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Original Article

Pathology Section

# Expression of Melan-A (MART-1) in Various Pigmented Melanocytic Nevi and Close Mimickers

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## ABSTRACT

**Introduction:** Melanocytic nevi are neoplasms resulting from the proliferation of melanocytes. Diagnosis of melanocytic tumours can be tricky, considering two factors, diagnosis of origin and determination of its benign or malignant nature. Visualisation of melanin or other pigments are non specific with Hematoxylin and Eosin (H&E) staining and specific with Melan-A Immunohistochemistry (IHC). Intensity and pattern of these reactions shows marked variability in different melanocytic lesions.

**Aim:** To study the intensity and pattern of Melan-A expression in various pigmented melanocytic nevi and to understand its utility to differentiate close mimickers.

**Materials and Methods:** A cross-sectional study of 50 lesions (45 cases and 5 controls) was conducted in the Department of Pathology of Dr. B.R. Ambedkar Medical College (tertiary hospital), Bengaluru, Karnataka, India, between September 2020 to October 2021. The skin biopsies received were fixed in formalin and paraffin embedded. Sections were stained with H&E and Melan-A IHC marker, using A103 antibody and a high temperature antigen retrieval was performed. Pattern and intensity of Melan-A expression were studied and its evaluation was done with normal skin (control).

**Results:** Melan-A showed varied intensity (+ weak; ++ moderate; +++ strong), pattern (patchy, diffuse), distribution and on various pigmented melanocytic lesions and mimickers were analysed. Dermal nevi (18) showed +++ intensity, diffuse pattern in dermis and two cases showed ++ intensity and patchy staining. Five cases of Compound nevi showed ++ intensity, diffuse pattern in Dermoepidermal Junction (DEJ) and dermis, Deep penetrating nevi (in one case) +++ intensity and diffuse pattern in DEJ, dermis. Two cases of pigmented Basal Cell Carcinoma (BCC) showed + intensity and no definitive pattern in dermis. Four cases in fibrohistiocytic lesions and Malignant Peripheral Nerve Sheath Tumour (MPNST) shows negative Melan-A. In control melanocytes show dendritic pattern of staining and melanophages were negative.

**Conclusion:** Melan-A is specific melanocytic marker as it stains only melanocytic lineage and no other cell types in background thus it is a marker of histogenesis instead of malignancy indicator. It highlights important architectural features and confirms the origin of lesions thus aiding the pathologist towards accurate diagnosis and differentiates from close mimickers.


**Keywords:** Dermal nevus, Dysplastic nevus, Fibrohistiocytic lesions, Malignant melanoma, Melanoma antigen recognised by T-cells

## INTRODUCTION

Melanocytic nevi occur due to proliferation of melanocytes, pigment producing cells in the skin. Nevi results due to clonal proliferation and growth arrest of melanocytes, stimulated by most commonly BRAF oncogenic mutation through Mitogen-Activated Protein Kinase (MAPK) pathway [1]. The incidence of Acquired Melanocytic Nevi (AMN) ranges from 15% to 40% [2]. The prevalence of nevi is related to the age, race, genetic and environmental factors. Melanocytic nevi usually present in childhood and adolescents with equal sex distribution. It has been estimated that the likelihood of any one nevus evolving into melanoma is roughly 1/1,00,000 and subsequent mortality is of the order of 1/500000 original nevi [3]. The size and number of AMN and presence of dysplastic nevi are the leading risk factors that should be recognised in the development of MM [4]. Diagnosis of melanocytic tumours poses two big glitches for a histopathologist: 1) To diagnose its origin for accurate diagnosis 2) determination of its benign or malignant nature [5]. In some cases, H&E staining cannot prove actual size of melanoma invasion, in these cases immunohistochemical examination with Melan-A can be a complementary method [6].

Many nonmelanocytic skin lesions have pigmented variants such as pigmented Basal Cell Carcinoma (BCC), fibro histiocytic lesions, Malignant Peripheral Nerve Sheath Tumour (MPNST) that can mimic melanocytic lesions. Histopathological examination helps to confirm the clinical diagnosis and helps in treatment of patients with pigmented skin lesions [7]. Many lesions create difficulty in histopathological analysis due to overlapping features and dilemma regarding origin of lesions hence, it is of prime importance to develop a diagnostic approach to confirm and differentiate melanocytic lesions and their mimickers by Melan-A IHC marker, this being the prime aim of this study.

Melan-A was cloned by Coulie et al., in 1985 from the human melanoma SK-MEL-29 cell line. Melan-A/MART-1 is an antigen recognised by tumour infiltrating cytotoxic T cells from a melanoma patient. This antigen is a gene product of MART-1 gene. In 1996, Chen YT et al., generated a monoclonal antibody against the recombinant Melan-A protein [8]. Immunoblotting and immunoprecipitation analysis exposed a 20-22 kDa doublet in Melan-A positive cell lines and this antibody worked on fixed tissue [9]. Two commercially available antibodies are:

1. A103: Mouse monoclonal antibody against Melan-A recombinant protein
2. M2-7C10: Mouse monoclonal antibody clone produced against MART-1 protein

Melan-A is a melanocyte lineage specific marker showing cytoplasmic staining and having no background staining. It is used for differentiating melanocytic nevi from other lesions with resembling H&E aspects, although some authors claim that MART-1 could be used in assessing the prognosis of the patients with melanoma [10].

In this study, the intensity and pattern of Melan-A expression in various pigmented melanocytic nevi were studied in detail to understand its utility to differentiate close mimickers.

## MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Pathology at Dr. BR Ambedkar Medical College (tertiary hospital), Bengaluru, Karnataka, India, from September 2020 to October 2021. A total of 50 lesions (45 cases and 5 controls) were analysed. Ethical Clearance was obtained from Institutional Research Board (reference No. EC-611).

**Inclusion and Exclusion criteria:** All patients aged between 10-70 years clinically diagnosed as pigmented lesions were included for the study and patient whose biopsy was scant or fragmented tissue bits, who are on steroid and were already on treatment for melanocytic nevi were excluded from the study.

### Study Procedure

The following spectrum of clinically diagnosed lesions were included in this study received from Dermatology Department were studied:

**Lesions:** Dermal nevus, compound nevus, deep penetrating nevus, dysplastic nevus, junctional lentiginous nevus with atypia, spitz nevus, Melanocytic Tumor of Uncertain Malignant Potential (MELTUMP), Malignant Melanoma (MM), Desmoplastic Melanoma (DMM).

**Pigmented lesion mimicker:** Pigmented BCC, fibro histiocytic lesions, Malignant Peripheral Nerve Sheath Tumour (MPNST).

All relevant clinical details and informed consent has been taken by explaining in his/her own understandable language. Skin biopsies were fixed in 10% formalin, grossed as per prescribed standards, embedded in paraffin, 4 to 5 microns thick sections were processed and stained with haematoxylin and eosin and subjected to histopathological analysis. Lesions were subjected for Melan-A immunohistochemistry, melanin bleaching was performed in selected lesions which showed abundant melanophages on histopathological examination by adding 3% $H_2O_2$ on the section for 10 minutes. Using A103 antibody and a high temperature antigen retrieval.

**Melan-A expression was interpretated under following headings:**

a. Intensity was graded as:

- Negative (-),
- weak (+) when <25% of positively stained cells,
- moderate (++) when 26-50% of positively stained cells,
- Severe (+++) when >50% of positively stained cells [11].

b. Pattern was graded as Patchy (P) and Diffuse (D)

c. In each lesion nevus cells and tumour cells were interpretated in locations such as epidermis, DEJ, dermis, periadnexal and perifollicular region.

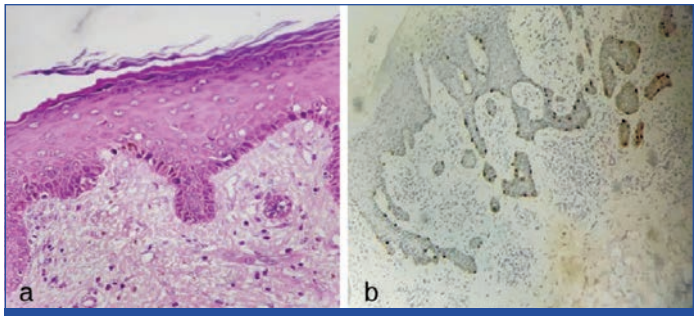
Data was tabulated and evaluated with normal skin (control) patterns of melanocytic expression.

## STATISTICAL ANALYSIS

The statistical software Statistical Package for the Social Sciences (SPSS) version 22.0 was used for analysis of data in Microsoft excel spreadsheet and word used to generate tables, graphs. Proportions were described as percentages. The data was reported as mean±standard deviation. The lesions were described using numbers and percentage. The basic demographic data was summarised using descriptive statistics.

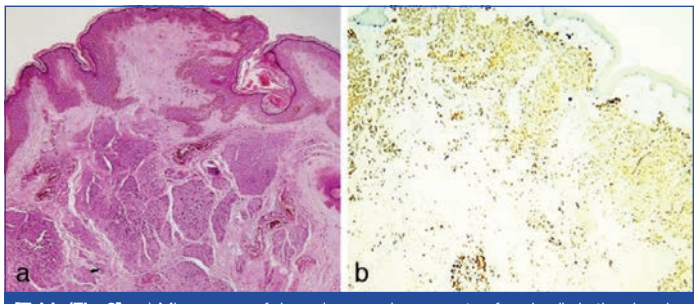
## RESULTS

The expression of Melan-A in normal skin used as control (five biopsies) showed dendritic pattern in normal melanocytes in the epidermis and it is negative in DEJ, dermis and perifollicular and periadnexal region H&E [Table/Fig-1a], IHC [Table/Fig-1b].




**[Table/Fig-1]:** a) Microscopy of skin (control) with epidermis and dermis showing normal melanocytes (H&E,400X); b) Microscopy of skin (control) staining with Melan-A shows dendritic pattern in normal melanocytes in epidermis (IHC, 100X).

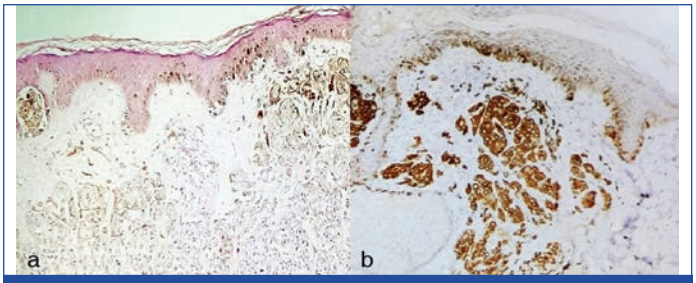
In the present study, there were 18 dermal nevi with nevus cells showing strong, diffuse pattern in the dermis and two cases where moderate and patchy staining was observed H&E [Table/Fig-2a], IHC [Table/Fig-2b]. Five cases of compound nevi showed moderate and diffuse positivity at DEJ and dermis H&E [Table/Fig-3a], IHC [Table/Fig-3b]. One case of deep penetrating nevus shows nests of nevi exhibiting downward streaming into the deeper dermis H&E [Table/Fig-4a], and Melan-A showed strong, diffuse positivity at DEJ, deep dermis with perifollicular and peri adnexal sparing IHC [Table/Fig-4b]. Two cases of dysplastic nevus showed strong, diffuse pattern of nests of nevi cells at DEJ, which extended greater than three rete ridges beyond the lateral margin of dermal component H&E [Table/Fig-5a], IHC [Table/Fig-5b]. One case of Junctional lengitneous nevus with atypia showed moderate intensity with cytoplasmic enhancement of atypical melanocytes in diffuse pagetoid pattern H&E [Table/Fig-6a], IHC [Table/Fig-6b]. One case of spitz nevus shows strong, diffuse pattern at junction and dermal region. MELTUMP showing nests of nevus cells with atypia in the dermis H&E [Table/Fig-7a], and Melan-A showed strong, irregular dermal diffuse positivity IHC [Table/Fig-7b]. All the cases of MM showed atypical melanocytes arranged in confluent nests invading deep dermis and prominent eosinophilic nucleoli H&E [Table/Fig-8a], and Melan-A showed strong, diffuse positivity in dermis IHC [Table/Fig-8b] except one case with additional moderate, patchy positivity in intraepidermis and perifollicular area showed moderate positivity IHC [Table/Fig-8c]. One case of DMM showed negative Melan-A staining H&E [Table/Fig-9a], IHC [Table/Fig-9b]. Two cases of pigmented BCC closely mimicking MM were stained with Melan-A which showed weak positivity for melanocytes with no definitive pattern between basaloid tumour cells H&E [Table/Fig-10a], IHC [Table/Fig-10b]. Negative staining was observed in both fibro histiocytic lesion H&E [Table/Fig-11a], IHC [Table/Fig-11b] and MPNST for Melan-A H&E [Table/Fig-12a], IHC [Table/Fig-12b]. All the data of melanocytic expression of Melan-A are presented in [Table/Fig-13-15].



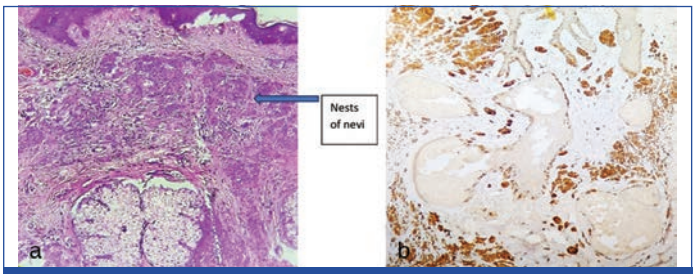

**[Table/Fig-2]:** a) Microscopy of dermal nevus shows nests of nevi cells in the dermis (H&E, 100X); b) Microscopy of dermal nevus staining with IHC Melan-A shows diffuse and strong cytoplasmic positivity for nevi cells in the dermis (IHC,100X).

## DISCUSSION

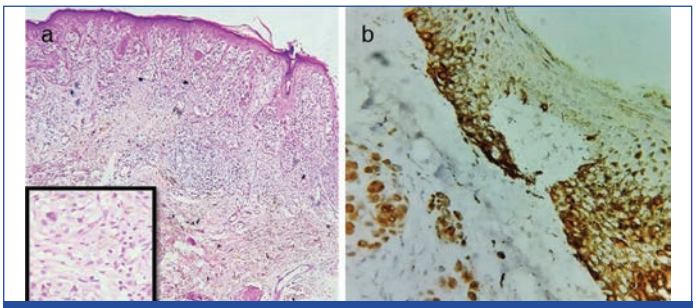
In this study, various spectrum of melanocytic lesions were encountered. Among the 50 lesions (45 cases and 5 control) in this study, benign lesions accounted for 60% of the cases, dysplastic nevi accounted for 4.4%, MELTUMP accounted for 6.6% of all the



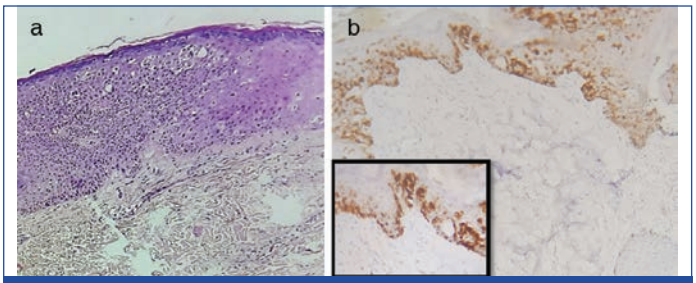

**[Table/Fig-3]:** a) Microscopy of compound nevus shows nests of nevi cells in the junction and dermis (H&E,100X); b) Microscopy of compound nevus staining with IHC Melan-A shows strong cytoplasmic positivity for nevi cells in the epidermis, junction and dermis (IHC,100X).



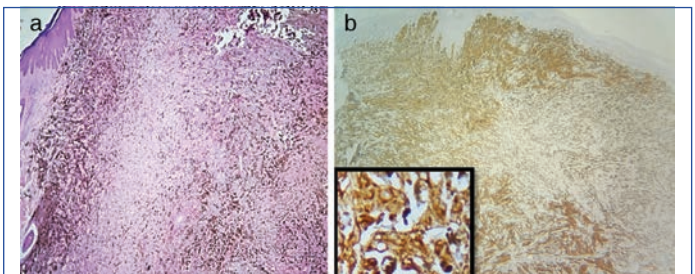

**[Table/Fig-4]:** a) Microscopy of deep penetrating nevus shows nests of nevi exhibiting downward streaming into the deeper dermis.Inset shows nests of nevi. (H&E,100X, inset 400X); b) Microscopy of deep penetrating nevi staining with IHC Melan-A shows strong cytoplasmic positivity for nevi cells in the dermis with perifollicular and peri adnexal sparing (IHC,100X).



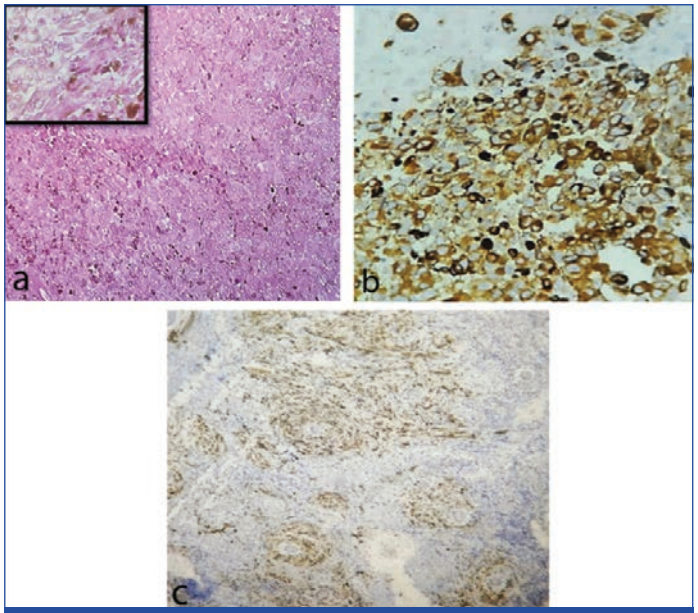

**[Table/Fig-5]:** a) Microscopy of dysplastic nevi shows nests of nevi cells shows bridging of adjacent rete ridges and papillary dermal fibrosis with mild atypia. Inset shows atypical cell (H&E,100X,inset 400X); b) Microscopy of dysplastic nevi staining with IHC Melan-A shows strong cytoplasmic positivity for nevi cells in the junction and dermis (IHC,100X).



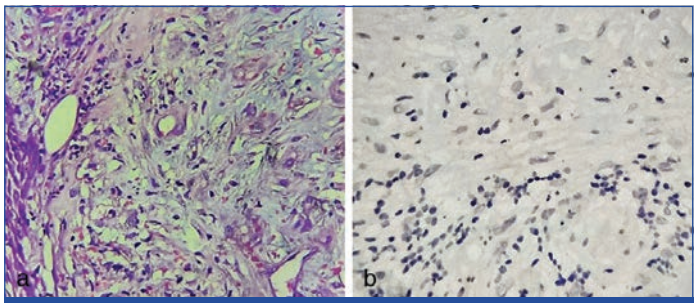

**[Table/Fig-6]:** a) Microscopy of Junctional lengitneous nevi with atypiashows atypical melanocytes in the epidermis in an ill-defined 'pagetoid' pattern (H&E,100X); b) Microscopy of Junctional lengitneous nevi with atypia staining with IHC Melan-A shows diffuse positivity for atypical melanocytes in the pagetoid patternInset shows diffuse pattern (IHC, 100X and insert-400X).



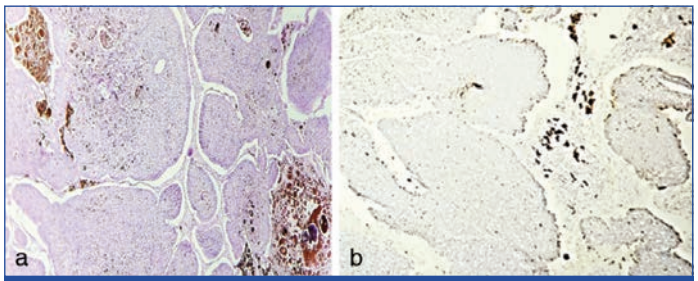

**[Table/Fig-7]:** a) Microscopy of MELTUMP showing nests of nevus cells with atypia in the dermis (H&E,100X); b) Microscopy of MELTUMP staining with IHC Melan-A showing diffuse and strong cytoplasmic positivity for nevi cells in the dermis Inset: shows strong intensity (IHC, 100X and insert-400X).



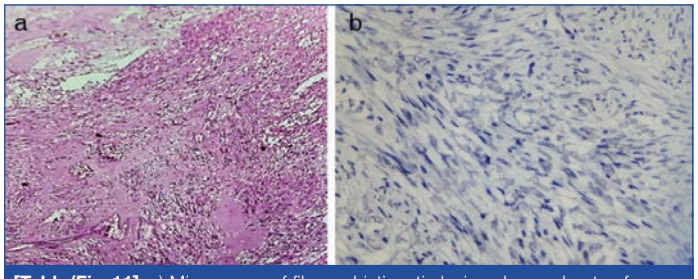

**[Table/Fig-8]:** a) Microscopy of malignant melanoma shows atypical melanocytes arranged in sheets invading deep dermis(H&E, mag 100x). Inset show prominent eosinophilic nucleoli (H&E, 100X and insert-400X); b) Microscopy of malignant melanoma staining with IHC Melan-A showing diffuse and strong cytoplasmic positivity for melanoma cell in the dermis (IHC,400X); c) Microscopy of malignant melanoma staining with IHC Melan-A showing diffuse and strong cytoplasmic positivity for melanoma cell in the dermis invading perifollicular region (IHC,100X).




**[Table/Fig-9]:** a) Microscopy of desmoplastic melanoma shows scattered epithelioid and spindle cells in dermis and myxoid change in stroma (H&E,100X); b) Microscopy of desmoplasticmelanoma negative with IHC Melan-A (IHC, 100X).

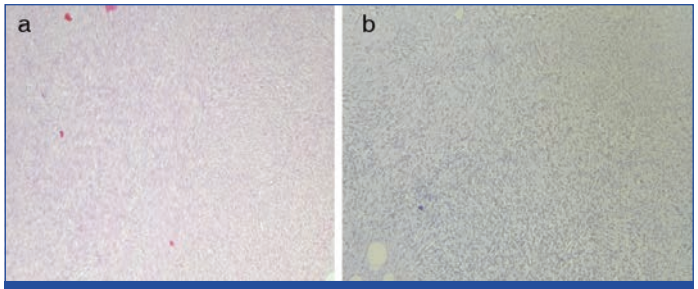



**[Table/Fig-10]:** a) Microscopy of Pigmented BCC shows tumour complex with melanocytes and stroma shows melanophages (H&E,100X); b) Microscopy of Pigmented BCC staining with IHC Melan-A showing weak positivity for melanocytes between tumour nest and negative for stromal melanophages (IHC,100X).




**[Table/Fig-11]:** a) Microscopy of fibrous histiocytic lesion shows sheets of fibro histiocytes with pigment deposit (H&E,100X); b) Microscopy of fibrous histiocytic lesions showing negative staining of melanophages with IHC Melan-A (IHC, 400X).

cases, lentiginous nevi with mild atypia accounted for 2.2% of all the cases, Malignant tumours accounted for 11.1% of the cases and pigmented lesion mimickers accounted for 15.5% of the cases.




**[Table/Fig-12]:** a) Microscopy of MPNST shows sheets of spindle cells with pigment deposit (H&E,100X); b) Microscopy of MPNST showing negative staining of melanophageswith IHC Melan-A (IHC, 100X).

| Lesions | Number of cases (n=38) | Melan-A | | | |
|---|---|---|---|---|---|
| | | Epidermis | Dermoepidermal junction | Dermis | Periadnexal and perifollicular |
| Dermal nevi | 20 | Dendritic pattern in normal melanocytes | Negative | +++, Diffuse (18) and ++, Patchy (2) | - |
| Compound nevi | 5 | Dendritic pattern in normal melanocytes | +++, Diffuse | ++, Diffuse | - |
| Deep penetrating nevus | 1 | Dendritic pattern in normal melanocytes | +++, Diffuse | +++, Diffuse | +++, Diffuse with adnexal sparing |
| Dysplastic nevus | 2 | Dendritic pattern in normal melanocytes | +++, Diffuse | +++, Diffuse | - |
| Junctional lentiginous nevus with atypia | 1 | ++, Diffuse in basal layer of epidermis with pagetoid pattern | ++, Diffuse in rete ridges | - | - |
| Spitz nevus | 1 | Dendritic pattern in normal melanocytes | +++, Diffuse | +++, Diffuse | - |
| Melanocytic tumour of uncertain malignant potential | 3 | Dendritic pattern in normal melanocytes | - | +++, Diffuse | - |
| Malignant melanoma | 4 | ++, Patchy in intraepidermal (1) | - | +++, Diffuse (4) | Perifollicular: ++, Diffuse (1) |
| Desmoplastic melanoma | 1 | Dendritic pattern in normal melanocytes | Negative | Negative | Negative |

**[Table/Fig-13]:** Intensity (+, weak; ++ moderate; +++ strong), and pattern (diffuse and patchy) of Melan -A IHC stain in diverse nevi ǂMELTUMP- melanocytic tumour of uncertain malignant potential.

| Pigment mimickers lesions | Number of cases (n=7) | Melan-A | | | |
|---|---|---|---|---|---|
| | | Epidermis | Dermoepidermal junction | Dermis | Periadnexal and perifollicular |
| Pigmented basal cell carcinoma | 2 | Negative | Negative | +, no definitive pattern | Negative |
| Fibro histiocytic lesions | 4 | Negative | Negative | Negative | Negative |
| Malignant peripheral nerve sheath tumour | 1 | Negative | Negative | Negative | Negative |

**[Table/Fig-14]:** Intensity (+, weak; ++ moderate; +++strong), and pattern (diffuse and patchy) of Melan -A IHC stain in pigment mimickers.

| Lesions | Number of cases (n=45) | Percentage |
|---|---|---|
| Dermal nevi | 20 | 44.40% |
| Compound nevi | 5 | 11.10% |
| Deep penetrating nevus | 1 | 2.20% |
| Dysplastic nevus | 2 | 4.40% |
| Junctional lentiginous nevus with atypia | 1 | 2.20% |
| Spitz nevus | 1 | 2.20% |
| Melanocytic tumour of uncertain malignant potential | 3 | 6.60% |
| Malignant melanoma | 4 | 8.80% |
| Desmoplastic melanoma | 1 | 2.20% |
| Pigmented basal cell carcinoma | 2 | 4.40% |
| Fibrohistiocytic lesions | 4 | 8.80% |
| Malignant peripheral nerve sheath tumour | 1 | 2.20% |

**[Table/Fig-15]:** Spectrum of lesions with percentage.

The lesions were most commonly seen in females with male to female ratio being 1:1.9 and the most common age range was 20-29 years.

The global incidence of non melanomatous skin lesions is 6.2% and MM is 1.6% with mortality for MM is 0.6%.In a study done in northern region of India by Labani S et al., showed incidence of melanoma of 1.62 and 1.21, in male and female, respectively [12].

Melanocytic nevi usually present in childhood and adolescents with equal sex distribution [3].The incidence of AMN ranges from 15% to 40% [2]. The prevalence of atypical melanocytic nevi was 7% in all children and was age dependent (age 4-5years, 1%; 9-10 years, 4%; 14-15 years, 16%) [13].The size and number of AMN and presence of dysplastic nevi are the leading risk factors that should be recognised in the development of MM [4].

Melanocytic nevi are benign neoplasms, although may progress into MMs. Nevi are growth arrested, clonal neoplasms of melanocytes initiated by well-defined oncogenic mutations in the MAPK pathway, most commonly by BRAF activating mutation [1]. BRAF-p is associated with Low-CSD melanomas (low degree of cumulative sun damage) whereas NF1, NRAS, other BRAF predominate in High-CSD melanoma of skin [14].

In young children and adolescents in whom lesions are at an early stage of development, increased pigmentation is to be anticipated. However, in adults, evidence of junctional activity is to be viewed with caution; it is those nevi with increasingly marked pigmentation, or appearing de novo in the older age groups, which are often excised for histologic evaluation. Intermittent intense sunlight is of greater importance than chronic exposure [14].

Melan-A/MART-1 is a melanocytic differentiation marker, which is recognised as an antigen on melanoma cells by cytotoxic T-lymphocytes. It is of interest for clinicians as potential immunotherapeutic target and it is relevant for pathologists as a novel diagnostic marker [15]. Melan-A is useful in the differential diagnosis of melanocytic tumours, especially metastatic tumours and it also helps to distinguish between melanocytes and melanophages (melanin containing macrophages) [16].

Studies by Busam KJ et al., also tested Melan-A expression in benign melanocytic nevi and primary cutaneous melanoma. MART-1 reactivity indicates an increased immunogenicity and tumour containment by immune system [17].

A study by Orosz Z, demonstrate most of dermal nevi show strong and diffuse pattern and the staining intensity was similar in junctional and dermal components in case of spitz, compound, atypical junctional nevi [9].These findings were similar to the present study.

Deep penetrating nevus was first described in 1989 by Seab et al., Clinically this lesion is seen as a solitary darkly pigmented lesion most commonly on the extremities. It frequently affects young female patients [18]. The DPN can be challenging to differentiate from cellular blue nevi, Spitz nevi, and MM. Histologically it is

characterised as a well demarcated, wedge-shaped lesions reaching down into the reticular dermis/subcutis exhibiting epithelioid/spindle-cell melanocytic nests with low-grade cytological atypia and possible mitotic figures.

Spread to regional lymph nodes and metastasis with atypical features are classified as "borderline" (B-DPN) which pose a diagnostic challenge, even to experts of the dermatopathology. DPN has a good prognosis demonstrating benign behaviour [19]. In a study conducted by van Ipenburg JA et al., showed diffuse expression of Melan-A with perifollicular extension [20] which is similar to the present study which showed nevus cells exhibiting downward streaming into the deeper dermis showing normal maturation. Melan-A Immunohistochemistry showed strong and diffuse positivity in DEJ, dermis, with perifollicular and peri adnexal sparing.

Dysplastic nevi showed that Melan-A had a strong intensity and diffuse staining pattern which were similar with Patrascu OM et al., study. The study showed Melan-A positivity in 80% of cases. Difference in pattern of staining was not observed with different grades of dysplasia [10].

Agusti-mejias A et al., did study of atypical lentiginous nevus shows predominance of proliferating melanocytes in the basal layer of the epidermis with focal pagetoid invasion [21] asserting the finding in the current study where Melan-A showed moderate positivity with pagetoid spread.

Elder D and Xu X, showed that Melan-A is positive in MELTUMP and strong intensity and diffuse pattern positivity which correlates to findings in the present study [22].

**Major criteria that distinguish melanoma from common acquired nevi include:** Size, symmetry, circumscription, ulceration, cellularity, pagetoid scatter, continuous basal proliferation, cytological atypia, mitotic activity, failure of dermal cell maturation and lympho vascular and perineural invasion [14].

Primary MM with Melan-A showed diffuse pattern in dermis in four cases and one of the cases showed moderate, patchy pattern in intraepidermal region. A case of melanoma showed a rare combination of MM and Squamous Cell Carcinoma (SCC) which have unknown biological potential, on histopathology the tumour was pigmented with atypical keratinisation and showed two morphologically distinct population of cells. The first population showed large, eosinophilic cells with signs of keratinisation. The second population was arranged in cords and nests, composed of small, pigmented, spindle and epithelioid cells.Immunohistochemical stains such as Cytokeratin 5/6 showed positivity for the pleomorphic keratinocytes, whereas Melan-A stained the atypical melanocytes [23].

When pathologist makes a diagnosis of primary MM, a series of differential diagnosis should be taken under consideration [9].

a. Benign melanocytic lesions like spitz, dysplastic nevi etc.,

b. Pigmented Soft tissue lesions

Pathologists come across a pigmented lesion should first characterise it as a melanocytic, keratinocyte, reactive process or mesenchymal. Melanocytic proliferations pose a task to the pathologist for several reasons.

1. Low-power evaluation, pigmented keratinocytes can easily be mistaken for melanocytes, so higher-power evaluation is necessary for correct morphological distinction.
2. Melanocytic proliferations are heterogenous, consideration of architectural and cytological features is needed to differentiate between diagnoses, which may be managed differently.
3. On H&E staining, interpretation can be mistaken by pigmentation secondary to either abundant melanophages or melanin/haemosiderin pigment incontinence. For such lesions bleaching techniques may be needed to clarify the cellular morphology.
4. Immunohistochemical studies may be useful adjuncts to confirm melanocytic differentiation and evaluate maturation pattern and degree of cellular proliferation [18].

Desmoplastic melanoma is a rare melanoma variant. Histologically it is made up of spindle cell component and epithelioid component. The spindle component closely mimickers sclerotic/desmoplastic nevi, non pigmented blue nevi, scar, and neural tumours. The diagnosis of DMM can be challenging due to its deceptively bland cytology. However, Melan-A along with other IHC panel can aid in diagnosis. In contrast majority of DMMs are negative for Melan-A in their spindle cell compartment [24]. The current study showed negative Melan-A expression in spindled melanoma cells in DMM. In Amelanotic melanoma Melan-A, shows granular localisation in the cytoplasm [25].

Basal cell carcinoma histologically consists of nest of basaloid cells and intervening cells such as melanocytes and Langerhans cells. These melanocytes can deposit abundant pigment. They can co-exist with another tumour including MM. IHC staining with Melan-A shall rule out existence of collision tumour (pigmented BCC and MM). Brankov N et al., demonstrate that no specific pattern of melanocyte cytomorphology, configuration, or distribution was observed histologically in pigmented BCC [26], while the present study shows weak intensity for Melan-A in intervening melanocytes and was negative in tumour nests.

Study done by Luzar B and Calonje E shows that Immunohisto chemistry is thus useful in Epithelioid Benign Fibrous Histiocytoma (EBFH) to exclude its potential histological mimics. In contrast to Spitz naevus, no nesting is present in EBFH, and the cells are consistently negative for Melan-A melanocytic markers. Spitz nevus shows that Melan-A is positive and having strong intensity and diffuse pattern [27]. In a study conducted by Gaspard Met al., showed Melan-A positivity in 14% cases of MPNST, whereas one case of MPNST in our study which showed negative reaction to Melan-A. MPNST caused due to sporadic causes or NF1 mutation can show positivity for Melan-A. MPNST can closely mimic spindle cell/DMM and lead to hindrance in diagnosis [28].

MPNST can morphologically resemble type C nevi cells and spindle cell melanoma. Some tumour cells can show pigmented deposition in intradermal location. Hence, are diagnosed as on histopathology as MM-nodular variant or spindle cell melanoma on morphology. IHC MelanA show negative staining helps to rule out melanocytic origin. Another study conducted by Tanas M and Rubin B Rubin showed Melan-A negative in MPNST in accordance to the present study [29]. These data show the possible useful role of Melan-A in differentiating tumours that are able to mimic MM and pigmented lesions. Present results confirm and extend the findings of other researchers, that Melan-A may be a potential useful marker in correct diagnosis of melanocytic lesions and helps in differentiating from close mimicker lesions.

### Limitation(s)

The limitation of this study is restricted sample size and hence significance of Melan-A staining pattern in differentiating non neoplastic and neoplastic nevi could be improvised by considering increased sample size.

## CONCLUSION(S)

Present study shows Melan-A is a useful marker in diagnosis of melanocytic lesions as it stains cells of melanocytic lineage and no other cell types in background. It confirms the origin of the lesion and helps to differentiate these lesions from its close mimickers aiding accurate diagnosis of melanocytic nevi.

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