Original Research Article

Clinicopathological correlation of hyperpigmented skin lesions with special emphasis on alkaline Congo red stain for amyloid detection

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Abstract

Introduction: Hyperpigmented skin disorders comprise a group of diseases of extreme heterogeneity of epidermal and dermal origin. Histopathology along with clinical features are sufficient for confirmation of clinical diagnosis in majority cases. Confirmation of amyloidosis is required by special stain for amyloid.Aims : In view of this scientific knowledge, the present study is undertaken to find out occurrence, morphological variants and clinico-pathological correlation of different pigmented skin lesions with an emphasis on the detection of the amyloid material by alkaline congo red stain under light microscopy. **Study design** : Observational study with cross sectional design. The study was performed in Department of Pathology of a state run medical college of northern part of West Bengal.52 patients were taken in the study maintaining the inclusion and exclusion criteria. **Results** : 52 patients with different hyperpigmented lesions were studied. The most common lesions were Lichen planus pigmentosus (28.8%). Most common lesions were found in this study are in the age group of 3rd and 4th decade. The patients arrived with hyper pigmented lesions had female predominance except Lichen amyloidosis. 3.8% cases were Lichen amyloidosis & 9.6% cases were macular amyloidosis & showed eosinophilic amorphous substance in hematoxylin and eosin stain in 100% cases. Apple green birefringes under polarized microscopy seen in 100% cases in this study.**Conclusion:** Out of 52 cases, 84% cases correlated clinically and histopathologically. So a good histopathological diagnosis is necessary for the accurate diagnosis and definite treatment of patients with pigmented skin lesions.

Keywords : Hyperpigmentation, skin lesions , alkaline Congo red stain ,amyloid detection

Introduction

Hyperpigmented skin disorders comprise a group of diseases of extreme heterogeneity of epidermal and dermal origin and subdivided into various types according to etiology, underlying pathology, and the nature of the pigment[1]. Several causes of hyper-pigmentation are there, for instances lichen planus, post inflammatory pigmentary alteration, primary cuteneous amyloidosis, discoid lupus erythematosus, frictional melanosis, morphea, drug induced hyperpigm-entations,acanthosis nigrans, ochronosis, melanocytic nevus and melanoma etc[2]. Primary localized cutaneous amyloidosis (PCA) is characterized by extracellular amyloid deposition in upper dermis without systemic involvement[3]. The term primary cutaneous amyloidosis (PCA)usually includes macular amyloid-osis, lichen amyloidosis and nodular amyloidosis [3,4]. Lichen amyloidosis and macular amyloidosis are best considered as different manifestations of the same disease process. Lichen amyloidosis is characterized by closely set, discrete, brown-red papules that often show some scaling and are most commonly located on the legs, especially the shins. Usually the lesions of lichen amyloidosis itch severely[4]. Macular amyloidosis is characterized by pruritic macules showing pigmentation with a reticulated or rippled pattern. Although, macular amyloidosis may occur anywhere on the trunk or extremities, the upper back is a fairly common site[5,6]. Primary cutaneous amyloidosis is a cause of hyperpigmentation of skin.Histopathology along with clinical features are sufficient for confirmation of clinical diagnosis in majority cases. Confirmation of amyloidosis is required by special stain for amyloid. Amyloid will be demonstrated as cyanophilic to orangophilic acellular material under heamatoxilin-eosin stain and looks salmon pink when stains with alkaline Congo red stain under light microscope & Apple green birefringence in Congo red with polarized microscopy, which is confirmatory[5,7]. Pathologic examination of the biopsied specimen serves as a

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confirmative part of the diagnosis and to assess evolution of skin lesions into different stages as the diseases progress[8]. In view of this scientific knowledge, the present study is undertaken to find out occurrence, morphological variants and clinico-pathological correlation of different pigmented skin lesions with an emphasis on the detection of the amyloid material by alkaline congo red stain under light microscopy.

Aims & objectives

The present study aims at finding out histopathological features of hyperpigmented skin lesion in a wide areas northern part of West Bengal.The study also aims to find out the utility of alkaline Congo red stain for detection of amyloid under light microscope and its correlation with Polarised microscopy in suspected cases.

Materials and methods

The study was performed after approval from the Institutional Ethics committee.

Study type &design: Observational study with cross sectional design.

Study setting: The study was performed in a state run medical college of northern part of West Bengal.

Inclusion Criteria : All clinically diagnosed skin disease cases presented with hyperpigmented skin lesions and gave consent for the study.

Exclusion criteria:-1.Patients not giving consent for the study (Many of the patients with hyperpigmentation on face are excluded from study due to this reason) .

2.Congenital hyperpigmented lesions.

3.Malignant hyperpigmented lesions.

4.Unsuitable for punch biopsy (Presence of large ulceration, infection etc).

Study Plan: Patients were selected from the study population satisfying the inclusion and exclusion criteria. Patient's history and clinical findings were noted in a predesigned standard proforma. Skin biopsy of 4-6 mm size were taken from the most representative lesion with the help of metal biopsy punch under local anesthesia. Biopsy materials were brought to Department of Pathology & processed. Specimens were examined under light microscope. Alkaline Congo red stain were done of those lesions, which showed eosinophilic amorphous acellular substance histologically. Those lesions which showed salmon pink or brick red appearance in congo red, send for polarized microscopy to look for apple green birefringence.

Sampling:Skin biopsy of 4-6 mm size was taken from the most representative lesion from patients . The biopsy material was processed routinely and multiple sections of 3-4 microns thickness was obtained from the paraffin blocks for the staining with hematoxylin and eosin (H&E) and few sections of 8-10 microns thickness taken out for staining with Alkaline Congo red. Congo red staining was done by Puchtler et al. (1962) method[7,9].

Tissue sections were examined for- i. Hyperkeratosis, Parakeratosis, Orthokeratosis, ii. Proliferation of melanocytes in epidermis or spreading to dermis. iii. Acanthosis, Spongiosis, Exocytosis, Acantholysis. iv. Amount distribution of melanin and melanophages in the dermis of basal layer. v. Changes in rete ridges. vi. Lymphocytic infiltration in dermis. vii. Amyloid material and Provitional Diagnosis was made by histopathological examination.

Results

Table 1: Distribution of cases

	Lesion	No.of cases	Percentage
1.	Macular Amyloidosis	5	9.60%
2.	Lichen Amyloidosis	2	3.80%
3.	Lichen planus pig.	15	28.80%
4.	Lichen planus	5	9.60%
5.	Lichen simplex	3	5.80%
6.	Dowling Degos	1	1.90%
7.	Morphea	4	7.7%
8.	Discoid lupus	1	1.9%
9.	Post Inflammatory hyperpigmentation	14	26.9%
10.	PrurigoNodularis	2	3.8%

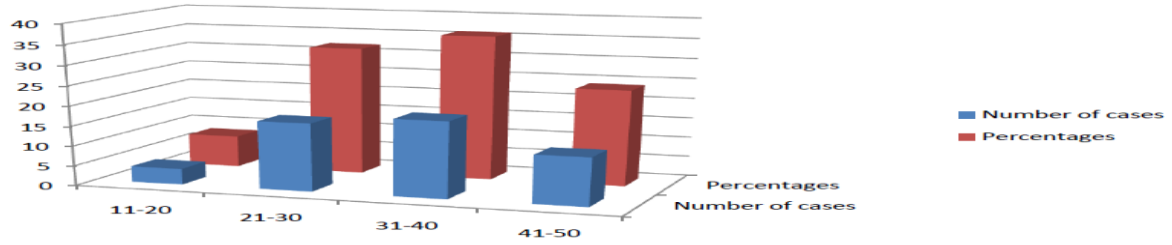

Fig 1: Age wise distribution

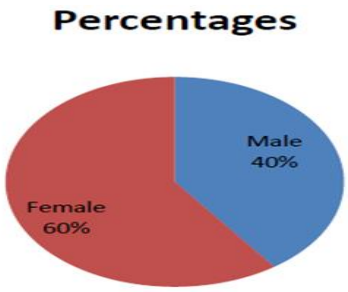

Fig 2: Gender distribution of Hyper pigmented diseases

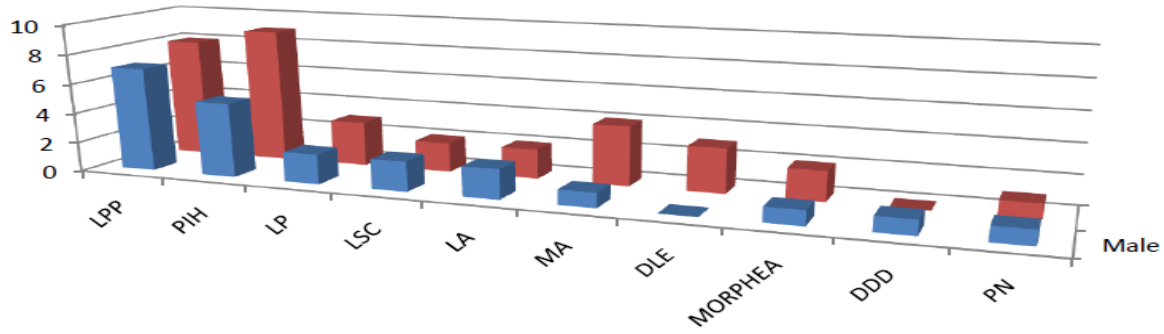

Fig 3: Disease wise distribution of gender

DDD - Dowling Degos Disease, DLE - Discoid lupus erythematosus, LPP -Lichen planus pigmentosus, LP - Classical lichen planus ,LSC - Lichen simplex chronicus, MA - Macular Amyloidosis, PN - Prurigonodularis, PIH- Post inflammatory hyperpigmentation , LA - Lichen Amyloidosis

In this study, 52 cases of hyperpigmented lesions diagnosed clinically, of which 44 cases was confirmed histopathologically. Overall parity between clinical & histopathological cases seen in 44(84%) cases and disparity in 18(16%) cases. Maximum(100%) cases of clinico-pathological correlation seen in most of the diseases like Classical Lichen Planus, Discoid lupus erythematosus, Lichen Simplex Chronicus, Lichen Amyloidosis, Macular Amyloidosis, Prurigonodularis , Dowling degos disease.

Table 2: Parity and disparity between clinical and histopathological diagnosis

Diseases	No.of cases	No.of cases clinically diagnosed	No. of cases confirmed by histo pathologically	% of Clinico pathological Parity	% of Clinico pathological Disparity
Macular Amyloidosis	5	5	5	100%	0%
Lichen Amyloidosis	2	2	2	100%	0%
Lichen planus pigmentosus	15	12	15	80%	20%
Lichen Planus	5	5	5	100%	0%
Lichen simplex chronicus	4	2	4	50%	50%
Dawling Dagos	1	1	1	100%	0%
Morphea	3	3	3	100%	0%
Discoid lupus erythematosus	1	1	1	100%	0%
Prurigonodularis	2	2	2	100%	0%
Post inflammatory hyperpigmentation	14	11	14	78%	22%
Total	52	44	52	84%	16%

The skin lesions were typed based on the classification suggested by Mosher et al[10] into epidermal and dermal hyper pigmentation.

Table 3 :Histopathological features in epidermis of different hyperpigmented lesions

	Lichen planus pigmentosus (n=15)	Lichen Planus(5)	Postinflammatory hyperpigmentation (n=14)	Macular Amyloidosis(5)	Lichen simplex chronicus (n=3)
Hyperkeratosis		3(60)	14(100)	2(40)	2(50)
Parakeratosis	0	0	0	0	0
Orthokeratosis	15(100)	5(100)	14(100)	5(100)	3(750
Acanthosis	1(6)	4(80)	9(56)	2(40)	4(100)
Thinning	10(67)	1(20)			
Pseudo epitheliomatous hyperplasia	1(60				
Irregular elongation of rete ridges	15(100)	5(100)		2(40)	4(100)
Saw tooth reteridges		2(40)			
Basal cell degeneration	15(100)	5(100)			
Basal cell pigmentation			10(62)	1(20)	
Civette body		1(20)			

	Lichen amyloidosis (n=2)	Morphea (n=3)	Dawlinbg dagos (n=1)	Prurigonodularis(n=2)	Discoid lupus erythmatosius (n=1)
Hyperkeratosis	2(100)	1(33)	1(100)	2(100)	1(100)
Para kratosis	0	0	0	0	0
Orthokeratosis	2(100)	1(33)	1(100)	2(100)	0
Acanthosis	2(100)			2(100)	
Thinning		2(66)			1
Irregular elongation of rete ridges	2(100)		1(1000		
Basal cell degeneration					1(100)
Basal cell pigmentation	2(100)		1(100)		

Table 4 :Histopathological features in dermis of different lesions

	Lichen planus pigmentosus (n=15)	Lichen Planus(5)	Postinflammatory hyperpigmentation (n=14)	Macular Amyloidosis (5)	Lichen simplex chronicus (n=3)
Dermis					
Band like infiltrate		5(100)			
Lymphocyte infiltrate		5(100)	14(100)		
Peri vascular infiltrate	15(100)	5(100)	14(100)		3(100)
Pigment incontinence	15(100)	5(100)		1(20)	
Collagen bundle					Vertical collagen bundle 3(100)
Amyloid deposits				5(100)	

	Lichen amyloidosis (n=2)	Morphea (n=3)	Dawlinbg dagos (n=1)	Discoid lupus erythmatosius (n=1)	Prurigonodularis (n=2)
Lymphocytic infiltrate	2(100)		1(100)	1(100)	
Perivascular infiltrate			1(100)	1100)	
Collagen bundles		3(100)			
Pulled up adenexal structures		3(100)			
Amyloid deposits	2(100)				
Nerve hypertrophy and thickenng					2(100)

Table 5:Incidence of Primary cutaneous amyloidosis

Total hyperpigmented lesion	Macular amyloidosis	Lichen amyloidosis
52	5	2
Percentages	9.6%	3.8%

Discussion

Mruthyunjayappa, et al. studied 200 pateint of hyper-pigmentation; among them there were classical lichen planus 26%, Hypertrophic lichen planus 6%, Lichenoid drug eruption 3%, Lichen planus pigmentosus 23%, Lichen simplex chronicus 8% Post inflammatory hyperpigmentation 9%, & only 1.5% cases of hyper-pigmented patients having DLE[11]. Sushan Shweta Jayker et al[12] found that, 11(13%) cases were Classical Lichen planus , 6(7%) were Lichen planus pigmentosus, 5(6%) cases of Psoriasis, 5(6%) cases of Prurigonodularis, 4 (4.7%) cases of Morphea, 4(4.7%) cases of Lichen simplex chronicus, 3 cases of Lichen striatus, 3 cases of Verruca plana, 2 cases of Macular amyloidosis, 1 cases of Post inflammatory hyper pigmentation & no case of lichen amyloidosis among 85 cases[13]. There is appreciable similarity between present study & the above two studies. (Table1) Whereas Lichen planus pigmentosusis the highest number of cases of hyper pigmentory disorder in our study, it was the second highest case in the studies of Sushan Shweta Jayker et al[13] &Mruthyunjayappa et al [11] However Classical lichen planus cases are a little low (10%) and Post inflammatory hyper pigmentation (27%), lichen amyloidosis (4%), Macular amyloidosis (10%) lesions are high in North Bengal Population in compare to above two studies.Hyper pigmented lesions may occur at different age groups ranging from first decade to 8th decade. Maximum incidence is seen at the age group of 31-40 years. Total 19 cases (36%) are seen at this age group. At age group 21 to 30 year, 17 cases(32%), 12(24%) cases on age group 41 to 50 years, 4 cases (8%) seen at age group of 11 to 20 years. No case seen below 11 years or above 47 years of age.(Figure 1)Sovernakar et at. found that benign skin were common in age group 21 to 40 year(66.7%)(49). Studies by AsmitaPrihar et al. &Sushan Shweta Jayker et al. also stated that most common age group of hyper pigmented skin lesion was 21 to 30 years[12,13]. Mruthyunjayappa, et al. found 110 (55%) were female, and the remaining 90 (45%) were male out of 200 patients[11]. Sushan Shweta Jayker et al. showed, among the 85 patients, 47 were females and 38 were males[13]. According to Salim T, Shenoi SD, Balachandran C, Mehta VR, on a study on Lichen amyloidosus of the 30 patients, 19 (63.3%) were males and 11 (36.7%) females with a mean age of 43.13 years[14]. So there is a concordance with the present study.(Figure 2,3)It is worthy to mention that in the present study all cases of lichen amyloidosis occurred at lower limbs & history of using scrub for bathing was there. Similar observation noticed by T Salim et al. They were found that the shin was the initial site of involvement in 86.7%, the arms in 10% and the back in 3.3% cases. 56.7% patients had used scrubs for bathing for more than 2 years[14]In this study, In Lichen planus pigmentosus , out of 15 cases all(100%) showed orthokeratosis, irregular elongation of reteridges, basal cell degeneration. 10(67%) patients were found to have thinning of epidermis. Only 1(6%) lesions showed acanthosis & pseudo epitheliomatous changes. Dermis showed all cases having pigment incontinence & perivascular lymphocytic infiltration [15,16]. All 5 cases of Classical lichen planus showed orthokeratosis, irregular elongation reteridges, basal cell degeneration. 4(80%)cases showed acanthosis, 3(60%) cases showed hyperkeratosis. 2(40%) cases were having saw tooth reteridges.(Table 3)In dermis, 5(100%) lesions showed lymphocyticinfiltration with band like lymphocytic infiltration, perivascular lymphocytic infiltration & pigment incontinence. (Table 4) All 14(100%) cases of PIH showed hyperkeratosis, orthokeratosis. 9(56%) cases found to have acanthosis, 10(62%) lesions showed basal cell pigmentation. 100% cases in dermis were displayed lymphocytic infiltrates along with perivascular lymphocytic infiltrates. Lichen simplex chronicus showed hyperkeratosis in 2(50%) cases, Orthokeratosis in 3(75%) cases, acanthosis & irregular elongation of reteridges in 4(100%) lesion.(Table 7 b) In dermis all lesions showed perivascular lymphocytic infiltration & vertically oriented collagen bundles. Out of 3 cases of Morphea, 2(66%) cases showed hyperkeratosis & ortho keratosis.1(33%) case showed thinning of epidermis. 3(100%) case showed deep dermal collagen bundles & pulled up adnexal structures. Discoid lupus erythmatosus showed hyperkeratosis, orthokeratosis, thinning of epidermis, basal cell degeneration & follicular plugging in 1(100%) cases. Lymphocytic infiltration & perivascular lymphocytic infiltration in all lesions dermis. In Dowling degos disease, single case was showed hyperkeratosis, orthokeratosis, basal cell pigmentation & antler horn type irregular elongation of reteridges.(Table 4) Histologically, Prurigonodularis was presented with marked hyperkeratosis, focal parakeratosis, and marked irregular acanthosis or pseudoepitheliomatous changes. In dermis there was hypertrophy and proliferation of dermal nerves in 100% cases[3]. Lichen Amyloidosis histologically presented with hyperkeratosis, orthokeratosis, acanthosis, irregular elongation of reteridges in 100% cases. In dermis lymphocytic infiltration & Amorphous eosinophilic amyloid like material was seen in all cases[3]. In Macular amyloidosis 2(40%) cases showed hyperkeratosis, acanthosis, irregular elongation of reteridges. 5(100%) lesions were presented with orthokereatosis. Epidermal basal cell degeneration or dermal pigment incontinence was seen only 1(20%) case. All lesions showed slight amorphous eosinophilic amyloid like material in dermis. Amyloid suspected on routine hematoxilin& eosin stain, had to be confirmed by congo red stain, as hyalinised collagen also may visualized as eosinophilic. On alkaline congo red staining, all patients (100%) of lichen amyloidosis & macular amyloidosis, showed salmon pink or brick red colour in place of amorphous eosinophilic amyloid like material that was seen in the dermis in haematoxylin & eosin section. These lesions later found to be shown apple green birefringence under polarized microscope. (Table 5) Mruthyunjayappa, et al& Asmita Parihar et al. showed similar findings in histopathological examination [11,12]. Mruthyun-jayappa, et al was detected Amyloid deposits in 28 out of 30 patients as uniformly stained pink globules occupying the dermal papilla. B. Vijaya et al. also stated that sensitivity of congored staining in amyloid detection was 100% in his study[4,17,18].Study of Mruthyunjayappa, et al. found that, positive correlation between clinical diagnosis and histopatho-logical diagnosis was in 95% of cases and negative correlation in 5% of case(14)[11]. Sushan Shweta

Jayker et al. stated that 85 cases were clinically presented as hyperpigmented lesions, of which 67 (78.9%) cases were confirmed on histopathology and 18(21.1%) cases were diagnosed only on histo-pathology[13].(51) So, there is variation &similarity withthe present study due to different criteria used to select the cases and difference in number of cases of each type.(Table 2)

Conclusion

Present study mainly focused on acquired non malignant diseases & covers most common diagnoses of Pigmentary disorders in North Bengal Population. Utility of histopathology was demonstrated as clinical presentation overlap significantly. Specific diagnosis of hyperpigmented lesions were based on possible histopathologic findings and interpretation in context of its clinical presentation. Lichen planus and its variants were the most common hyperpigmented skin lesions encountered in this study. Most of the lesions were of epidermal type which responds well to treatment than its dermal counterpart. Incidence of Primary Cutaneous Amyloidosis was fairly high in the present study. Proper histopathological study with haematoxylin-eosin stain along with Puthler's modification of Alkaline Congo red stain & Polarised microscopy helped to diagnose all these diseases. In the present study the sensitivity of Alkaline Congo red staining in detecting amyloid was 100%. So in low equipped hospital setup, where sophisticated Polarized microscope not available, Alkaline congo red stain may be used as sole method of confirmation of tissue amyloid. Out of 52 cases, the diagnosis of 44(84%) cases correlated clinically and histopathologically, which emphasizes a correlation between clinical diagnosis and histopathological diagnosis.

List of abbreviation used A – Acanthosis, DDD - Dowling Degos Disease, DLE - Discoid lupus erythematosus, F – Female, LPP -Lichen planus pigmentosus, H/E – Haematoxylin & Eosin, HK – Hyperkeratosis, LP - Classical lichen planus, LSC - Lichen simplex chronicus, M – Male, MA-Macular Amyloidosis,No.–Number,PN – Prurigo-nodularis,P –Parakeratosis, PIH- Post inflammatory hyper-pigmentation,O-Orthokeratosis, LA - Lichen Amyloidosis ,T – Thinning of epidermis, ZN- Ziehl Neelson stain ,X-Number of multiplication under microscope

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