DOI: 10.7860/JCDR/2025/78168.22100

Original Article

Pathology Section

# Clinicopathological Features of Malignant Melanoma with Emphasis on S-100, HMB-45, and BRAF Expression: A Cross-sectional Study

SAYANTAN DE[1], MAMATA GUHA MALLICK SINHA[2]




## ABSTRACT

**Introduction:** Malignant melanoma is one of the most lethal forms of skin cancer. While it predominantly originates from the skin, it can also develop in extracutaneous sites, including ocular, mucosal, and leptomeningeal regions. S-100 and HMB-45 immunohistochemical markers are widely used for melanoma detection. Additionally, the V600E missense mutation, which changes valine to glutamic acid, is a common BRAF alteration in melanoma and serves as a target for therapeutic interventions.

**Aim:** To evaluate the clinico-epidemiological and histopathological features of malignant melanoma, with emphasis on the immunohistochemical expression of S-100, HMB-45, and BRAF.

**Materials and Methods:** The present prospective cross-sectional study was conducted in the Department of Pathology, IPGME&R, Kolkata, West Bengal, India, from January 2020 to June 2023. A total of 26 specimens diagnosed as malignant melanoma were analysed. Only excision specimens were included in this study. Histopathological parameters, including type, pigmentation, Breslow thickness, lymph node status were recorded. Subsequent immunohistochemical testing of paraffin-embedded blocks was performed using S-100, HMB45, and BRAF markers. Collected detailed epidemiological and histopathological and immunohistochemical data were entered into Microsoft excel sheet. From that tabulated descriptive measures obtained including percentages and frequencies. Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) Statistics software (version 25). A Chi-square test of independence was used to assess the association between melanoma type, pigmentation, subtype, lymph node involvement and BRAF expression levels. A p-value of less than 0.05 was considered statistically significant.

**Results:** Among the 26 cases, nineteen were of cutaneous origin, six were mucosal, and one was congenital. All cutaneous melanomas were located on the lower extremities. Superficial Spreading Melanoma (SSM) was the most common type (13/19 cases), followed by Nodular Melanoma (NM) (5/19 cases). Lymph node involvement was observed in six cases, all from the cutaneous group, with inguinal lymph nodes being the primary site. All cases showed diffuse S-100 and HMB-45 positivity. BRAF positivity was detected in 26.92% of cases (7/26 cases).

**Conclusion:** A thorough assessment of histopathological parameters, supplemented by immunohistochemical analysis of markers such as S-100 and HMB-45, is essential for precise diagnosis, prognostication, and the formulation of effective therapeutic strategies. Although BRAF immunoexpression was identified in approximately one-fourth of the cases, its detection holds significant therapeutic relevance by facilitating the implementation of targeted molecular therapies, thereby potentially improving overall patient survival.


**Keywords:** Amelanotic melanoma, Immunohistochemistry, Mucosal melanoma

## INTRODUCTION

Melanoma is a malignant tumour that arises from melanocytes, the pigment-producing cells located in the epidermis. While melanoma is predominantly of cutaneous origin, it can also occur in various extracutaneous sites, such as ocular melanomas, mucosal melanomas, and leptomeningeal melanomas [1].

Malignant melanoma is known for its aggressive behaviour. However, it remains highly curable if detected early, with an overall five-year survival rate of approximately 90% [2]. Recent advances in targeted therapies, immunotherapies, and radiation treatments have significantly improved survival, extending it to several years in some cases [3]. Mucosal melanomas, though rare, tend to behave more aggressively and have a poorer prognosis compared to cutaneous melanomas [4].

The S-100 family of calcium-binding proteins, known for their solubility in 100% ammonium sulfate, plays a crucial role in the aetiology, progression, manifestation, and treatment of neoplastic disorders, including malignant melanoma [5]. Another important marker, HMB-45 is widely used in immunohistochemistry for the detection of both primary and metastatic melanoma [6].

The BRAF protein (B-raf murine sarcoma viral oncogene homolog B1) is a serine-threonine protein kinase consisting of 766 amino acids and three domains: two regulatory domains and one catalytic domain responsible for MEK phosphorylation [7]. Aberrant activation of the MAPK pathway by melanoma leads to increased cell proliferation, invasion, metastasis, migration, survival, and angiogenesis [8]. The most common BRAF mutation in melanoma is the V600E missense mutation, which replaces valine with glutamic acid. This mutation is present in approximately 50% of all metastatic melanomas [9].

This study provides a detailed examination of the various subtypes of malignant melanoma, emphasising their distinct histopathological characteristics. Immunohistochemical analysis was employed by utilising markers such as S-100 and HMB-45 for diagnostic confirmation, along with BRAF immunohistochemistry expression in various types of melanomas and its association with different histological findings such as pigmentation, lymph node involvement and tumour subtypes.

## MATERIALS AND METHODS

The present prospective cross-sectional study was conducted from January 2020 to June 2023 in collaboration with the

Departments of Surgery and Plastic Surgery at IPGME&R, Kolkata. (IEC: IPGMER/IEC/2020/357). A total of 26 patients undergoing surgery for suspected melanoma were included. Data on age, sex, clinical presentation, radiological and dermatological findings, and histopathological features were documented.

**Inclusion criteria:** All cases that Were diagnosed and confirmed by histological and immunohistochemical examination. Only excision specimen was included.

**Exclusion criteria:** Cases where detailed clinicoepidemiological data were not available. Incision and tru-cut biopsies were also excluded. Review cases were also not included.

### Study Procedure

**Histopathological analysis:** Specimens were grossly examined, and representative sections were stained with haematoxylin and eosin. Histopathological features, including tumour type, melanin pigmentation, Clark level, Breslow thickness, lymphovascular invasion, Tumour-Infiltrating Lymphocytes (TIL) , mitotic activity, ulceration, lymph node status, and TNM stage, were assessed.

**Immunohistochemistry:** Paraffin-embedded blocks were subjected to immunohistochemical analysis using S-100 (EP32-Rabbit monoclonal antibody), HMB-45 (HMB-45-Mouse monoclonal antibody), and BRAF (VE1-Mouse monoclonal antibody) markers.

Slides were coated for 4-5 times with concentrated poly-L-lysine and air dried for 20 minutes at room temperature and then three-micron sections on slides were incubated for 30 minutes at 60° Celsius. Then the slides were taken out and air dried for 20 minutes. Deparaffinisation, rehydration, antigen retrieval, Tris buffer wash and Peroxidase blocking were done. Then slides were covered with primary antibody for 60 minutes. Again, after tris buffer wash, sides were covered with Horseradish Peroxide (HRP) labelled secondary antibody for 30 minutes. DAB chromogen is added to the sections and kept covered to avoid light for 10 minutes. The slides are counterstained with H&E for 15-30 seconds. The slides were rehydrated in graded alcohol in reverse order. Air drying is done for 30 minutes and mounted with coverslips using Distyrene, Plasticizer, and Xylene (DPX).

**S-100 immunostaining:** S-100 staining was observed as brown cytoplasmic as well as nuclear stain also. Schwannoma was taken as positive control. Diffuse nuclear and cytoplasmic staining was scored positive and others like only nuclear or cytoplasmic scored negative [10].

**HMB-45 immunostaining:** HMB-45 staining showing diffuse brown cytoplasmic positivity was scored as positive and other staining pattern scored as negative [10]. It is found in varying proportions of benign melanocytic tumours like junction naevus and compound naevus. Appendix was used as negative tissue controls.

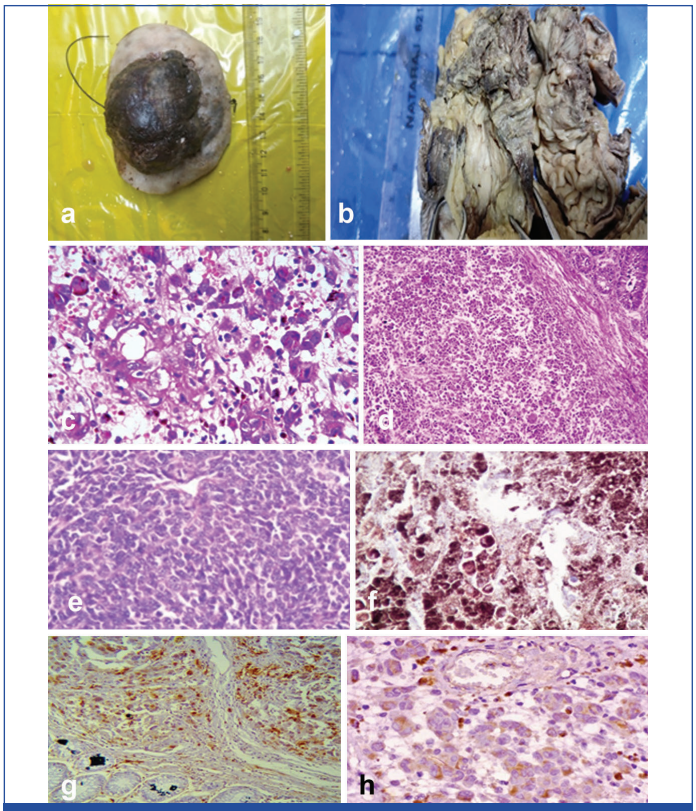
**BRAF immunostaining:** The intensity of staining of BRAF expression in tumour cells was recorded on a 0-3 scale. Strong cytoplasmic staining was scored as 3, medium cytoplasmic staining as 2, weak cytoplasmic staining as 1 and the absence of staining was scored as 0. Score with 2+(Grade 2) and 3+(Grade 3) were only taken as positive [11]. Papillary carcinoma of thyroid was used as positive control.

## STATISTICAL ANALYSIS

Collected detailed epidemiological and histopathological and immunohistochemical data were entered into Microsoft excel sheet. From that tabulated descriptive measures obtained including percentages and frequencies. Statistical analysis was performed using IBM SPSS Statistics software (version 25). A Chi-square test of independence was used to assess the association between melanoma type, pigmentation, subtype, lymph node involvement and BRAF expression levels. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

Gross picture of NM depicted in [Table/Fig-1a] showing blackish nodular mass in a wide local excision specimen. In [Table/Fig-1b] depicted a case of mucosal melanoma in low anterior resection specimen where growth identified in anorectal region. The tumour cells were large, mostly polygonal or round having epithelioid morphology with abundant eosinophilic cytoplasm and vesicular nuclei and prominent nucleoli with granular pigmentation of cytoplasm in melanotic type [Table/Fig-1c]. Histological image of amelanotic melanoma presented in [Table/Fig-1d,e]. Cells can have spindle morphology also [Table/Fig-1e]. S-100 shows strong cytoplasmic and nuclear positivity [Table/Fig-1f] and HMB-45 shows cytoplasmic positivity in a case of mucosal melanoma [Table/Fig-1g]. BRAF shows moderate granular cytoplasmic staining (grade 2) [Table/Fig-1h].




**[Table/Fig-1]:** a) Gross picture of cutaneous melanoma (nodular type); b) Gross picture of mucosal melanoma in colectomy specimen; c) HE (400X) show large epithelioid cells with prominent nucleoli and pigmented cytoplasm; d) HE(200X) Mucosal melanoma (amelanotic type); e) HE(400X)-Amelanotic melanoma-spindle cell type; f) 400X-S-100 shows strong cytoplasmic and nuclear positivity; g) 200X-HMB 45 shows cytoplasmic positivity in mucosal melanoma; h) 400X-BRAF shows moderate granular cytoplasmic staining (grade 2).

The patient population demonstrated a wide age range, with the majority of individuals (14 out of 26 cases, accounting for 53.85%) falling within the 41 to 60-year age group. This was followed by eight patients (30.77%) who were between the ages of 61 and 80 [Table/Fig-2]. In terms of gender distribution, there was a slight predominance of female patients, with 14 females comprising 53.85% of the total study population, while the remaining 12 patients (46.15%) were male [Table/Fig-2].

Types and anatomical sites of melanoma: Among the various subtypes of melanoma identified in this study, cutaneous melanoma was the most prevalent, accounting for 19 out of 26 cases (73.08%). Notably, all cutaneous melanomas were localised to the lower extremities, specifically involving the foot, heel, and sole regions. SSM was most common type (13/19 cases, 68.42%) followed by NM (5/19 cases,26.32%) [Table/Fig-2]. Mucosal melanoma represented the second most common type, observed in six cases (23.08%), and was primarily localised to the anorectal region [Table/Fig-2] and A single case (3.85%) of congenital melanoma was identified, which involved the facial area [Table/Fig-2].

| Age group (years) | Male | Female | Cutaneous | | | Mucosal (%) | Congenital (%) | Total (%) |
|---|---|---|---|---|---|---|---|---|
| | | | Superficial spreading (%) | Nodular (%) | Acral lentiginous (%) | | | |
| 0-20 | | 1 | | | | | 1 (3.85) | 1 (3.85) |
| 21-40 | | | | | | | | |
| 41-60 | 4 | 10 | 8 (30.77) | 1 (3.85) | 1 (3.85) | 4 (15.38) | | 14 (53.85) |
| 61-80 | 5 | 3 | 4 (15.39) | 2 (7.69) | | 2 (7.69) | | 8 (30.77) |
| >80 | 3 | | 1 (3.85) | 2 (7.69) | | | | 3 (11.54) |
| Total | 12 | 14 | 13 | 5 | 1 | 6 | 1 | 26 |
| | | | 19 | | | | | |

**[Table/Fig-2]:** Distribution of melanoma cases by age group, gender, type, and histological subtype.

**Histopathological findings:** Pigmentation was common histological characteristic, present in 88.46% of all melanoma cases-23 cases out of which 19 were cutaneous and four were mucosal [Table/Fig-3]. Rest four were amelanotic type. Ulceration was a frequent finding in cutaneous melanomas, observed in 89.47% of the cases [Table/Fig-3]. Analysis of tumour thickness revealed that 68.42% of cutaneous melanomas exhibited a Breslow thickness greater than 4 millimeters, which is indicative of more advanced-stage tumours [Table/Fig-3]. Regarding tumour depth, Clark level ≥IV was assigned in 89.47% of cutaneous melanoma cases, signifying invasion into the reticular dermis and subcutaneous tissue [Table/Fig-3]. Lymphovascular invasion was identified in 15.39% of all melanoma cases [Table/Fig-3]. The presence of TILs, which may suggest an immune response against the tumour, was documented in 78.95% of the cutaneous melanomas [Table/Fig-3]. Furthermore, all cases of cutaneous melanoma demonstrated mitotic activity greater than one mitosis per square millimeter [Table/Fig-3]. Lymph node involvement was confirmed in six cases, all of which were cutaneous melanomas with metastasis to the inguinal lymph nodes [Table/Fig-3].

| Parameters | Present (%) | | Absent (%) | |
|---|---|---|---|---|
| Melanin pigment (n=26) | 23 (88.46) (19 cutaneous+4 mucosal) | | 3 (11.54) (2 mucosal+1congenital) | |
| Ulceration in cutaneous melanoma (n=19) | 17 (89.47) | | 2 (10.53) | |
| Lymphovascular invasion (n=26) | 4 (15.39) | | 22 (84.61) | |
| Tumour infiltrating lymphocytes in cutaneous melanoma (n=19) | 15 (78.95) | | 4 (21.05) | |
| Breslow thickness >4mm in cutaneous melanoma (n=19) | 13 (68.42) | | 6 (31.58) | |
| Clark level ≥IV in cutaneous melanoma (n=19) | 17 (89.47) | | 2 (10.53) | |
| Mitotic activity >1 per mm2in cutaneous melanoma (n=19) | 19 (100) | | 0 (0) | |
| Lymph node involvement in melanoma(n=14) | 6 (42.86) | | 8 (57.14) | |
| S-100 positivity in melanoma (n=26) | 26 (100) | | 0 (0) | |
| HMB-45 positivity in melanoma (n-26) | 26 (100) | | 0 (0) | |
| BRAF positivity in melanoma (n=26) | 2+score-2 (7.69) | 7 (26.92) | 0 score-2 (7.69) | 19 (73.08) |
| | 3+score-5 (19.23) | | 1+score-17 (65.39) | |

**[Table/Fig-3]:** Distribution of different parameters along with S-100, HMB-45 and BRAF positivity in melanoma.

**Immunohistochemical findings:** Immunohistochemical staining revealed universal positivity for both S-100 and HMB-45 markers across all cases examined, affirming the melanocytic origin of the tumours [Table/Fig-3]. In terms of BRAF expression, which is relevant for prognostic and therapeutic considerations, two cases (7.69%) demonstrated strong (Grade 3) BRAF positivity. Moderate expression (Grade 2) was noted in five cases (19.23%) [Table/Fig-3], while the remaining 19 cases (73.08%) showed either low (Grade 1) or absent (Grade 0) BRAF expression [Table/Fig-3]. We studied BRAF immunohistochemical expression association with types of melanomas, pigmentation, subtype of cutaneous melanoma and lymph node involvement but none of the parameters were statistically significant (p-value >0.05) [Table/Fig-4].

| BRAF | | Positive (Percentage) | Negative (Percentage) | $\chi^2$ | p-value |
|---|---|---|---|---|---|
| Type | Cutaneous | 6 (31.58) | 13 (68.42) | 0.899 | 0.638 |
| | Mucosal | 1 (16.67) | 5 (83.33) | | |
| | Congenital | 0 (0) | 1 (100) | | |
| Pigment | Melanotic | 7 (30.43) | 16 (69.57) | 1.249 | 0.264 |
| | Amelanotic | 0(0) | 3 (100) | | |
| Subtype of cutaneous melanoma | Superficial spreading | 5 (38.46) | 8 (61.54) | 1.057 | 0.590 |
| | Nodular | 1 (20) | 4 (80) | | |
| | Acral lentiginous | 0 (0) | 1 (100) | | |
| Lymph node | Involved | 3 (50) | 3 (50) | 0.933 | 0.334 |
| | Not involved | 2 (25) | 6 (75) | | |

**[Table/Fig-4]:** Association of BRAF immunohistochemical expression with types of melanomas, pigmentation subtype of cutaneous melanoma and lymph node involvement.

## DISCUSSION

Melanoma is a malignancy originating from the uncontrolled proliferation of melanocytes, which arise from pluripotent neural crest stem cells. It can develop on cutaneous and mucosal surfaces, the uveal tract, and leptomeninges, and is the most lethal form of skin cancer [1,12]. A study in Spain by Nagore E et al., enrolled 1,571 cases over 18 years [13], while Sharma K et al., and Panda S et al., reported 72 and 182 cases from India over 12 and 6 years, respectively [12,14]. The present study included 26 cases of malignant melanoma over 2.5 years. The variability in case numbers among studies may reflect differences in incidence rates, particularly higher rates in fair-skinned populations [3].

Patient ages in melanoma studies ranged from 23 to 86 years, with a mean age of 57.6 years [15]. Men are more prone to melanoma, with higher prevalence in whites compared to blacks [1,16]. The main risk factors include unprotected UV exposure, indoor tanning, immunosuppression, family history, moles, and obesity [16]. In the present study, 12 (46.15%) patients were male, and 14 (53.85%) were female. Fourteen cases (53.85%) occurred in the 41-60 age group and eight cases (30.77%) were in the 61-80 age group.

Cutaneous melanomas accounted for 82% and 78.57% of cases in studies by Chang JW and Mukhopadhyay S et al., respectively [17,18]. Western studies reported melanoma more frequently on the backs and shoulders of men and the lower limbs of women.

However, in the Indian subcontinent, the most common site for cutaneous melanoma is the lower extremities, irrespective of sex [1,15,17,19]. In the present series, 19 out of 26 cases (73.08%) were cutaneous, all involving the lower extremities, predominantly around the heel, toes, and dorsum of the foot.

Mucosal melanoma most frequently occurs in the anorectal canal, followed by the stomach, small intestine, and colon. This rare malignancy is aggressive, has a poor prognosis, and is predominantly noted in females over 50 years. BRAF mutations are absent in most cases [20]. In our study, six cases of mucosal melanoma were of the anorectal variety, with a female preponderance. Two cases lacked pigmentation, and five of six showed no significant BRAF activity. Paediatric malignant melanoma is rare, comprising 1%-4% of all melanoma cases [21]. Surgical excision is the primary treatment. Genetic pathways differ between adult and childhood melanomas, with BRAFV600E mutations occurring in only 5-15% of congenital cases [22]. We described a case of malignant melanoma in a one-year-old girl with a giant congenital melanocytic nevus on her face and nape, along with nodules on the trunk and extremities. Histology showed undifferentiated "blastic" morphology with no melanin or BRAF activity. Immunohistochemistry confirmed the diagnosis with S-100 and HMB-45 positivity.

Amelanotic melanoma constitutes about 8% of all melanomas, predominantly affecting females under 50 years. Prognosis is worse compared to melanotic melanoma [23]. We reported three cases of amelanotic melanoma: one congenital and two mucosal, all confirmed by S-100 and HMB-45 positivity, with no significant BRAF activity.

Prognosis, recurrence, and treatment are now more accurately determined using the American Joint Committee on Cancer (AJCC) staging, tumour thickness, ulceration, mitotic index, and lymph node status [24]. Tumour thickness, measured by Clark Level and Breslow thickness, is a critical prognostic factor. Melanomas with a Breslow index >4 mm show a ten-year survival rate of only 39% [25]. NMs are more invasive and ulcerated than SSMs [23]. In Western populations, SSM is most frequent, often linked to sun exposure. However, NM predominates in Nepal (82.8%) [26]. In the present study, 13 of 19 cutaneous melanomas were SSM (68.42%), followed by NM (26.32%). Most cases were Clark Level IV, with Breslow thickness >4 mm in 68.42% and ulceration in 89.47% of cases.

Sentinel lymph node (SN) biopsy is a powerful prognostic tool for primary cutaneous melanoma-lymph node metastasis increases recurrence and mortality risk [27]. Regional lymph node metastasis is common in lower extremity melanomas [12]. In our series, 14 cases had lymph nodes examined, and six showed metastatic deposits, all originating from cutaneous melanomas involving the inguinal nodes.

Melanoma's histological variability necessitates immunohistochemistry for accurate diagnosis. S-100 and HMB-45 markers are reliable, with S-100 exhibiting 97-100% sensitivity and HMB-45 specifically identifying melanoma [28,29]. In the present study, all 26 cases were positive for these markers. BRAF mutations, particularly the V600E variant, are common and influence targeted therapies [30]. BRAF mutations are less frequent in Asian populations compared to Western populations [31,32]. Immunohistochemistry serves as a rapid screening tool for BRAF status, with 89.2% sensitivity and 96% specificity [11]. In The current study, BRAF positivity was observed in 26.92% of cases, predominantly in cutaneous melanomas (31.58% vs. 16.67% in mucosal cases). Despite a lack of statistically significant association (p-value >0.05), 38.46% of SSMs and 50% of cases with lymph node metastasis showed BRAF positivity.

### Limitation(s)

Since the present study was single institution-based and the number of cases were small, it is not possible to give a generalised result to comment on the whole population. The present study may be reviewed as a component of a large multicentric study to reach a definite conclusion. As the study period was short, survival analysis could not be done as follow up was not possible in the short time limit. The authors could not evaluate any marker of the present study by molecular technique because of financial constraints.

## CONCLUSION(S)

This study highlights the clinicopathological diversity of malignant melanoma. S-100 and HMB-45 immunohistochemical markers are indispensable for diagnosis. While BRAF positivity was observed in a subset of cases, its association with clinical parameters was statistically insignificant. These findings underscore the importance of comprehensive diagnostic and therapeutic approaches tailored to individual patient profiles.

## REFERENCES


[1] Matthews NH, Li WQ, Qureshi AA, Weinstock MA, Cho E. Epidemiology of Melanoma. In: Ward WH, Farma JM, editors. Cutaneous Melanoma: Etiology and Therapy [Internet]. Brisbane (AU): Codon Publications; 2017 Dec 21. Chapter 1.
[2] Ross MI, Gershenwald JE. Evidence-based treatment of early-stage melanoma. J Surg Oncol. 2011;104:341-53.
[3] Domingues B, Lopes JM, Soares P, Pópulo H. Melanoma treatment in review. Immunotargets Ther. 2018;7:35-49.
[4] Wu E, Golitz LE. Primary noncutaneous melanoma. Clin Lab Med. 2000;20:731-44.
[5] Sedaghat F, Notopoulos A. S-100 protein family and its application in clinical practice. Hippokratia. 2008;12(4):198-204.
[6] Baisden BL, Askin FB, Lange JR, Westra WH. HMB-45 immunohistochemical staining of sentinel lymph nodes. A specific method for enhancing detection of micrometastases in patients with melanoma. Am J Surg Pathol. 2000;24:1140-46.
[7] Charpin C, Martin M, Balandraud N, Roudier J, Auger I. Autoantibodies to BRAF, a new family of autoantibodies associated with rheumatoid arthritis. Arthritis Res Ther. 2010;12(5):R194.
[8] Inamdar GS, Madhunapantula SV, Robertson GP. Targeting the MAPK pathway in melanoma: Why some approaches succeed and other fail. Biochem Pharmacol. 2010;80(5):624-37.
[9] Liu Y, Sheikh MS. Melanoma: Molecular pathogenesis and therapeutic management. Mol Cell Pharmacol. 2014;6(3):228.
[10] Ricci C, Dika E, Ambrosi F, Lambertini M, Veronesi G, Barbara C. Cutaneous melanomas: A single center experience on the usage of immunohistochemistry applied for the diagnosis. Int J Mol Sci. 2022;23(11):5911.
[11] Schirosi L, Strippoli S, Gaudio F, Graziano G, Popescu O, Guida M, et al. Is immunohistochemistry of BRAF V600E useful as a screening tool and during progression disease of melanoma patients? BMC Cancer. 2016;16(1):905. Published 2016 Nov 18. doi:10.1186/s12885-016-2951-4.
[12] Sharma K, Mohanti BK, Rath GK. Malignant melanoma: A retrospective series from a regional cancer center in India. J Cancer Res Ther. 2009;5:173-80.
[13] Nagore E, Oliver V, Botella-Estrada R, Moreno-Picot S, Guillen C, Fortea JM. Clinicopathological analysis of 1571 cutaneous malignant melanomas in valencia, spain: Factors related to tumour thickness. Acta Derm Venereol. 2006;86:50-56.
[14] Panda S, Dash S, Besra K, Samantaray S, Pathy PC, Rout N. Clinicopathological study of malignant melanoma in a regional cancer center. Indian J Cancer. 2018;55(3):292-96.
[15] Hui SK, Lau KH, Leung CY, Tang WYM, Chong LY, Lo KK. Cutaneous melanoma: Clinical features of 32 Hong Kong Chinese patients. Hong Kong J Dermatol Venereol. 2005;13:130-40.
[16] Saginala K, Barsouk A, Aluru JS, Rawla P, Barsouk A. Epidemiology of Melanoma. Med Sci (Basel). 2021;9(4):63.
[17] Mukhopadhyay S, Ghosh S, Siddhartha D, Mitra PK. A clinicopathological study of malignant melanoma with special reference to atypical presentation. Indian J PatholMicrobiol. 2008;51:485-88.
[18] Chang JW. Cutaneous melanoma: Taiwan experience and literature review. Chang Gung Med J. 2010;33:602-12.
[19] Radhika K, Prayaga AK, Sundaram C. A clinicopathologic study of malignant melanoma based on cytomorphology. Indian J Cancer. 2016;53(1):199-203.
[20] Paolino G, Didona D, Macrì G, Calvieri S, Mercuri SR. Anorectal Melanoma. In: Scott JF, Gerstenblith MR, editors. Noncutaneous Melanoma [Internet]. Brisbane (AU): Codon Publications; 2018 Mar. Chapter 6.
[21] Tariq S, Shallwani H, Waqas M, Bari ME. Congenital and infantile malignant melanoma of the scalp: A systematic review. Ann Med Surg (Lond). 2017;21:93-95.
[22] Yang C, Gru AA, Dehner LP. Common and not so common melanocytic lesions in children and adolescents. Pediatr Dev Pathol. 2018;21:252-70.
[23] Thomas NE, Kricker A, Waxweiler WT, et al. Comparison of clinicopathologic features and survival of histopathologically amelanotic and pigmented melanomas: A population-based study. JAMA Dermatol. 2014;150(12):1306-14.
[24] Greenwald HS, Friedman EB, Osman I. Superficial spreading and nodular melanoma are distinct biological entities: A challenge to the linear progression model. Melanoma Res. 2012;22(1):01-08.

[25] Gershenwald JE, Scolyer RA. Melanoma Staging: American Joint Committee on Cancer (AJCC) 8th Edition and Beyond. Ann Surg Oncol. 2018;25:2105-10.
[26] Thapa S, Ghosh A, Ghartimagar D, Prasad T, Narasimhan R, Talwar O, et al. Clinicopathological study of malignant melanoma at tertiary care centre. JNMA J Nepal Med Assoc. 2017;56(205):132-36.
[27] Kretschmer L, Mitteldorf C, Hellriegel S, Leha A, Fichtner A, Ströbel P, et al. The sentinel node invasion level (SNIL) as a prognostic parameter in melanoma. Mod Pathol. 2021;34(10):1839-49.
[28] de-Andrade BAB, Toral-Rizo VH, León JE, Contreras E, Carlos R, Delgado-Azañero W, et al. Primary oral melanoma: A histopathological and immunohistochemical study of 22 cases of Latin America. Med Oral Patol Oral Cir Bucal. 2012;17(3):e383-e388.
[29] Ohsie SJ, Sarantopoulos GP, Cochran AJ, Binder SW. Immunohistochemical characteristics of melanoma. J Cutan Pathol. 2008;35(5):433-44.
[30] Arkenau HT, Kefford R, Long GV. Targeting BRAF for patients with melanoma. Br J Cancer. 2011;104(3):392-98.
[31] Ren M, Zhang J, Kong Y, Bai Q, Qi P, Zhang L, et al. BRAF, C-KIT, and NRAS mutations correlated with different clinicopathological features: An analysis of 691 melanoma patients from a single center. Ann Transl Med. 2022;10(2):31.
[32] Ahmad F, Avabhrath N, Natarajan S, Parikh J, Patole K, Das BR. Molecular evaluation of BRAF V600 mutation and its association with clinicopathological characteristics: First findings from Indian malignant melanoma patients. Cancer Genet. 2019;231-232:46-53.

**PARTICULARS OF CONTRIBUTORS:**
1. Senior Resident, Department of Pathology, IPGMER and SSKM Hospital, Kolkata, West Bengal, India.
2. Professor and Head, Department of Pathology, IPGMER and SSKM Hospital, Kolkata, West Bengal, India.

**NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:**
Mamata Guha Mallick Sinha,
Professor and Head, Department of Pathology, IPGMER and SSKM Hospital,
244 AJC Bose Road, Kolkata-700220, West Bengal, India.
E-mail: mgmsinha12345@gmail.com

**PLAGIARISM CHECKING METHODS:** [Jain H et al.]
- Plagiarism X-checker: Jan 20, 2025
- Manual Googling: May 19, 2025
- iThenticate Software: Jun 09, 2025 (8%)

**ETYMOLOGY:** Author Origin

**EMENDATIONS:** 7

**AUTHOR DECLARATION:**
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Jan 19, 2025**
Date of Peer Review: **Apr 17, 2025**
Date of Acceptance: **Jun 11, 2025**
Date of Publishing: **Dec 01, 2025**