ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 01 | January - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CLINICOPATHOLOGICAL STUDY OF HYPOPIGMENTARY DISORDERS OF SKIN: AN IMMUNOHISTOCHEMICAL APPROACH TO DIAGNOSIS.

Histopathology

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ABSTRACT

Introduction - Hypopigmentary disorders in darker skin individuals like Indians can be distressing to the patients and the family. These disorders cover a wide range of pathologies including infections, inflammatory disorders, autoimmune diseases, lymphoproliferative disorders, and sclerosing diseases. Histological diagnosis is very important because treatment and prognosis for these diseases are varied and specific. **Aim:** To study clinicopathological features of hypopigmentary skin disorders along with immunohistochemical expression. This article outlines the common Hypopigemented lesions which encountered in the Department of Dermatology and the biopsy received in Department of Pathology for further evaluation of the disease. **Method And Material**- This is an Ambispective observation study carried out on 83 cases whose specimen of biopsy received in Pathology Department, Sri Aurobindo medical college and Post Graduate Institute. Then paraffin embedded tissue sections were stained with routine haematoxylin and eosin and diagnosis was made on the basis of the histopathological findings. Special stain as per requirement like Masson Fontana, PAS and Fite Ferraco. Immunohistochemistry HMB– 45, MELAN -A, will be performed wherever required. Appropriate positive and negative controls will be added in each batch of immune staining. **Result**- Maximum numbers of patients had presented with clinical diagnosis of Vitiligo (11 cases) followed by Hansen's leprosy (BT-11 cases and LL-9 cases, BL -5, Type 1 and Type 2 ENL 13), 2 out of 83 cases were diagnosed on histopathology as lichen sclerosis et atrophicus, 8 as Morphea, Pityriasis Lichenoid Chronicus 1 case, post inflammatory and idiopathic Guttate Hypomelanosis 14 cases., Scleroderma and systemic sclerosis 4 cases with Mycosis Fungoides 1 case. 11 Cases with diagnosis of Vitiligo showed expression of Melan –A and HMB-45 Immunostain. **Conclusion**- Adequate clinical data and workup in combination with pathological resources can help in elucidation of specific etiology and good clinicopathological correlation.

KEYWORDS

Melan-A, HMB-45,IHC, IGH, PIH, BT, LL.

INTRODUCTION-

Hypopigmentary disorders in darker skin individuals like Indians can be distressing to the patients and the family. These disorders cover a wide range of pathologies including infections, inflammatory disorders, autoimmune diseases, lymphoproliferative disorders, and sclerosing diseases. Histological diagnosis is very important because treatment and prognosis for these diseases are varied and specific. These hypopigmentary disorders could be classified based on their etiology (congenital or acquired), age of onset (childhood or adulthood) and extent of lesions (localized or generalized). Further differentiation could be made on the basis of clinical findings, degree of pigment loss, and sites of involvement. The intention of this study is to correlate the histopathological findings with clinical findings of Hypopigemented disorders of skin to arrive at an accurate diagnosis[1,2,3]. The clinicopathological picture is determined by the equilibrium between the agent and the host resistance. Skin has different pathophysiological subunits which cause some local modulation of the central host response as a result of which there are different grades of resistance and hence different clinicopathological responses in different areas. The etiology of these disorders ranges from infections, autoimmune processes, sclerosing diseases to lymphoproliferative disorders. In this study, hypopigmentary disorders due to infections have contributed to 37.7%, autoimmune disorder contributed to 30.1%, sclerosing diseases for about 9.4%, post inflammatory conditions include 5.6%, and genetic disorder contributed 1.9%. Histological diagnosis is essential as treatment differs for these disorders[1,4,5] Chug et al had conducted clinicopathological correlation study of acquired hypopigmentary disorders in 50 patients. The overall clinicopathological correlation in their study was 80%. In this study, there was 86.7% correlation in 53 cases.[6] The principles of immunohistochemical analysis were first conceptualized by Coons et al. Generally, Immunohistochemistry is done for confirming the Hypopigemented disorders in cases with diagnostic dilemmas. But, here it was done to classify the Hypopigemented disorders as melanopenic or melanocytopenic in addition to confirming the diagnosis. The melanocytopenic disorders were devoid of melanocytes and the current study showed Vitiligo and DLE to be melanocytopenic. Seleit l et al and Franca AFE da C et al showed Vitiligo and DLE were melanocytopenic with HMB 45 marker in their studies respectively. Therefore clinical diagnosis of Hypopigemented disorders alone is never specific and cannot be used as a single diagnostic tool for confirmation. Histopathological examination helps in arriving at a specific etiology and good clinicopathological correlation. Furthermore, Immunohistochemistry helps in differentiating between melanopenic or melanocytopenic and in confirming diagnosis. Hence, a systematic approach of clinical, histopathological examination and Immunohistochemistry will provide an accurate diagnosis of Hypopigemented disorders and thereby reducing the patient distress.[1, 7, 8, 9] The purpose of this study was to investigate the usefulness of the Melan-A marker for identifying and estimating melanocyte content in different zones of non-segmental Vitiligo lesions: the zone of depigmented skin, marginal zone (bordering the area), and zone of perilesional normally pigmented skin.[10,11].

Methodology (Material & Methods) –

- All the skin biopsies with hypopigmentation disorders reported from June 2022 to November 2023 will be included in present study. (June 2022 to May 2023 retrospective, June 2023 to November 2023- prospective cases.
- All Punch biopsy will be processed as per standard operating procedure.
- After conventional processing and embedding in paraffin wax, sections of 5um thickness will be cut and stained using haematoxylin and eosin (H&E) for histopathological study. These H&E stained slides will be studied as per the standard reporting protocol.
- Special stain as per requirement like Masson Fontana, PAS and Fite Ferraco. Immunohistochemistry HMB– 45, MELAN -A, CD-8 will be performed wherever required. Appropriate positive and negative controls will be added in each batch of immune staining.
- Data will be filled in the proforma and master chart and will be analyzed.
- The observations and results will be compared with previous

similar studies.

It is estimated that approximately 5-10 cases of Hypopigemented Skin lesion are diagnosed every month in the Department of pathology.

Hence, this study is feasible for a sample size of 83 cases which include data collection for duration of 18 months.

Descriptive statistics of quantitative traits was presented as medians and quartiles (Me [Q1; Q3]). The Mann-Whitney U-test was used for comparison of unrelated groups by both quantitative and ordinal traits. Correlation analysis was performed using the Spearman rank correlation coefficient.

Inclusion Criteria-

The study will include all the Hypopigemented skin disorders undergoing skin biopsy during study period.

Exclusion Criteria-

1. Biopsy inadequate for IHC study.
2. Cases with generalized Hypomelanossis will also be excluded from the study.

Data Collection And Methods-

All newly diagnosed cases of Hypopigemented Skin lesion proven on histopathology examination will be included. Clinical details including age, family history will be recorded in the proforma. The sectioned tissue pieces will be processed as per the standard operating procedure to obtain the slides. The slides will be stained with routine haematoxylin and eosin stain and will be examined microscopically, special stain as per requirement like Fite Ferraco. Immunohistochemistry HMB– 45, MELAN -A, will be performed wherever required. Appropriate positive and negative controls will be added in each batch of immune staining. In testing hypotheses, the differences were considered statistically significant at (p **<0.001**).

DISCUSSION-

This article mainly emphasizes on the clinicopathological study of Hypopigmented lesion in a broad classified age group encountered in our Institute in Department of Dermatology whose biopsy were received in our Histopathology Department for Further Diagnosis and evaluation. The categorization of Hypopigmentated disorders is generally based on etiology (Congenital or acquired), Age, of onset (Childhood or Adulthood) and the extent of the lesion (Local or Generalized). Localized hypopigmentation disorders are a common presentation in adults. The commonest cause of depigmented lesions is vitiligo. Vitiligo is characterized clinically by depigmented macules and patches that correspond histologically to a decrease or absence of melanocytes in the epidermis and less often the hair follicles. In general, Vitiligo can be categorized into localized, generalized anduniversal forms. Localized forms can be segmental, focal or mucosal. The generalized forms are more common, and can be acrofacial or the vulgaris type. Post-inflammatory hypopigmentation and chemical leucoderma are other common cause of localized hypopigmentation. However, there are some inflammatory diseases in which the lesions are Hypopigmented from the onset and preceding inflammatory changes may be clinically absent. Examples include Pityriasis versicolor, mycosis Fungoides (MF),lichen Sclerosus (LS),morphoea(localized scleroderma). Distribution of cases with Histomorphological diagnosis encountered are as follows had presented with clinical diagnosis of Vitiligo (11 cases, 14.45%) followed by Hansen's leprosy (BT-11 cases and LL-9 cases, BL -5, Type 1 and Type 2 ENL 13(42.14%), 2 out of 83 cases were diagnosed on histopathology as lichen sclerosis et atrophicus 2(2.0 %), 8(9.6%) as Morphea, Pityriasis Lichenoid Chronicus 1 case, post inflammatory and idiopathic Guttate Hypomelanosis 14 cases (15.66%). Scleroderma and systemic sclerosis 4 (4.72%) cases with Mycosis Fungoides 1 case. 11 Cases with diagnosis of Vitiligo showed expression of Melan –A and HMB-45 Immunostain, Positive and Negative reporting were done on the basis of Intensity and Pattern of IHC Distribution in Dermoepidermal junction , Epidermis and Dermis (Table 1).

Cases with Vitiligo were applied with Immunohistochemistry Melan-A and HMB – 45had significant correlations in & cases with Positive expression. (Table 3 and Image No 1 and 2), Correlation analysis was performed using the Spearman rank correlation coefficient. In testing hypotheses, the differences were considered statistically significant at (p **<0.001**).There is a statistically significant correlation between No of Cases and Percentage (p **<0.001**).

Table .2

In the present study the Histopathological diagnosis showed parity in 99.88% cases for Vitiligo and Idiopathic Guttate Hypomelanosis, Hansen's disease and PIH. Vitiligo cases 11 cases IHC was applied with HMB- 45 and Melan – A which showed the expression in 7 cases with both as Positive as shown in (Table 4) and Images 1 and 2 . In Dermis and Dermo-Epidermal junction and Epidermis with Melanocytes . Regarding melanin granules, in the zone of depigmented skin they were found in individual regions of the basal layer, in the marginal zone they were found in almost the entire basal layer, and in the zone of perilesional normally pigmented skin they were found throughout the basal and suprabasal layers of the epidermis. Seleit et al. demonstrated the presence of HMB-45+ cells (indicating differentiated and active melanocytes) in 44% of cases in interfollicular epidermis and in 46.7% of cases in follicular epidermis in lesional Vitiligo skin.

Table 1 Distribution Of Histopathological Lesions Proved Cases On Hypo Pigmented Lesions

Table 1 Clinical Distribution of Cases N = 83		
Clinical Diagnosis for skin Diseases	**No. of Cases**	**Percentage**
Vitiligo	11	14.45
Morphea	8	9.6
Scleroderma	3	3.61
DLE	6	7.22
BT HANSEN'S	10	12.04
BL Hansen's	5	6.02
LL Hansen's	9	10.84
HANSEN WITH type 1 with ENL	7	8.43
Hansen's with type 2 with ENL	4	4.81
IGH	13	15.66
PIH	1	1.2
Systemic sclerosis	1	1.2
Lichen Simplex at atrophicus	2	2.4
Pityriasis Lichenoid Chronica	1	1.2
Cutaneous Lymphoma (Mycosis Fungoides)	1	1.2
Total	83	99.88

Tables 2 (Statistic Evaluation of Number of Cases with Percentage)

Descriptive Analysis

Quantitative variables

	mean (sd)	**median [Q25-75]**	**min**	**max**	**n**
No of Cases	10.4 (19.8)	5.50 [1.75; 9.25]	1.00	83.0	16
Percentage	12.5 (23.8)	6.62 [2.10; 11.1]	1.20	99.9	16

Univariable Analysis

By No of Cases

	correlation coefficient	**n**	**p**	**test**
Percentage	1.00	16	**<0.001**	Spearman

There is a statistically significant correlation between No of Cases and Percentage (p **<0.001**).

Expression of HMB- 45 (Human Melanoma Black -45) in Vitiligo cases

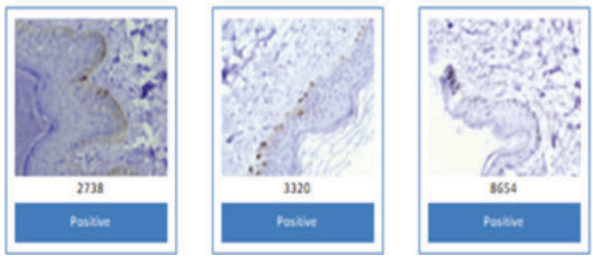

Image 1

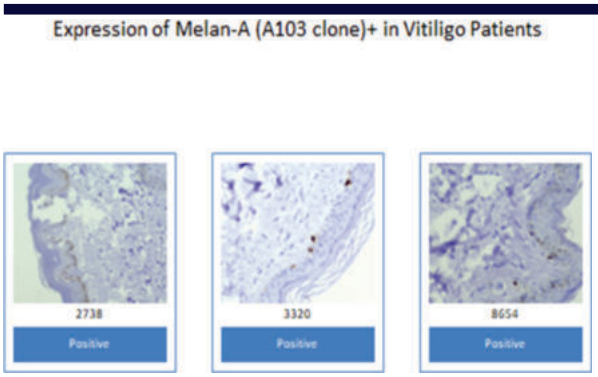

Image 2

Table 3 IHC Distribution Location Of The Hypopigmentated Lesion

HPE N0. Vitiligo Vulgaris	Location	HMB 45	Melan- A
6825/22	Right Leg	Positive	Positive
7087/22	Abdomen	Positive	Positive
8654/22	Left side abdomen	Positive	Positive
8953/22	Right leg Patch	Negative	Negative
8982/22	Left leg	Positive	Positive
2738/23	Right hand dorsal	Positive	Positive
3020/23	Right side abdomen	Positive	Positive
3222/23	Left leg	Negative	Negative
3778/23	Hypopigemented macule chest	Negative	Positive
4183/23	Hypopigemented macule chest	Negative	Negative
4899/23	Left foot	Positive	Positive

Table 4 Histopathological Findings In Vitiligo Cases

Vitiligo Cases	11
Epidermal spongiosis	11
Basal cell layer vacuolization	0
Dermal melanophages	3
Dermal lymphocytic infiltrate	6
Immunohistochemistry (hpf)	11
HMB-45	Positive = 07
MELAN - A	Negative = 08

Table -5 Intensity And Pattern Of Melan – A IHC Stain On Vitiligo Cases At Various Extend Of Skin

HPE NO.	MELAN-A IHC		
Case number	Epidermis	D-E junction	Dermis
2738	Dendritic pattern in normal melanocytes	Negative	Negative
3222	Negative	negative	negative
3320	Dendritic pattern in normal melanocytes	diffuse positive	negative
3778	Dendritic pattern in normal melanocytes	diffuse positive	negative
4183	Negative	negative	diffuse positive
4899	Dendritic pattern in normal melanocytes	focal positive	negative
6825	Dendritic pattern in normal melanocytes	diffuse positive	negative
7087	Dendritic pattern in normal melanocytes	diffuse positive	negative
8654	Dendritic pattern in normal melanocytes	focal positive	focal positive
8953	Negative	negative	negative
8982	Dendritic pattern in normal melanocytes	diffuse positive	negative

CONCLUSION-

Adequate clinical data and workup in combination with pathological resources can help in elucidation of specific etiology and good clinicopathological correlation.

This study indicates the usefulness of the Melan-A marker for identifying melanocytes in non-segmental Vitiligo patients' skin and for estimating their content in different zones of the Vitiligo lesions. Expression of melanosome protein Melan-A and melanin granules was found in all three zones of Vitiligo lesions. For some patients, including those with long-standing disease (up to 40 years), Melan-A+ cells and melanin granules were detected in depigmented skin as an indication that the residual melanocytes are preserved in Vitiligo lesions, and these results are consistent with the data of other authors.

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