ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 10 | October - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

STUDY ON THE SIGNIFICANCE OF BRAF V600E MUTATIONS IN MELANOMA PATIENTS AT A TERTIARY CANCER CARE CENTRE IN SOUTHERN INDIA

Oncology

Dr. Suresh Babu M. C.	DM Medical Oncology, Professor, Kidwai Memorial Institute of Oncology.
Dr. Darshan R. S.*	Senior Resident, DM Medical Oncology, Kidwai Memorial Institute of Oncology. *Corresponding Author
Dr. Guruprasad C. Shenoy	Senior Resident, DM Medical Oncology, Kidwai Memorial Institute of Oncology.
Dr. Lokesh K. N.	DM Medical Oncology, Professor, Kidwai Memorial Institute of Oncology.
Dr. Rudresha A. H.	DM Medical Oncology, Associate Professor, Kidwai Memorial Institute of Oncology.
Dr. L. K Rajeev	DM Medical Oncology, Associate Professor, Kidwai Memorial Institute of Oncology.
Dr. Smitha C. Saldanha	DM Medical Oncology, Associate Professor, Kidwai Memorial Institute of Oncology.
Dr. GIRI G V	DM Medical Oncology, Associate Professor, Bangalore Medical College and Research Institute.

ABSTRACT

Background: Melanoma arises from the malignant transformation of melanocytes which are derivatives of neural crest cells. The most common location of melanoma is hair follicle bearing skin giving rise to cutaneous melanomas. There is enough evidence to suggest that MAPK activation by point mutations in at least 70% of melanomas. According to WHO the number of Melanoma cases is increasing faster. Melanomas are relatively rare in Indian context. The literature on BRAF mutation analysis is rarer in both local disease and metastatic disease context. **Methods:** We conducted a prospective observational study for duration of one year from January 2024 till December 2024 at a tertiary cancer centre in southern India. A total of 54 patients in local and metastatic disease were included in the study. After taking ethical committee clearance and informed consent clinical and pathological characteristics were noted. BRAF V600E mutation analysis was done by PCR method. Mutation positive and negative patienst were correlated for various parameters. **Results:** Of the 54 patients majority belonged to less than 60 years with median age at diagnosis being 49 years. There was a slight Male predominance in our study. The most common type of disease was cutaneous melanoma followed by mucosal melanoma (22 and 20). 31 cases presented with stage 4 disease comprising about 57.4%. The most common IHC marker positive on the specimens was S100 (94.4%). On analysis BRAF V600E Mutation analysis majority a total of 14 patients tested positive. On correlation of Ki67 status and BRAF mutation analysis, 8 patients who were positive for mutation had higher ki67 that is >40%, this was statistically significant. **Conclusion:** Melanoma is a rare subset of cancer among Indian subcontinent which usually present at advanced stages and is associated with poorer prognosis. BRAF mutation is present in about 1 in every 4 diagnosed cases of melanoma. BRAF mutation is more common in advanced stage and has higher Ki67. Immune checkpoint inhibitors and Targeted therapy form main stay of treatment.

KEYWORDS

Cutaneous Melanoma, Mucosal Melanoma, BRAF Mutation, IHC marker, Ki67

INTRODUCTION

Melanoma arises from the malignant transformation of melanocytes which produces Melanin. These melanocytes are derivatives of neural crest cells and during fetal life they migrate to different parts of body. These cells can lead to development of melanoma due to malignant transformation of these. The most common location of melanoma is hair follicle bearing skin giving rise to cutaneous melanomas[1]. As per Global cancer observatory 2022 data Melanoma ranks 17 in overall incidence and rank 22 in cause specific mortality. It also found that melanoma is more common in European and North American population among others[2].

There is enough evidence to suggest that MAPK activation by point mutations in at least 70% of melanomas which results in constitutive signalling and oncogenic cell proliferation and evasion of apoptosis[3]. The majority of the mutations in the MAPK pathway occurs in BRAF and vast majority of these BRAF mutations involve substitution of Valine at 600th amino acid position to Glutamine[4,5].

The BRAF V600E mutation frequency is inversely corelated with age as it is seen more in patients of melanoma without chronic sun exposure and skin damage than older adults[4]. BRAFV600K constitutes the second most common mutation with incidence increasing with age. Other mutations like V600D and V600R are less common[6].

According to WHO the number of Melanoma cases is increasing faster on comparison to other cancer, especially in countries like Australia and USA. Also, the number of deaths is also increasing in these population[7]. Melanomas are relatively rare in Indian context and it also carries poor prognosis due to presentation at late stages and limited treatment option due to affordability and availability issues[8].

The literature on BRAF mutation analysis is rarer in both local disease and metastatic disease context as per our knowledge till date. The recent development of anti-BRAF targeted therapies has been a major improvement in the management of BRAF mutated melanoma patients. This study was done to look into the clinical and pathological features of melanoma patients in Indian context.

AIMS AND OBJECTIVES

To study the significance of BRAF V600E mutations in melanoma patients. The primary objective was to study the clinicopathological features of melanoma patients with and without BRAF mutations. Secondary objective being to correlate BRAF mutation status with the clinical and pathological features and also to look for response to treatment based on BRAF mutation status.

MATERIALS AND METHODS

This was an observational study conducted at Kidwai memorial Institute of Oncology, Bengaluru, Karnataka from a period from January 2024 to December 2024. All the patients who were diagnosed with Melanoma were eligible for the study. Patient's demographic data, site of primary disease, metastatic sites. Diagnosis was made based on histopathological examination of tissue sample and BRAF mutation analysis was done by PCR.

INCLUSION CRITERIA

1. Age >18 years

2. All newly diagnosed melanoma cases
3. All melanoma cases who present in metastatic state

Exclusion Criteria

1. Patient not willing to give informed consent
2. Severe Cardiac, Renal or Hepatic illness

Statistical Analysis

The data was analysed using SPSS Version 23. Count data was summarised as proportions and represented as pie charts or bar graphs. Difference between proportions was analysed using chi-square test. The level of significance was kept at 95% for all statistical analyses.

ETHICS

The study was done after taking clearance from Institutional Review Board and in accordance with the principles of Helinski's declaration (1960). Patients were explained in detail regarding the study protocol in their understandable language along with investigations and the right to withdraw from the study if not willing to participate.

RESULTS

Total of 54 patients with melanoma including local and metastatic disease were included in the study. A total 35 of the patients belonged to age group less than 60yrs comprising about 64.8% of the cases and the rest were above 60yrs and the median age of presentation was 49 years (25-71yrs). We found a slight male preponderance than female (1.16:1) in diseased population. Diabetes mellitus was the most common comorbidity observed among the study group followed by Hypertension. Majority of the patients had ECOG PS of 1 (61.2%)

In our study the most common type of disease was cutaneous melanoma followed by mucosal melanoma (22 and 20 respectively). 5 patients had conjunctival melanoma. 7 of the patients who were diagnosed with metastatic disease had no discernible primary even after extensive evaluation for primary. We did not find acral or lentigo melanomas in our study.

Of the total cases 31 cases presented with stage 4 disease comprising about 57.4%. Rest of the patients were in either Local or locally advanced stage. The most common IHC marker positive on the specimens was S100 (n=51,94.4%) which is the sensitive marker followed by HMB45 (n=49, 90.7%). 38.9% of the patients had a Ki67 of <20%. Detailed baseline characteristics is depicted in Table 1.

Table 1: Baseline Characteristics

		Number (n)	Percentage (%)
Age	>60	19	35.2
	<60	35	64.8
Sex	Male	29	53.7
	Female	25	46.3
Comorbidities	DM	13	24
	HTN	10	18.5
	CAD	5	9
	Others	3	5
	Nil	46	85
ECOG PS	0	21	38.8
	1	33	61.2
Location of primary	Mucosal	20	37
	Conjunctival	5	9.3
	Cutaneous	22	40.7
	MM of unknown 1'	7	12.9
Stage at presentation	I/II	12	22.2
	III	11	20.4
	IV	31	57.4
IHC	HMB-45	49	90.7
	MelanA	45	83.3
	S100	51	94.4
	SOX10	47	87.03
Ki67	<20%	21	38.9
	20-40%	17	31.5
	>40%	16	29.6

On analysis BRAF V600E Mutation analysis majority a total of 14 patients tested positive for the mutation (Fig. 1). Of the positive patients 10 belonged to less than 60 years of age and remaining 4 in more than 60 years and also 9 patients were male and 5 patients were female. Both Age and Sex were not associated with significance on statistical analysis.

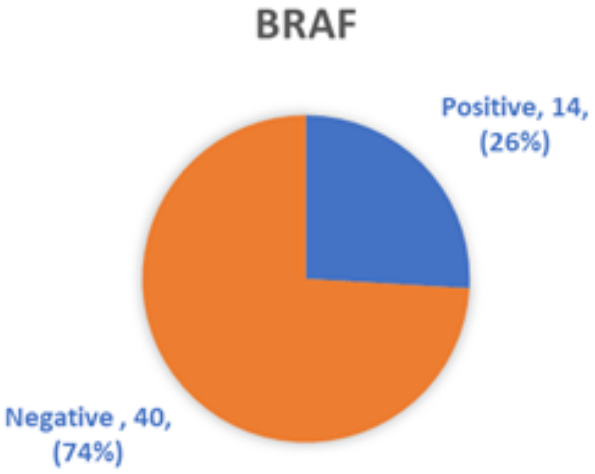

Figure 1: BRAF Mutation status

7 patients were BRAF mutation positive in mucosal melanoma and 6 in cutaneous melanoma group. The association was not statistically significant(p=0.3321). Also, on an analysis of mutation based on stage, majority patients with BRAF mutation positivity had stage 4 disease which was not statistically significant. There was no much difference among different IHC markers and BRAF mutation status.

On correlation of Ki67 status and BRAF mutation analysis, 8 patients who were positive for mutation had higher ki67 that is >40%, this was statistically significant (p=0.020), indication BRAF mutated melanoma patients have higher proliferation index (Fig. 2). Detailed correlation of mutation status and different parameters in given in Table 2.

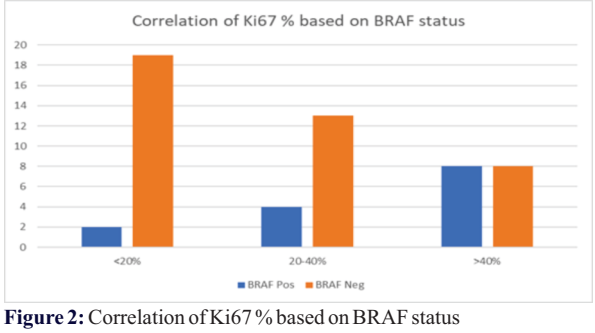

Figure 2: Correlation of Ki67 % based on BRAF status

Table 2: Correlation of BRAF Alteration With Clinical Features

Characteristics		BRAF positive (n=14)	BRAF negative (n=40)	Total (n=54)	χ^2, degree of freedom	p value
Age	>60	4	15	19	0.36, 1	0.547
	<60	10	25	35		
Sex	Male	9	20	29	0.85, 1	0.356
	Female	5	20	25		
Location of primary	Mucosal	7	13	20	3.41, 3	0.331
	Conjunctival	1	4	5		
	Cutaneous	6	16	22		
	MM of unknown 1'	0	7	7		
Stage at presentation	I/II	0	12	12	5.68, 2	0.058
	III	3	8	11		
	IV	11	20	31		
IHC	HMB-45	13	36	49	0.12, 3	0.998
	MelanA	11	34	45		
	S100	14	37	51		
	SOX10	12	35	47		
Ki67	<20%	2	19	21	7.82, 2	0.020
	20-40%	4	13	17		
	>40%	8	8	16		

On analysis of BRAF positivity based on metastatic site 7 patients with mutation had distal lymph node metastasis and 6 patients had regional Lymph node metastasis. 5 patients with local recurrence had positive mutation status (Table 3).

Table 3: BRAF Positivity Based On Metastatic Site

Metastatic site	Overall	BRAF Positive	Percentage
Regional LN	18	6	42.8
Distant LN	20	7	50
Brain	5	2	14.3
Bone	11	3	21.4
Viscera (lung/liver)	15	3	21.4
Local recurrence	9	5	35.7

Out of 11 patients positive for BRAF mutation in metastatic stage only 2 patients received BRAF inhibitors. Of the patients who were negative for any mutation 5 patients received immunotherapy, rest of the patients receiving either form of chemotherapy majority being recipients of Dacarbazine (n=18). Detailed treatment regimens received on first line in metastatic setting is given in Table 4.

Table 4: First Line Therapy In Metastatic Disease

Regimen	Number of patients
BRAF Inhibitors	2
Immunotherapy	5
Dacarbazine	18
Temozolamide	2
Paclitaxel	4

DISCUSSION

Melanoma arises from the malignant transformation of melanocytes which are pigment producing cells derived during embryonic period from the neural crest cells. The three most common types of melanomas are cutaneous, mucosal and uveal based on the location of melanocytes[9].

There was slight male preponderance in our study i.e.,1.16:1 which was also seen in a study conducted by Kuldeep Sharma et al[10], where they had male to female ratio of 1.25:1. Studies done by Wanebo et al[11] and Castel et al[12] had a female preponderance. The median age of presentation in their study was 46.5years which was slightly earlier on comparison with the previous study[10].

The predominant type of melanoma in our study was cutaneous melanoma (40.7%) followed by mucosal melanoma (37%). Though the numbers were not as similar as that reported in studies done by Chang et al[13] and Mukhopadhyay et al[14] where 82% and 78.5% cases being cutaneous melanoma, the most common type was cutaneous type in these studies. Among the cutaneous melanoma the most common primary was seen in extremities that is lower limb, this was also seen in studies done by Radhika et al[8] and Tjarta et al[15].

In the present series majority of the patients were in stage IV comprising about 57.4%, this was also seen in the previous studies done by Mukhopadhyay et al[14] and Kuno et al[16].

Melanoma has a wide spectrum of histologic features and can mimic hematological, epithelial, mesenchymal and neural lesions. To establish the correct diagnosis the most important primary tool is Immunohistochemistry (IHC), where HMB45, S100 and Melan A are typically positive leading to characterization of melanomas[17].

S100 is the most sensitive marker for detection of melanoma on IHC which can be positive in as many as 95% of the cases. In our study we found S100 to be the most common IHC marker to be positive (94.4%), the second most common positive IHC marker was HMB45 (90.7%). These trends were also observed by Prieto et al[18].

Ki67 is the proliferation index, detected by IHC method and higher values are associated with aggressive nature of the disease. It may also indicate earlier rates of metastatic disease or patient outcomes[19]. This study had most of the tumors having a Ki67 of less than 20% (38.8%) and least number was seen in Ki67 more than 40% group (29.6%). Gimotty et al concluded higher Ki67 values are associated with increased metastatic disease on longer followup[20].

BRAF mutation analysis were done in all the patients who were included in the study. A total of 14 (25.9%) patients were positive for BRAF V600E mutation, which was lesser in incidence on comparison to western data where the incidence is in range of 50-60% as per Ascierto et al[21]. The values were also lesser as compared to those reported from Japan (41.4%)[22] and Russia (43.3%)[23]. Another earlier study from India had reported an incidence of 30% for BRAF mutations[24] whereas another study found an incidence of 16.21%[25].

Of the BRAF mutation positive patients' majority were Males (9/14). We observed BRAF mutation was not correlated with age or sex of the patients diagnosed with melanoma. This was in discordance with the earlier results which suggested BRAF mutations are more common among females and age less than 60 years[26].

BRAF mutations are suggested to be associated with early stages of disease in melanoma patients[27]. The findings in our study were not consistent with the model as only 3 out of 14 patients were in locally advanced disease and none in local disease. On contrary 11 patients were having stage IV disease. There was no correlation between expression of different IHC markers and BRAF mutation status

Higher Ki67 values are seen in patients with BRAF mutation who are diagnosed with papillary thyroid carcinoma on comparison with BRAF negative tumors[28]. In another study patients with higher Ki67 were found to be associated with poor prognosis and reduced survival[29]. In our study we observed higher Ki67 among BRAF positive patients on comparison with negative patients which was statistically significant indicating BRAF mutant patients have higher Ki67 or aggressive tumor histology which was a new finding as per our knowledge.

Most common site of metastasis in overall population was Lymph nodes distal followed by regional (22 and 18 respectively) and same was also seen in BRAF positive patients (7 and 6 respectively). This trend was also seen in study done by Lian et al, where metastatic cells are considered to spread through dermal lymphatics to regional and then non regional nodes[8,14,30].

Only few of the patients received standard of care treatment that is Immune checkpoint inhibitors or BRAF inhibitors due to financial constraints. This being a real-world data depicts the actual practice in the management of Melanoma patients which is among the rarer subset of cancers in Indian population and also has a poorer outcome without standard of care.

Meaningful responses can be achieved in few by using other systemic therapy options like taxanes or dacarbazine[31-33]. In this study the most common systemic therapy option used was Dacarbazine followed by Paclitaxel. We could not analyse the progression free survival and overall survival based on BRAF mutation as majority of the patients were not affordable for standard of care management.

CONCLUSION

Melanoma is a rare subset of cancer among Indian subcontinent which usually present at advanced stages and is associated with poorer prognosis. We concluded the incidence of melanoma is earlier and is slightly more common among males. BRAF mutation is present in about 1 in every 4 diagnosed cases of melanoma. BRAF mutation is more common in advanced stage and has higher proliferation index. ICI and targeted therapy form the standard of care based on presence or absence of targetable mutations.

Conflict Of Interest: Nil

Funding: Nil

Acknowledgement: Nil

REFERENCES

1. DeVita Jr VT, Rosenberg SA, Lawrence TS. DeVita, Hellman, and Rosenberg's Cancer: eBook without Multimedia. Lippincott Williams & Wilkins; 2022 Sep 21.
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2024 May;74(3):229-63.
3. Gray-Schopfer V, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. Nature. 2007 Feb 22;445(7130):851-7.
4. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N. Mutations of the BRAF gene in human cancer. Nature. 2002 Jun 27;417(6892):949-54.
5. Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Barford D, Marais R. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004 Mar

19;116(6):855-67.
6. Menzies AM, Haydu LE, Visintin L, Carlino MS, Howle JR, Thompson JF, Kefford RF, Scolyer RA, Long GV. Distinguishing clinicopathologic features of patients with V600E and V600K BRAF-mutant metastatic melanoma. Clinical cancer research. 2012 Jun 15;18(12):3242-9.
7. Lens MB, Dawes M. Global perspectives of contemporary epidemiological trends of cutaneous malignant melanoma. British Journal of Dermatology. 2004 Feb 1;150(2):179-85.
8. Radhika K, Prayaga AK, Sundaram C. A clinicopathologic study of malignant melanoma based on cytomorphology. Indian Journal of Cancer. 2016 Jan 1;53(1):199-203.
9. Ali Z, Yousaf N, Larkin J. Melanoma epidemiology, biology and prognosis. European Journal of Cancer Supplements. 2013 Sep 1;11(2):81-91.
10. Sharma K, Mohanti BK, Rath GK. Malignant melanoma: A retrospective series from a regional cancer center in India. Journal of cancer research and therapeutics. 2009 Jul 1;5(3):173-80.
11. Wanebo HJ, Cooper PH, Young DV, Harpole DH, Kaiser DL. Prognostic factors in head and neck melanoma. Effect of lesion location. Cancer. 1988 Aug 15;62(4):831-7.
12. Castel T, Baradad M, Castro J, Mascaro JM, Garcia-Valdecasas JC, Grande L, Fuster J, Visa J, Estapé J. Primary malignant melanoma of the skin. Retrospective study of 375 cases. Clinical aspects and histology. Medicina clinica. 1990 Feb 24;94(7):246-9.
13. Chang JW. Cutaneous melanoma: Taiwan experience and literature review. Chang Gung Med J. 2010 Nov 1;33(6):602-12.
14. Mukhopadhyay S, Ghosh S, Siddhartha D, Mitra PK. A clinicopathological study of malignant melanoma with special reference to atypical presentation. Indian J Pathol Microbiol 2008; 51:485-8
15. Tjarta A, Kanoko M, Ueda M, Hamzah M, Cipto H, Ichihashi M, et al. rare case of melanoma studied for its histopathological features in Indonesia. Med J Indones 2000; 9:93-9.
16. Kuno Y, Ishihara K, Yamazaki N, Mukai K. Clinical and pathological features of cutaneous malignant melanoma: a retrospective analysis of 124 Japanese patients. Japanese journal of clinical oncology. 1996 Jun 1;26(3):144-51.
17. Ohsie SJ, Sarantopoulos GP, Cochran AJ, Binder SW. Immunohistochemical characteristics of melanoma. Journal of cutaneous pathology. 2008 May;35(5):433-44.
18. Prieto VG, Shea CR. Use of immunohistochemistry in melanocytic lesions. Journal of cutaneous pathology. 2008 Nov; 35:1-0.
19. Menon SS, Guruvayoorappan C, Sakthivel KM, Rasmi RR. Ki-67 protein as a tumour proliferation marker. Clinica chimica acta. 2019 Apr 1; 491:39-45.
20. Gimotty PA, Van Belle P, Elder DE, Murry T, Montone KT, Xu X, Hotz S, Raines S, Ming ME, Wahl P, Guerry D. Biologic and prognostic significance of dermal Ki67 expression, mitoses, and tumorigenicity in thin invasive cutaneous melanoma. Journal of Clinical Oncology. 2005 Nov 1;23(31):8048-56.
21. Ascierto PA, Kirkwood JM, Grob JJ, Simeone E, Grimaldi AM, Maio M, Palmieri G, Testori A, Marincola FM, Mozzillo N. The role of BRAF V600 mutation in melanoma. Journal of translational medicine. 2012 May 3;10(1):85.
22. Yamazaki N, Kiyohara Y, Uhara H, Uehara J, Fujimoto M, Takenouchi T, Otsuka M, Uchi H, Ihn H, Minami H. Efficacy and safety of nivolumab in Japanese patients with previously untreated advanced melanoma: a phase II study. Cancer science. 2017 Jun;108(6):1223-30.
23. Aksenenko MB, Kirichenko AK, Ruksha TG. Russian study of morphological prognostic factors characterization in BRAF-mutant cutaneous melanoma. Pathol Res Pract 2015;211(07):521–527
24. 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 genetics. 2019 Feb 1; 231:46-53.
25. Vengurlekar V, Shetty O, Gurav M, Bapat P, Karnik N, Wagh G, Epari S, Rekhi B, Ramadwar M, Desai S. BRAF V600E Mutations and Beyond: A Molecular Perspective of Melanoma from a Tertiary Cancer Referral Center of India. South Asian Journal of Cancer. 2023 Oct;12(04):359-70.
26. Shinozaki M, Fujimoto A, Morton DL, Hoon DS. Incidence of BRAF oncogene mutation and clinical relevance for primary cutaneous melanomas. Clinical cancer research. 2004 Mar 1;10(5):1753-7.
27. Omholt K, Platz A, Kanter L, Ringborg U, Hansson J. NRAS and BRAF mutations arise early during melanoma pathogenesis and are preserved throughout tumor progression. Clinical cancer research. 2003 Dec 15;9(17):6483-8.
28. Nakayama H, Yoshida A, Nakamura Y, Hayashi H, Miyagi Y, Wada N, Rino Y, Masuda M, Imada T. Clinical significance of BRAF (V600E) mutation and Ki-67 labeling index in papillary thyroid carcinomas. Anticancer research. 2007 Sep 1;27(5B):3645-9.
29. Jurmeister P, Bockmayr M, Treese C, Stein U, Lenze D, Jöhrens K, Friedling F, Dietel M, Klauschen F, Marsch W, Fiedler E. Immunohistochemical analysis of Bcl 2, nuclear S100A4, MITF and Ki67 for risk stratification of early stage melanoma–A combined IHC score for melanoma risk stratification. JDDG: Journal der Deutschen Dermatologischen Gesellschaft. 2019 Aug;17(8):800-8.
30. Lian B, Cui CL, Zhou L, Song X, Zhang XS, Wu D, Si L, Chi ZH, Sheng XN, Mao LL, Wang X. The natural history and patterns of metastases from mucosal melanoma: an analysis of 706 prospectively-followed patients. Annals of Oncology. 2017 Apr 1;28(4):868-73.
31. Pflugfelder A, Eigentler TK, Keim U, Weide B, Leiter U, Ikenberg K, Berneburg M, Garbe C. Effectiveness of carboplatin and paclitaxel as first-and second-line treatment in 61 patients with metastatic melanoma. PloS one. 2011 Feb 16;6(2):e16882.
32. Anderson CM, Buzaid AC, Legha SS. Systemic treatments for advanced cutaneous melanoma. Oncology (Williston Park, NY). 1995 Nov 1;9(11):1149-58.
33. Young AM, Marsden J, Goodman A, Burton A, Dunn JA. Prospective randomized comparison of dacarbazine (DTIC) versus DTIC plus interferon-alpha (IFN-α) in metastatic melanoma. Clinical oncology. 2001 Dec 1;13(6):458-65.