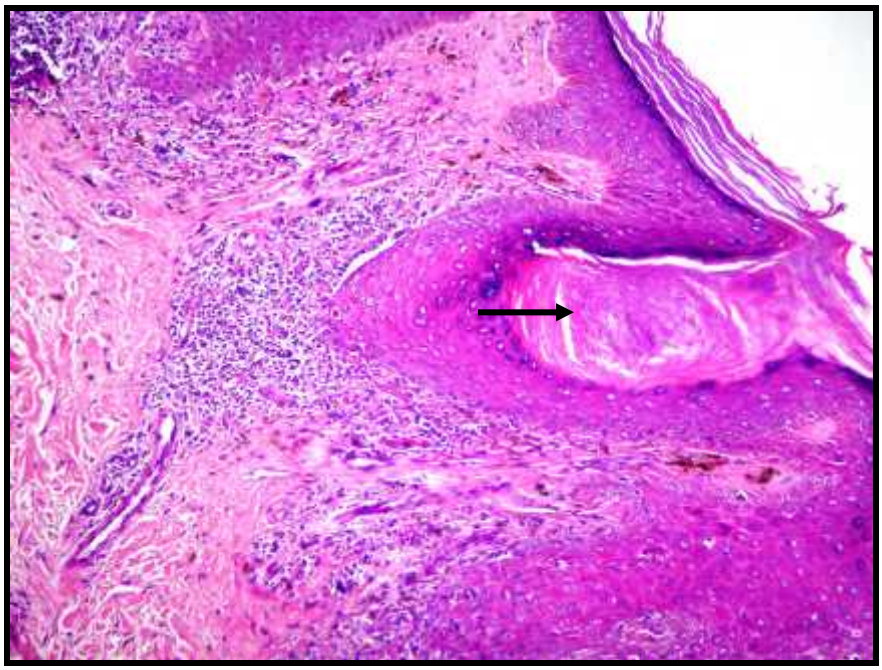


FIG. 13 : Microscopy showing classical lichen planus showing hyperkeratosis , keratotic plug (arrow) , acanthosis, and band of chronic inflammatory infiltrate at dermoepidermal junction (100x, H&E)

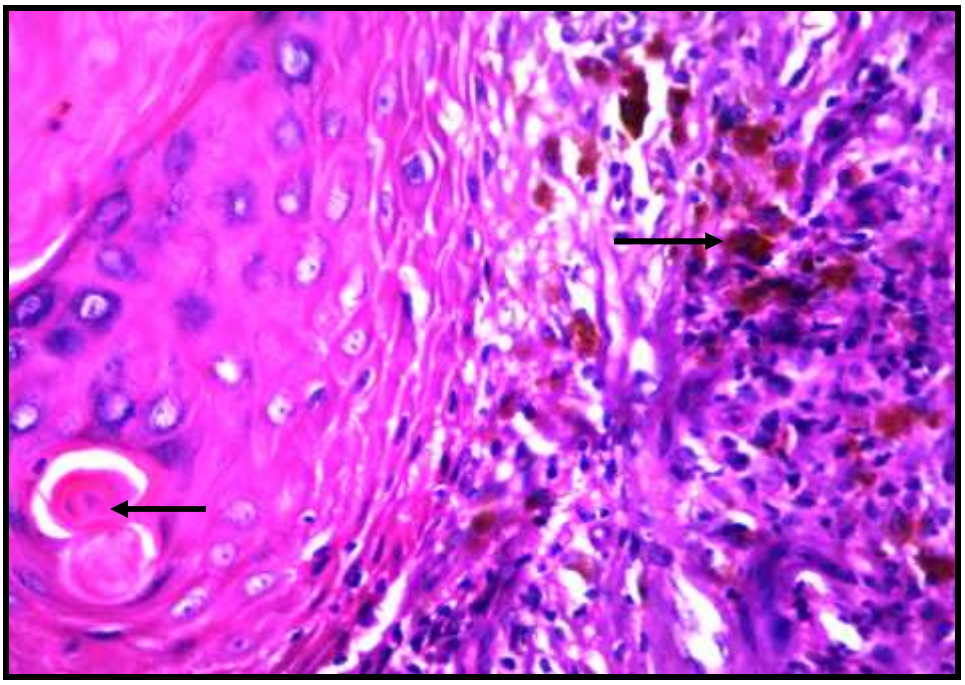


FIG. 14 : Microscopy showing pigment incontinence, colloid body (400x, H&E)

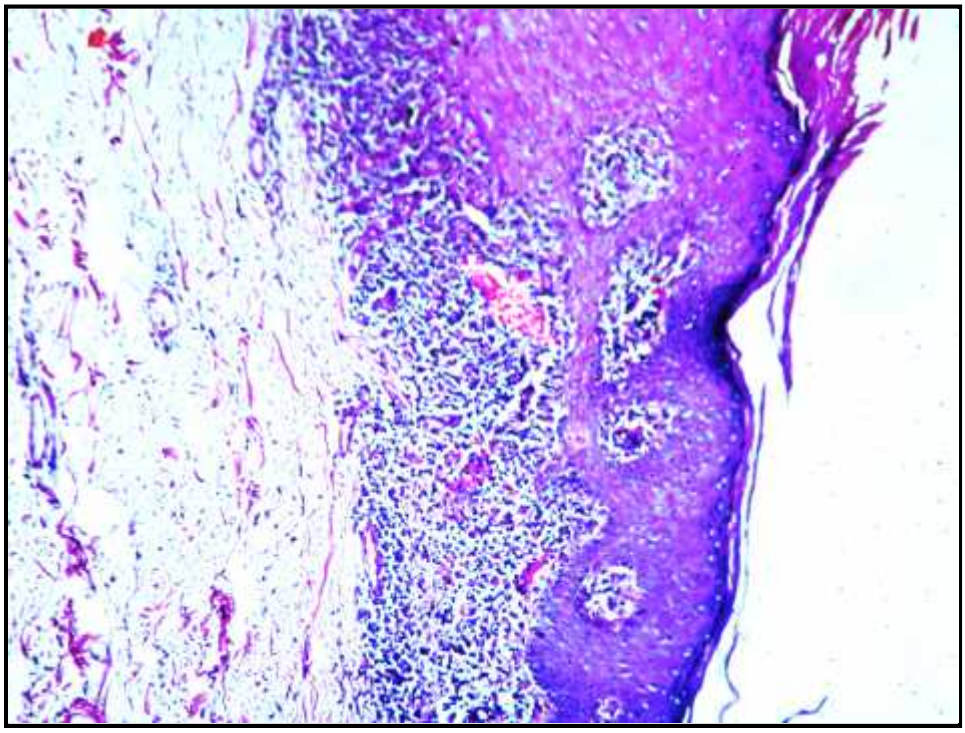


FIG. 15 : Microscopy showing basal cell degeneration (arrow), band of inflammatory cells at dermo-epidermal junction. (100x, H&E)

DISCUSSION

Prevalence:

The present study was conducted over a period of 12 months from November 2007 to October 2008 in the OPD of Dermatology, Venereology and Leprosy in KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum. A total number of 21071 skin patients were treated during this period, out of which 50 cases were taken.

The overall prevalence is believed to be somewhat less than 1% of general population.[1] In the present study the prevalence of Lichen planus is 0.23%.

The prevalence reported by Anbar et al.[50] was 0.28%.

Age distribution:

In the present study, maximum number of cases belonged to the age group of 21-40 years, which is 46%.

In the study of Sehgal et al[11] 46%, and Kacchawa et al[10] 47% of cases were in the age group of 21-40 yeras.

The mean age in the study of Beaird et al[35] was 55years and Lodi et al[31] was 61.7years, were in range of 28-88 years.

Sex distribution:

In majority of the studies females were more frequently affected. In the present study males were 62% and females 38%, with male to female ratio of 1.6:1.

Predominance of males in our study was in accordance with study of Kachhawa et al[10] with 58.6% males, while female predominance has been reported by Lodi et al[31] and Daramola et al.[33] respectively.

Occupation

In the present study housewives and agriculturists constituted to 28% and 26% respectively. Students 24%, business category 4%, officers 14%, nurse 2%, driver 2% were the other category.

Naldi et al[38] also reported 46.3% cases to be manual workers.

Duration of illness

In the present study the maximum duration was 2-3months seen in 36% of the cases, which was similar to that reported by Sehgal et al[11] and Kachhawa et al.[10]

Symptoms :

Itching was the major symptom in 98% of the patients out of which 42% had moderate itching. 2% of the patients were asymptomatic.

Sehgal et al[11] and Kachawwa et al[10] reported moderate to severe itching in 95.91% and 72.8% of cases. Fine et al [15] opined that severity of pruritus varies with extent of involvement.

4% of patients with Oral LP had burning sensation in the oral cavity.

Risk factors

In the present study, risk factors like drugs 18% followed by alcoholism 16%, previous surgery 12%, stress 10% and smoking 8%.

The study by Naldi et al[38] reported smoking in 36.5% and alcoholism in 67.6% cases.

Previous medical history

In the present study, lichen planus was associated with diabetes mellitus in 18% cases, hypertension in 12%, and jaundice in 10%.

Hypertension was reported in Naldi et al[38] in 11.5% cases but by Kachawwa et al[10] in 2.4% cases

Boyd et al[4] and Naldi et al[38] reported diabetes mellitus in 1.6% and 4.9% cases.

Clinical forms

In the present study, 96% of patients had cutaneous involvement and 8% had cutaneous and mucosal lesion. 4% patients had only mucosal involvement.

The studies of Stojanovic et al[35], Sehgal et al[11], Kachhawa et al[10] reported 98%, 70.6%, 67.3% with only skin involvement, 68%, 20.4%, 10% with skin and mucosal involvement and 7%, 12.2%, 72% with only mucosal involvement.

Clinical variants :

The present study, classical type constituted 86%, hypertrophic type 6%, follicular type 2% and actinic type 2%.

The study by Sehgal et al[11] showed classical type in 75.2% cases and follicular type in 1.5% cases.

Distribution of lesions

The extremities were most commonly affected in the present study. Lower limbs 78%, upper limbs 64% , trunk 34%, face and neck 2%, oral cavity 12%, nail 16%. In 4% of cases there was generalized involvement.

The study was comparable to Kachawwa et al[10], which reported involvement of lower limbs in 61.9% cases.

Das et al[39] reported generalized lichen planus in 1.92% cases.

Types of oral lesions :

Involvement of oral mucosa was seen in 12% of the cases. Buccal mucosa was involved in 100% cases, in which 83.3% had reticulate lesions, 16.6% had plaque type lesions. Lips were involved in 16.6% of cases, tongue in 33.3% cases with plaque type lesions.

Sehgal et al[11] also reported oral lichen planus in 12.2% cases, with 89% on buccal mucosa, 12.5% on lips, 6.2% on tongue. Reticulate lesions in 89.6% and erosive type in 22.9% cases.

Stojanovic et al[35] reported in 3.5% cases, with involvement of buccal mucosa only, with reticular and erosive type.

Koebner's phenomenon:

In the present study 6% cases had Koebner's phenomenon. However it was a frequent finding in study by Sehgal et al[11] and Boyd et al.[4]

Nail changes :

In the present study none of the patients had isolated nail involvement. 6% patients showed nail changes, out of which 3 had longitudinal ridges, 1 of them had pitting and discoloration.

Kachawwa et al[10] also reported nail changes in 6.4% of cases, whereas Sehgal et al[11] reported absence of nails changes.

Histopathology of LP :

In this study, epidermis showed hyperkeratosis in 90% cases, hypergranulosis in 54% , parakeratosis in 6%, acanthosis in 64%, basal cell degeneration in 84%, saw toothing of rete ridges in 14%, max joseph space and colloid bodies each seen in 2 % cases.

In the dermis, band of inflammatory infiltrate were seen in 96%, melanin incontinence in 12% of cases.

Out of 43 cases of Classical LP, 100% of the cases showed hyperkeratosis, hypergranulosis, acanthosis, saw-toothing of rete ridges, basal cell degeneration and band of lymphocytic infiltration.

Our results are similar to the observations by Boyd et al[4] and Hamid et al.[3]

Out of 3 cases of Hypertrophic LP, hyperkeratosis, hypergranulosis and acanthosis was seen in 100% of cases. This was almost consistent with the findings of Hamid et al. [3]

Follicular LP was observed in one patient, and it showed marked hyperkeratosis, acanthosis and perifollicular infiltrate, apart from classical features.

Actinic LP was seen in 1 case showing, hyperkeratosis, basal cell degeneration and band of lymphocytic infiltrate was seen, with absence of acanthosis. These findings were similar to that observed by Hamid et al[3] and Salman et al.[16]

Histopathology of Oral LP showed parakeratosis, acanthosis, basal cell degeneration and band of lymphocytic infiltrate in 100% of the cases. This was correlating with the findings of Sehgal et al.[11]

Correlation of LP with HCV :

Lichen Planus occur in association with a variety of systemic disorders and drug therapies. Recent studies have confirmed a significant association between LP and chronic liver disease, in particular HCV infection.

Table 14 : The prevalence of HCV antibodies in LP in different studies:

S.No	Studies	Place	Year of study	% of anti-HCV antibodies
1	Daramola et al[33]	Nigeria	2000	15.8
2	Michelle et al[32]	Italy	2000	28.89
3	Smitha et al[8]	Calicut	2002	0
4	Lodi et al[31]	Italy	2004	20.8
5	Das et al[39]	Kolkata	2006	1.92
6	Present study	Belgaum	2009	0

In our study no association was established between LP and HCV infection.

CONCLUSION

The present study shows that Lichen planus commonly occurs in young adults, with male predominance. Itching was the most common symptom.

Most of the cases were of classical LP, with only cutaneous involvement. The lower limb was the most common site affected. Few patients had only oral lesions, with involvement of buccal mucosa. Nail changes was not a consistent finding.

The histopathology findings of most of the patients showed typical features of classical LP.

None of the patients were tested positive for anti-HCV antibodies.

In the present study no association was established between LP and HCV.

SUMMARY

The present study is a one-year time bound cross-sectional study from November 2007 to October 2008. The source of data for the study includes all cases of LP attending dermatology OPD and referred cases from other departments, at KLES Dr Prabhakar Kore Hospital and MRC, Belgaum.

The objectives of the study were to find out the association of Hepatitis C Virus infection with mucocutaneous Lichen Planus and to study various clinical and histopathological manifestations of LP.

1. In the study, 50 cases were clinically and histopathologically diagnosed to have LP
2. The prevalence of Lichen planus is 0.23%.
3. The age group ranged from 10- 70 years. Maximum cases i.e. 46% belonged to the age group of 21-40 years.
4. Males were more frequently affected than females in the ratio of 1.6:1
5. Most common symptom was itching seen in 98% of cases, 2% of the patients were asymptomatic.
6. No significant differences had been observed in relation to previous medical history.
7. 96% of cases had only cutaneous involvement, 8% cases had cutaneous and mucosal involvement and 4% cases had only oral lesion.
8. Classical LP was seen in 86% cases followed by hypertrophic variant in 6%, follicular type in 2% and actinic type in 2%.

9. Lower limbs were involved in 78% cases and upper limbs in 64%. Trunk was involved in 34%, face and neck in 2%. Nails in 16%, oral cavity 12% cases. Koebner's phenomenon was seen in 6% cases.
10. Reticular type of OLP on buccal mucosa was most common.
11. Nail changes were seen in 6% of cases, longitudinal ridging being the most common change.
12. In the histopathology, hyperkeratosis, acanthosis, hypergranulosis, basal cell degeneration and band like inflammatory infiltrate in dermo-epidermal junction were consistent findings.
13. None of the patients were tested positive for anti-HCV antibodies.

Finally our result showed no association of mucocutaneous LP with HCV infection.

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ANNEXURE - I

PROFORMA

"ASSOCIATION OF MUCOCUTANEOUS LICHEN PLANUS WITH HEPATITIS C VIRUS INFECTION IN A ONE YEAR CROSS SECTIONAL STUDY AT K.L.E.S PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM."

Case No.
OP /IP No.

Name: First name ☐☐☐☐☐☐ Middle name ☐☐☐☐☐☐☐

Last name ☐☐☐☐☐☐

Age: ☐☐

Sex:

1. Male ☐
2. Female

Occupation:

1. Labourer
2. Housewife ☐
3. Officer
4. Any other

Address:

Presenting complaints and duration:

History of present illness:

Site of lesion:

Face

1. Present ☐
2. Absent

Upper limb

1. Present ☐
2. Absent

Lower limb

1. Present ☐
2. Absent

Trunk

1. Present ☐
2. Absent

Itching:
1. Present
2. Absent

History of photosensitivity:
1. Present
2. Absent

History of burning sensation in mouth:
1. Present
2. Absent

History of trauma:
1. Present
2. Absent

History of drug intake:
1. Present
2. Absent

History of emotional stress:
1. Present
2. Absent

Aggravating factors:
1. Present
2. Absent

Constitutional symptoms:
1. Present
2. Absent

Any associated disease:
1. Present
2. Absent

Past History:

History of similar illness:
1. Present
2. Absent

History of blood transfusion:
1. Present
2. Absent

History of Janudice:
1. Present
2. Absent

History of Surgery:
1. Present
2. Absent

History of Diabetes Mellitus:
1. Present
2. Absent

History of Hypertension:
3. Present
4. Absent

Family History:
1. Present
2. Absent

Personal History:

Diet
1. Veg
2. Mixed

Appetite
1. Normal
2. Poor

Bowel/ Bladder
1. Normal
2. Altered

Sleep
1. Normal
2. Altered

Smoking
1. Normal
2. Altered

Alcohol intake
1. Normal
2. Altered

General Physical Examination:

Built
1. Poor
2. Moderate
3. Good

Vitals

Pulse

BP: Systolic ☐
mmHg Diastolic

Temperature ^{0}F ☐

Weight Kg ☐

Pallor
1. Present
2. Absent ☐

Icterus
1. Present
2. Absent ☐

Cyanosis
1. Present
2. Absent ☐

Clubbing
1. Present
2. Absent ☐

Lymph nodes
1. Palpaple
2. Non palpable ☐

Edema
1. Pitting
2. Non Pitting
3. Absent ☐

Mucocutaneous Examination:

Type of lesion:

Papules
1. Present
2. Absent ☐

Plaques
1. Present
2. Absent ☐

Vesicle
1. Present
2. Absent ☐

Bulla
1. Present
2. Absent ☐

Ulcer
1. Present
2. Absent ☐

Erosion
1. Present
2. Absent ☐

Morphology of lesion:
1. Hypertrophic
2. Atrophic
3. Vesiculobullous
4. Erosive
5. Ulcerative
6. Follicular
7. Actinic
8. Pigmentosus
9. Perforating
10. Guttate
11. Classical ☐

Configuration:
1. Annular
2. Linear ☐

Site:
1. Face
2. UL
3. LL
4. Trunk ☐

Distribution:
1. Localized
2. Generalized ☐

Wickhams striae:
1. Present
2. Absent ☐

Koebner's Phenomena:
1. Present
2. Absent ☐

Secondary changes:
1. Present
2. Absent ☐

Appendages:

Nail:

Longitudinal ridges:
1. Present
2. Absent ☐

Pterygium:
1. Present
2. Absent ☐

Subungual hyperkeratosis:
1. Present
2. Absent ☐

Nail dystrophy:
1. Present ☐
2. Absent

Thinning:
1. Present ☐
2. Absent

Atrophy:
1. Present ☐
2. Absent

Onychoschizia:
1. Present ☐
2. Absent

Onycholysis:
1. Present ☐
2. Absent

Anonychia:
1. Present ☐
2. Absent

Hair: Cicatricial Alopecia
1. Present ☐
2. Absent

Mucosal Examination:

Genital lesion:
1. Present ☐
2. Absent

Oral lesion:

Ulcer
1. Present ☐
2. Absent

Erosion
1. Present ☐
2. Absent

Bullous
1. Present ☐
2. Absent

Papule
1. Present ☐
2. Absent

Plaque
1. Present ☐
2. Absent

Reticular
1. Present ☐
2. Absent

Atrophic
1. Present ☐
2. Absent

Systemic Examination:

Cardiovascular system: Heart sounds
1. Normal ☐
2. Abnormal

Respiratory system: Breath sounds
1. Normal
2. Abnormal

Per abdomen: ☐

Spleenomegally
1. Present ☐
2. Absent

Hepatomegally
1. Present ☐
2. Absent

Any other
1. Present ☐
2. Absent

Central nervous system: Neurological examination
1. Normal ☐
2. Abnormal

Investigations:-

-Hb:

-TLC:

-DLC:

-Urine Routine:

- Random blood sugar

- HCV ELISA:

- Skin Biopsy:

Diagnosis:-

Signature:

Guide's Signature:

ANNEXURE –II

INFORMED CONSENT FORM

"ASSOCIATION OF MUCOCUTANEOUS LICHEN PLANUS WITH HEPATITIS C VIRUS INFECTION IN A ONE YEAR CROSS SECTIONAL STUDY."

You are invited to participate in a research study being conducted by Department of Dermatology, Venereology and Leprosy, J. N. Medical College, Belgaum, supervised by Dr. B. S. Manjunathswamy, Professor of Dermatology.

We would like to learn more about Lichen Planus and its associations so that we can provide better treatment to patients affected with Lichen Planus.

Patients diagnosed as Lichen Planus will be asked to participate. If you agree to participate in this study, we will ask you some questions regarding the onset of disease, complaints associated with the lesions, any history of trauma following which lesions develops, history of alcoholism, history of jaundice .We will also be asking about any similar complaints in family. It will take 30 minutes to collect this information. After which you will be asked to undergo few investigation.

You have the right to refuse or may choose not to answer any question that makes you feel uncomfortable. You may stop your participation in the study at any time.

This study may not benefit you, but may be beneficial to the other patients with Lichen Planus. The results of this study may help us to find out the association of Hepatitis C Virus infection with mucocutaneous Lichen Planus.

If you choose not to participate in this study, it will not affect the care given to you .You are free to stop participation in this study at any time and for any reason.

You will not be paid anything for participating in this study and you do not have to pay or give anything to participate in this study except for time.

Every effort will be made to protect the confidentiality of the information you provide. This means that the researchers will be careful not to let anyone who is not working on the study see the information you provide. Your name will not be written on any paper that includes information about you. Instead, you will be given a special number when you agree to participate in the study. This number will be written on all papers that contain information about you in place of your name. Only Dr. B. S Manjunathswamy, Professor, Department of Dermatology, Venereology and Leprosy and Dr. Deepti Rana Post Graduate (Dermatology) , J.N.M.C Belgaum will have access to the information collected. Results of this study may be published for scientific purposes, but your name will not be used.

If you have any questions about this study, you may please call, visit or write to Dr. B. S. Manjunathswamy , Professor Dept. of Dermatology, J.N.M.C ,Belgaum-590010, Tel. No. 98453282198. If you have any questions regarding your rights as study participant, you may contact Dr. V.D.Patil, Principal & Chairman of Ethical Committee, J. N. Medical College, Belgaum, Tel. No. 9448190231.

Statement of Consent: I volunteer and consent to participate in this study. I have read the consent or it has been read to me. This study has been fully explained to me and I was given an opportunity to ask questions and receive answers.

Signature/ Thumb impression: Participant –

Signature/ Thumb impression: Witness –

Date : | | | | | | | | | | |

ANNEXURE - III

ERBA LISA HEPATITIS C

USE ALL READY TO USE REAGENTS. SAVE TIME AND USE QUALITY HEPATITIS C ELISA KIT FOR DETECTION OF ANTIBODIES TO HEPATITIS C VIRUS IN HUMAN SERUM AND PLASMA.

INTRODUCTION : Hepatitis C is a disease caused by viral infection, which is primarily a result of blood transfusions. According to reports studied prior to 1980, the risk of post-transfusion hepatitis was estimated to be 7 to 12%, with approximately 90% of post transfusion hepatitis being caused by the NANB hepatitis agent. Other reports estimated that 5 to 10% of transfused individuals would develop acute NANB hepatitis, with 40% to 60% progressing to become chronic NANB hepatitis and carriers.

ERBA L1SA HEPATITIS C uses synthetic peptides and recombinant proteins of Hepatitis C virus as coating materials.

PRINCIPLE : The ERBA's ELISA HEPATITIS C test kit is a solid phase immunoassay, utilising a mixture of synthetic peptides and recombinant proteins of HCV i.e. CORE, NS3, NS4 and NS5 for detection of HCV antibodies present in human serum and plasma.

When human serum or plasma is added to the well, the bound antigen present in well will form a stable complex with the anti-HCV present in test or positive control specimen. After washing, anti-human IgG with conjugate is added to the wells. Only the bound antigen-antibody complex present in the well, will react with the conjugate

molecule. A second washing step will remove the unbound conjugate molecule. Addition of color reagent, will develop color only in positive control wells and wells containing anti-HCV in test specimen. The intensity of development of color is directly proportional to the presence of bound anti-HCV in the respective wells.

	CONTENTS OF THE KIT	96 test	480 test
1	HCV antigen coated Plate	1	5
2	Conjugate	6.0 ml	30.0 ml
3	Anti HCV Positive Control	0.5 ml	2.0 ml
4	HCV Negative Control	0.5 ml	2.0 ml
5	Color Reagent	6.0 ml	30.0 ml
6	Sample Diluent	11.0 ml	55.0 ml
7	Stopping Solution	12.0 ml	60.0 ml
8	Washing Solution	30.0 ml	150.0 ml
9	Black Cover	1	5
10	Adhesive Strips	2	10

Items 1 to 8 should be stored at 2-8°C and rest items i.e.,

MATERIALS REQUIRED BUT NOT PROVIDED

1. 0-20µl and 50-200µl micropipettors and disposable tips.
2. Automatic microplate washing instrument.
3. ELISA reader.
4. Disposable gloves.
5. Timer.
6. Measuring cylinder - 500.0ml.

STORAGE

1. The kit should be stored at 2-8°C . Do not FREEZE.
2. Immediately after use, return the reagents at 2-8°C.

PRECAUTIONS

1. Disposable gloves should be worn throughout the procedure.
2. For in vitro diagnostic use only.
3. The positive control sera have been inactivated. These does not ensure the complete absence of viable HCV, and there fore, these sera should be treated as infectious materials.
4. Prior to disposal, all waste materials should be col lected and kept in 5% sodium hypochloride solution for 30 minutes.
5. Do not use the kit beyond its labeled expiry date.
6. Do not interchange reagents between lots
7. Use clear serum. Particulate matter should be removed by centrifugation.
8. Use separate tips for controls and individual test specimens.
9. Do not expose color reagent to sunlight.
10. Distilled or deionised water to be used for dilution of washing buffer
11. **After using required number of strips, rest of the strips along with activated silica gel should be kept in sealed condition into the polythene zip lock bag.**

SPECIMEN COLLECTION AND STORAGE

1. Handle all test specimens as potentially biohazardous.
2. Early separation of serum from the clot prevents haemolysis of serum. Specimen should be collected aseptically.
3. Undiluted serum should be stored at 2-8°C.
4. Frozen specimen must be thawed properly.

MICROPLATE WASHING PROCEDURE

Dilute washing solution (1 + 19) in distilled or deionised water. Washing solution may be crystallized at cool storage condition. If so, use it after thawing at 37°e water bath. We suggest that 6 cycles with at least 0.35ml wash buffer per well per wash and a soak time of 30 seconds. **Invert the plate and tap it on absorbent pad to remove the remaining washing solution.**

TEST PROCEDURE

1. Bring all the reagents and test specimens at room temperature before use.
2. Except the blank, add 100µl of Sample Diluent F to each well. In each run, there will be one blank, three negative controls and one positive control. Add 10µl of control and test specimens to the respective wells. Mix properly with pipettor. Cover the plate with black cover and incubate 45minute at 20-30°C.
3. Wash the plate as per microplate washing procedure.
4. Add 50µl 1 conjugate to each well (except blank well). Cover the plate with black cover and incubate 15 minute at 20-30°C.
5. Repeat step 3.

6. Add 50µl of color reagent to each well. Cover the plate with black cover and incubate for 15minutes in dark at 20-30°C.
7. Add 100µl of stopping buffer to each well.
8. Read absorbancy at 450nm.
9. Deduct blank absorbancy from the control and test wells.

CALCULATION FOR CUT-OFF VALUE DETERMINATION

Blank value : Absorbancy of blank value should be less than 0.10.

Positive Control : Absorbancy of individual positive control should be greater than 1.0

Negative Control : Absorbancy of individual negative control should be less than 0.20.

NCx : Average value of negative controls.

Calculation of NCx:

Example:

NC	Absorbance
1	0.095
2	0.096
3	0.094

NCx : (0.095+0.096+0.094)/3 = 0.095

Cut-Off Value Formula: 0.30 + NCx

Cut-Off value: 0.30 + 0.095 = 0.395

Interpretation of the result :

Non-Reactive : If the absorbancy of the test serum is less than the cut-off value, then the sample is considered as nonreactive.

Reactive : If the absorbancy of the test serum is equal or greater than the cut-off value, then it is considered as **initial reactive.** This sample should be retested as duplicate. If the absorbancy of duplicate retest results are less than cut-off value, then the specimen is considered **as non-reactive.** If both of duplicate retest results are found reactive, then the specimen is considered as **repeatedly reactive.**

Repeatedly Reactive specimens found in **ERBA's LISA HEPATITIS C,** must be further confirmed with other tests e.g. Western Blot or Indirect Immunofluorescent assay.

Limitation of the test.

A non-reactive result with this test does not preclude the possibility of HCV infection.

HARRIS HAEMA TOXYLIN AND EOSIN STAIN (REGRESSIVE STAIN)*

SOLUTION

Harris Haematoxylin

Haematoxylin crystals	-----	5.0gm
Alcohol, 100%	-----	50.0ml
Ammonium or potassium alum	-----	100.0gm
Distilled water	-----	1000.0ml
Mecuric oxide (red)	-----	2.5gm

Dissolve the haematoxylin in alcohol, the alum in hot water. Remove from heat Mix the NO solutions together and heat to boiling. Remove from heat and add mercuric oxide slowly. Reheat until it becomes dark purple, Remove from heat immediately and plunge the vessel into a basin of cold water until it is cool. Add 2-4 m1 of glacial acetic acid per 100 m1 of solution and the stain is ready for use,

Acid Alcohol

Alcohol, 70%	-----	1000.0ml
Hydrochloric acid, conc.	-----	10.0ml

1 % Stock alcohol Eosin

Eosin Y, water soluble	-----	1.0ml
Distilled water	-----	20.0ml (Dissolve and add)
Alcohol, 95%	-----	80.0ml

Working Eosin solution

Eosin solution	-----	1 part
Alcohol, 80%	-----	3 parts

Just before use, add 0,5 m1 of glacial acetic to each 100 m1. of stain and stir.

*Culling C, F, A., Allison R.T., Barr W.T. (eds) Cellular Pathology technique 4th ed, London: Butterworth, 1985: 160-161.

STAINING PROCEDURE

Remove paraffin wax with xylene for 5 mins. Treat with absolute alcohol, 2 changes for 1 min. each. Wash in water and stain with Harris haematoxylin for 45 seconds, wash in water. Differentiate in 1 % acid alcohol, till only the nuclei remain blue. Wash in water and blue in running water, rinse in water and stain in 1% Eosin Y for 3 mins. Wash in running water for 1 min., dehydrate in 3 changes of absolute alcohol, clear in 2 changes of xylene. Mount in synthetic resin.

RESULTS

Nuclei	- blue black,	Cytoplasm	- varying shades of pink
Muscle fibers	- deep pinky red,	Fibrin	- deep pink

MASTER CHART

S.No	Name	Age	Sex	Occ	Duration	Itching	Risk Factors							Type of lesion						Morphology						
							Drugs	DM / HTN	Jaundice	Stress	Surgery	Sm / Al	BT	Papule	Plaque	Vesicle	Bulla	Ulcer	Erosion	Classical	Hypertrophic	Atrophic	VB	Erosive	Ulcerative	Actinic
1	Datagir M.H	38	M	Ag	2Y	S	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
2	Abdul M.M	66	M	Ag	2M	L	+	HTN	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
3	Duming I.G	50	M	Ag	1Y	S	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
4	Jakava A.S	32	F	HW	3M	S	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
5	Seema Y	22	F	N	2M	L	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
6	Tejpal N.L	22	M	B	6M	M	-	-	-	+	-	Sm + Al	-	+	+	-	-	-	-	-	+	-	-	-	-	-
7	Balgravayya G.K	42	M	O	5M	L	-	DM	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
8	Arjun B.H	50	M	Ag	2M	S	-	DM	-	-	-	Al	-	+	+	-	-	-	-	+	-	-	-	-	-	-
9	Ravalu M.P	31	M	Ag	15D	S	-	-	-	-	-	Al	-	+	+	-	-	-	-	+	-	-	-	-	-	-
10	Vaman Rao K	64	M	Ag	2M	S	+	HTN	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
11	Bharati C.T	38	F	HW	2M	S	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-
12	Rudrappa G.V	35	M	O	2M	M	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
13	Arati C.K	30	F	S	1M	S	-	-	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
14	Fayaz D.M	24	M	S	2M	L	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
15	Anand	26	M	O	5M	L	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
16	Praveen N.N	29	M	O	1Y	S	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
17	Motiram B.A	55	M	Ag	4M	S	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-
18	Pratibha	40	F	HW	6M	M	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
19	Remediana A.P	52	F	HW	10Y	S	-	-	-	-	-	Al	-	+	+	-	-	-	-	+	-	-	-	-	-	-
20	Ismail K.D	60	M	Ag	2Y	M	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
21	Sukri .M	37	F	HW	3M	S	+	DM	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
22	Prasanna K.G	10	M	S	1Y	S	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
23	Laxmi B.H	35	F	HW	1M	M	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
24	Savitri K.K	12	F	S	2M	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
25	Kallappa .C	53	M	Ag	5Y	M	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-

MASTER CHART

S.No	Name	Age	Sex	Occ	Duration	Itching	Risk Factors							Type of lesion						Morphology						
							Drugs	DM / HTN	Jaundice	Stress	Surgery	Sm / Al	BT	Papule	Plaque	Vesicle	Bulla	Ulcer	Erosion	Classical	Hypertrophic	Atrophic	VB	Erosive	Ulcerative	Actinic
26	Vinayak	14	M	S	3Y	M	-	-	+	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-
27	Meena .C.C	64	F	HW	1Y	S	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	Suresh	44	M	O	15D	M	-	+	-	-	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
29	Pooja M.B	7	F	S	3M	M	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
30	Nanda .V.B	42	F	HW	15D	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	Goudappa .B.K	57	M	Ag	4Y	L	-	-	-	-	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-
32	Razak .B.K	48	M	B	2M	M	-	DM	-	+	-	Al	-	+	+	-	-	-	-	+	-	-	-	-	-	-
33	Amit .R.K	25	M	S	1Y	S	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
34	Heena .F.A	37	F	HW	4M	M	-	DM	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
35	Balku.D.U	55	M	Ag	3M	L	-	DM	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
36	Sushila .C.K	45	F	HW	1M	M	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
37	Altaf	28	M	S	6M	M	-	-	-	+	-	Sm	-	+	+	-	-	-	-	+	-	-	-	-	-	-
38	Gangawwa.A.P	60	F	HW	5Y	L	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
39	Mallawwa.B.M	40	F	HW	3M	S	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
40	Kotrappa .S	70	M	D	4Y	M	+	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
41	Narayan.S.R	28	M	S	3M	S	-	-	-	-	-	Sm	-	+	+	-	-	-	-	+	-	-	-	-	-	-
42	Mallari .M.M	42	M	Ag	7Y	M	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
43	Rahmatbi .G.S	35	F	HW	2M	L	-	-	-	-	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
44	Pratibha .R.S	24	F	S	20D	M	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
45	Ratnamala.H.C	59	F	HW	1Y	M	-	HTN	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
46	Parshuram .M.R	55	M	Ag	2M	L	-	-	-	-	-	Al	-	+	+	-	-	-	-	+	-	-	-	-	-	-
47	Ramanna .J.B	40	M	O	2M	M	-	-	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
48	Sampat .K.G	27	M	S	4M	M	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
49	Akshay .k	13	M	S	1M	S	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
50	Aslam	48	M	O	6M	S	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-

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KEY TO MASTER CHART

Sex

M - Male

F - Female

Occ - Occupation

HW - Housewife

Ag - Agriculture

N - Nurse

B - Business

O - Officer

S - Student

D - Driver

Duration

Y - Years

M - Months

D - Days

Itching

L - Itching

M - Moderate

S - Severe

Risk factors

DM – Diabetes mellitus

HTN – Hypertension

SM – Smoking

Al - Alcohol

BT - Blood transfusion

Morphology

VB – Vesiculobullous

Site

UL – Upper limb

LL – Lower limb

WS – Wickham striae

KP – Koebner's phenomenon

Nail changes

LR – Longitudinal ridging

Disc – Discoloration

Oral lesions

Ret – Reticular

Histopathology

HK - Hyperkeratosis

HG - Hypergranulosis

AC – Acanthosis

STR – Saw toothed rete ridges

BCD – Basal cell degeneration

MJ – Max Joseph space

CB – Colloid body

BI – Band of inflammatory infiltrate

PI – Pigment incontinence

PAP – Papillomatosis

LP - Lichen planus

HCV – Hepatitis C virus