Journal of Pakistan Association of Dermatologists. 2020; **30(1)**: 128-136.

Original Article

A retrospective observational study on clinical and histopathological correlational analysis of malignant melanoma

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Abstract

Background Malignant melanoma derives from the neuroectodermal mucosa. Melanoma accounts for 1-3 percent of malignancies in children and adults. The incidence and clinical properties of the disease are multifactorial and have biological and environmental variables. Tumor thickness was correlated to age, blood supply, site and nerve supply separately.

Objective This study was made to determine the clinical and histopathological correlation of malignant melanoma.

Material and Methods A retrospective observational study was conducted at a tertiary care hospital, Urumqi in China to study the clinical and histopathological correlation of malignant melanoma. A detailed history and thorough physical examination of patients, lab tests including primary or secondary tumor biopsies, complete blood count and PET scans help in diagnosis. Clinical staging was done on the basis of regional lymph node involvement and distant metastasis.

Results The highest no. of patients (28.20%) was recorded for 60-69 years age group followed by (25.64%) in 70-79 years age group. The largest no. (30.76%) of cases comprised of acral lentiginous melanoma as well as nodular melanoma followed by lentigo maligna melanoma (20.51%) and superficial spreading melanoma (17.95%). The highest no. of patients (35.90%) had tumor thickness of 1-2 mm followed by (20.51%) cases had 2-4 mm, (17.95%) cases had <1 mm. However, not a single patient had tumor thickness of >4 mm in our study.

Conclusion This study investigated histopathological and clinical results. The main result we found was that the trunk was involved to major extent than the periphery. Frequently involved subtypes were nodular melanoma and acral melanoma. Female are more commonly affected in this study then male 2:1.

Key words
Malignant Melanoma, Skin Tumor, Tumor Staging, Clark Level, Breslow Thickness.

Introduction

A clear regional and racial divergence with increased incidence in western countries is shown by malignant melanoma.[1,2] Malignant melanoma is derived from the neuroectodermal mucosa.[3] Melanoma accounts for 1-3 percent of malignancies in children and adults.[4] This age group is < 2% of all patients with melanoma.[4,5] However, melanoma incidents across all age groups have increased in recent decades.[6,7]Initial

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diagnosis is carried out via morphological, clinical, dermoscopic, microscopic or immunohistochemical sizes: a strong tumor forecast depth in mm.[8,9] Melanoma is a type of melanocyte generated skin cancer. The incidence and clinical properties of the disease are multifactorial and have biological and environmental variables.[10,13] According to Ackerman's malignant melanoma study in the Caucasians, approximately 80 percent are novices, while only about 20 percent are linked to existing nevi melanocytes mainly in the trunk and proximity. Nevi is a combination of SSM and nodular (NM) melanomas, whilst Nevi is a result of Lentigo malignant (LMMs) and Acral Lentiginous (ALM) melanoma. In Asia, over 50% of melanomas in ALM not exposed to the sun as in the Caucasians are reported.[15,16] In this respect, ultraviolet radiation seems unconnected to black and Asian melanoma.[17] The lesion thickness is usually the most accurate of all the prime melanoma forecasts in Breslow.[18,20] In every patient: age, sex, anatomy, histogenetic type, the thickness of Breslow, the presence of ulceration, stage, and symptoms like bleeding, color, and dimensional variations, alterations, and previous trauma, all were chosen to determine clinical or pathological properties.

Materials and Methods

Study population / Sample size / Clinical data

The present study includes 39 patients of malignant melanoma of all different stages including 13 male and 26 female patients of melanoma.

The study was designed for current analysis after the institutional review board's permission prior to medical records and examination. The basic statistic data also included a database, diseases (anatomic sites, histologic subtypes, ulceration, Breslow thickness, treatment methods, operating methods, and system treatment) and published predictive factors. The patient's characteristic features like age, sex, and performance were reported. The database was designed and checked, re-examined and added to the database medical documents of every patient submitted to the pathology department if malignant melanoma had been confirmed. In addition, detailed history and physical examination of patients was included in the institutional protocol. Tests included tumor biopsy, complete blood count, serum electrolytes, metabolic or liver screen, chest CT, abdominal CT, ultra-reactive and PET scans. The PET-CT reporting was done to examine the foci on the skin or other tissues and organs carefully. Testing of the concentric tuft or other tissues was done by positron emission tomography PET-CT. Cases were treated with surgery and postoperative samples were sent for pathological assessment. In patients with skin melanoma in clinical stage I, local excision is performed without a biopsy. Further assessment tests were necessary for patients who were entitled to our future institutional trials. Either for clinical or pathological stages, the American Joint Cancer Committee (AJCC) stage-system (6^{th} edition) was used. Clinical stages were based on available categories, regional involvement of lymph nodes and distant metastasis without Breslow's pathological thickness analysis for referred patients.

Data analysis

The time period for regional or distant metastasis has been measured in documents for non-metastatic diseases from the end of treatment. Patients at stage IV were treated progression-free (SFF) in any area and they had documented their progression at any stage from the beginning of any therapy (systemic or local palliation). The total survival time for alive patients was calculated from the time of death or

Table 1 Distribution of patients among stages on the bases of Age, Gender, and Anatomic location

Characteristics	*Stages*					*Total (n = 39)*
	0	*1*	*2*	*3*	*4*	
Age group (in years)						
< 39	0	2	0	1	0	3 (7.69%)
40 – 49	1	1	4	1	0	7 (17.94%)
50 – 59	3	0	4	0	0	7 (17.94%)
60 – 69	2	4	1	4	0	11 (28.20%)
70 – 79	3	2	3	2	0	10 (25.64%)
>80	0	0	0	1	0	1 (2.56%)
Total (n=39)	9	9	12	9	0	39 (100%)
Mean Age	59. 92 years					
Median range	61 years					
Range	Min age: 33years Max age: 82 years					
Gender						
Male	3	4	4	2	0	13 (33.33%)
Female	6	5	8	7	0	26 (66.66%)
Total (n=39)	9	9	12	9	0	39 (100%)
Anatomic Location						
Head and Neck	0	1	0	1	0	2 (5.13%)
Face	1	1	0	0	0	2 (5.13%)
Trunk	1	2	1	2	0	6 (15.38%)
Upper Extremities						
Thumb	0	2	2	0	0	4 (10.26%)
Palm	1	0	0	0	0	1 (2.56%)
Lower Extremities						
Finger	1	0	4	0	0	5 (12.82%)
Heel	0	2	1	2	0	5 (12.82%)
Valve	0	0	1	0	0	1 (2.56%)
Waist	1	0	0	0	0	1 (2.56%)
Other	3	1	5	3	0	12 (30.76%)
Total (n=39)	10	7	14	8	0	39 (100%)

the final follow-up visit for melanoma. In each case, the tumor lesions were taken to the safety limits and sutured to the "per primam" and subsequently sent to the histopathologic analytics laboratory. The excised fragments were fixed to 10 percent of tamponed formalin and processed using the standard paraffin integration method. Paraffin blocks were first cut into usual stained sections of Hematoxylin-Eosin in the 3-4-micron thick segments for the immunohistochemical analysis of poly-L-lysine-coated glass slides the serial parts were then carried out and highlighted.

Histology and immunohistochemistry

Specimens of histology and immunohistochemistry for examination were subsequently processed by 4% formaldehyde, conventional dehydration paraffin-embedded hematoxylin and eosin used for staining and light microscopy was done. Envisions two-step method was used in immunohistochemical staining. The antibodies used were anti-melan anti-hmb45 anti-s100 anti-candanti-ki67 antibodies. Using Diamino-benzidine DAB, positive and negative controls have been

developed. HMB45 and Melan have a positive expression in the cytoplasm and S-100 in the nucleus. The cell membrane has a positive CK expression and the coloring of the nucleus or cell membrane of the brown tumor cell was considered beneficial for any coloration.

Statistical Analysis

Data was compiled in Microsoft Excel and statistical analysis was done using a statistical software PASW SPSS version 20. Medium range descriptive statistics, like mean ± standard deviation have been performed with demographic and epidemic characteristics at a 95% Confidence Interval. Hazard ratio was carried out at a significant P-value of 0.005 for inferential statistics, Chi-Square test, Kaplan-Merrier test, Mann-Whitney U test.

Observations and Results

See **Table 1**

Demographic and epidemic characteristics

In the current study 39 patients at various tumor stages were included, out of which 13 were male and 26 were female with male-female ratio 1:2. The mean age was 59.92 years for the 39 patients diagnosed. (Median age; 56 years, range; 17-79 years, 95% CI 49.37-59.14). The highest no. of patients (28.20%) were recorded for 60-69 years age group followed by (25.64%) in the age group of 70-79 years.

Tumor anatomic location and staging

Tumor was mainly located in lower extremities (61.52%), including finger (12.82%), heel (12.82%), valve (2.56%), waist (2.56%) and other extremes (30.76%), followed by upper extremities (12.85%) including thumb (10.26%) and palm (2.56%) while (5.13%) patients of melanoma had tumor in head and neck, (15.38%) patients of melanoma on the trunk **(Table 2)**.

Histopathological subtype & clinical diagnosis

Table 2 shows that the largest (30.76%) cases consisting of acral lentiginous melanoma as well as nodular melanoma followed by LentigoMaligna melanoma (20.51%), superficial spreading melanoma (17.95%). Most of the patients 37 out of 39 (94.87%) had clinically diagnosed malignant melanoma while only (2.56%) diagnosed nevus and (2.56%) verrucous nevus **(Table 3)**.

Table 2 Distribution of patients among stages according to histopathological subtype and clinical diagnosis

Characteristics	*Stages*					*Total*
	0	*1*	*2*	*3*	*4*	*(n = 39)*
Histopathological subtype						
Acral Lentiginous Melanoma	2	2	4	4	0	12 (30.76%)
LentigoMaligna Melanoma	5	2	0	1	0	8 (20.51%)
Nodular Melanoma	2	3	4	3	0	12 (30.76%)
Superficial Spreading Melanoma	0	2	4	1	0	7 (17.95%)
Total (n = 39)	9	9	12	9	0	39 (100%)
Clinical Diagnosis						
Malignant Melanoma (MM)	9	8	12	8	0	37 (94.87%)
Nevus	0	1	0	0	0	1 (2.56%)
Verrucous nevus	0	0	0	1	0	1 (2.56%)
Total (n = 39)	9	9	12	9	0	39 (100%)

Table 3 Classification of patients by age (in years), tumor thickness, Clark Level

Tumor Thickness	*No. (%)*
In Situ	10 (25.64%)
Less than 1 MM	7 (17.95%)
1 – 2 MM	14 (35.90%)
2 – 4 MM	8 (20.51%)
More than 4 MM	0 (0%)
Total	39 (100%)
Duration Age (Yr)	No. (%)
0 – 1	10 (25.64%)
2 – 4	19 (48.72%)
5 – 9	6 (15.38%)
More than 10	4 (10.26%)
Total	39 (100%)
Clark level	No. (%)
I epidermal layer	9 (23.08%)
II dermis layer	6 (15.38%)
III epidermis layer	12 (30.77%)
IV dermal layer	12 (30.77%)
V	0 (0%)
Total	39 (100%)

Tumor Thickness and Clark Level

Table 3 shows that according to the present study, the highest no. of patients 14 out of 39 (35.90 %) had tumor thickness of 1-2 mm followed by 10 out of 39 patients of melanoma (25.64%) had tumor thickness in situ melanoma, 8 (20.51%) patients had 2 - 4 mm tumor thickness, 7 (17.95%) had less than 1 mm tumor thickness. However, not a single patient had tumor thickness more than 4 mm in our study. The study focused that (48.72%) patients of melanoma had tumor for 2-4 years followed by (25.64%) for 0-1 year, (15.38%) for 5-9 years and only (10.26%) patients of melanoma had tumor for more than 10 years. (30.77%) patients had Clark level III (epidermis layer), (30.77%) had Clark level IV (dermal layer), followed by (23.08%) Clark level I (epidermal layer), (15.38%) had Clark level II (dermis layer), while no patients of melanoma were recorded for Clark level V.

The correlation between demographic and epidemic characteristics and staging

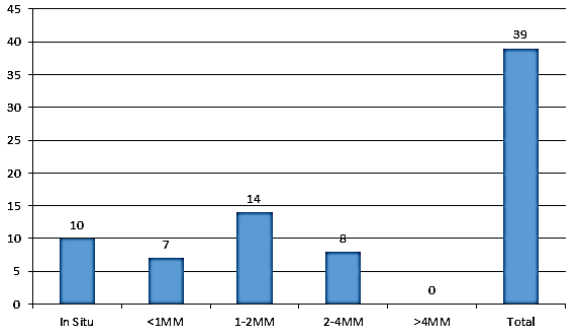

Figure 1 Classification of Patients on the basis of Tumor Thickness

Table 4 Relationship between histopathological subtypes with statistical analysis

Parameter	*Mann-Whitney U (P-value)*	*Significance*
ALM vs. LMM	0.378	NS
ALM vs. NMM	0.002*	S
ALM vs. SSM	0.602	NS
LMM vs. NMM	0.057	NS
LMM vs. SSM	0.512	NS
NMM vs. SMM	0.004*	S

ALM = Acral Lentiginous, LMM = LentigoMaligna, NMM= Nodular, SSM = Superficial Spreading S = statistically significant with P < 0.005, NS= Not Significant

Results indicated that there was no statistical difference between sex and staging (P=0.352), age and staging (P=0.389) and area and stage (P=0.399). There was however a strong association between histopathology subtype and staging (P=0.03). The study also showed that nodular melanoma was associated at higher levels **(Table 4)**.

Gender and age

Based on the mortality risk ratios among the men and women, women had a half death chance (HR=0.506), the studies showed that p-value (P=0.133) not significant. The results for old age groups were similar, and according to Kalpan-Meier analysis, the age was also not substantially related to different survival rates.

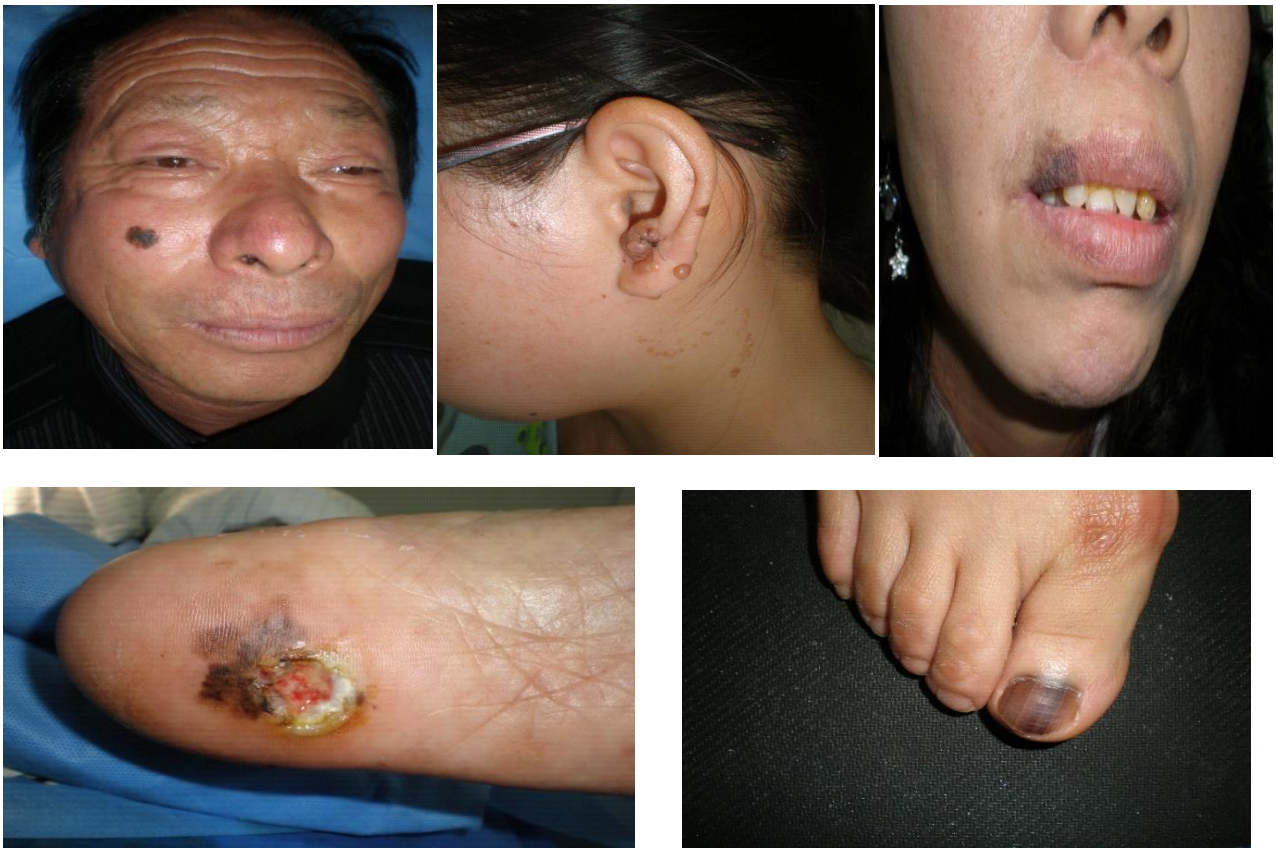

Figure 2

Table 5 Univariate analysis based on the model of Cox proportional hazard

Characteristics	*Mortality (%)*	*P-Value*	*Hazard Ratio*
Gender			
Male	3/13 (25%)	0.133	1
Female	4/26 (15.38%)		0.506
Age Group (in years)			
≤ 50	2/10 (16.7%)	0.789	1
50-69	3/18 (18.3%)	0.797	0.945
≤ 69	2/11 (20.2%)	0.833	1.212
Location			
Head and Neck	1/2 (50%)	0.259	1
Upper extremities	1/5 (9.7%)	0.323	0.356
Lower extremities	4/24 (15.7%)	0.100	0.387
Trunk	2/6 (30.6%)	0.513	0.599
Clark Level			
I – III	3/27 (9%)	0.029*	1
IV –V	3/12 (26.95%)		3.561
Histopathological subtypes			
Acral Lentiginous Melanoma	2/12 (17.4%)	0.780	1
LentigoMaligna Melanoma	0/8 (0%)	0.986	0.000
Nodular Melanoma	4/12 (29.9%)	0.223	1.999
Superficial Spreading Melanoma	2/7 (23.9%)	0.891	1.045

Location of primary tumors

According to the Kalpan - Merier analysis, we observed no significant survival differences based on the primary tumor location.

Invasion level

The Clark IV-V death rate was about three times that of a lower invasion rate, which was statistically significant (P=0.029). The mortality rate was three times higher. We were reported similar survival differences based on the level of invasion according to Kalpan-Merier analysis.

Tumor thickness

There was no big difference between the studies < 2 mm thickness and > 2 mm tumor survival rate (P=0.063). According to the analysis from Kalpan-Merier, no significance was discovered between the survival rate and the thickness of the tumor.

Histopathological subtypes

Based on the clinical histopathological classification, we found no distinction in the survival rates, according to Kalpan- Merier analysis.

Discussion

Studies on Melanoma were performed in the Southern European regions such as Italy,[8,10,13,15] some melanoma research studies were also performed in Spain[11,12] and have all been published in Spanish Literature.[12] Malignant Melanoma is a tumor that arise from the epithelium. (YU and LIU) Malignant melanoma usually involves the skin, but not frequently the mucosa. Rural communities also have high re-occurrence and metastasis (YU and LIU) and poor prognosis. However, cases of the malignant melanoma have increased nowadays, but still not sufficient to conclude about all the aspects of primary and recurrent Malignant Melanoma. For this reason, we took 39 patients (13 males and 26 females) of malignant melanoma of all different stages. The present study shows the sex ratio of the sample study population (1:2; male-to-female) which is unlike the one observed in Korean reports which were similarly designed.[11-13] Our results, however, are similar to the study conducted (chi et al) as sex ratio 1:1.8 (male-to-female) The mean age of our sample study population (39 cases) was 54.25 years, with a mean age of 55 years and a range (17-79 years) similar to the study given by Chi et.al. Also, it was found approximately the same as the study performed in South Korea having a mean age of 50s.[8,11,12]

Present study shows that most common location of primary melanoma was in lower extremes (61.52%), including finger (12.82%), heel (12.82%), valva (2.56%), waist (2.56%) and other extremities (30.76%), followed by upper extremities (12.85%), including thumb (10.26%) and palm (2.56%). (5.13%) cases had a tumor at head and neck, (15.38%) in the trunk which was near about the same to the study of South Korea.[8,11,12] In the current study, the histopathological subtypes of malignant melanoma showed the largest (30.76%) cases of acral lentiginous melanoma as well as nodular melanoma followed by lentigo malignant melanoma (20.51%), superficial spreading melanoma (17.95%), which differs from the results of Won et al[14], Lee et al[11] and Chun et al.[13]

In our study, 10 out of 39 cases (25.64%) had tumor thickness in situ melanoma. The highest no. of patients (35.90%) had tumor thickness 1-2 mm followed by (20.51%) cases had 1-2 mm, (17.95%) cases had <1 mm however not a single patient had tumor thickness >4 mm in our study which differs with the study observed by (Kyuangwook Nam et. al.) according to this, other study.

Our study showed no statistically meaningful difference between sex and stage (*P*=0.352), age

and stages (*P*=0.389) and location and stage (*P*=0.399) in relation to demographic and epidemic properties. There was however a major link between histopathology and staging (*P*=0.03). Studies have also demonstrated that nodular melanoma is seen more frequently than acral lentiginous melanoma (*P*=0.002) and superficial spreading melanoma (*P*=0.004). Approximately the same results were observed in the study (Kuang Wook Nam et. al.) In this study, the Clark IV-V-level death rate was about three times as high as the low invasive tumor which was statistically significant (*P*=0,029). According to the Kalpan-Merier analysis, we demonstrated a significant difference in the survival rate depending on the level of invasion that the KuangWook Nam et al study assumes.

Conclusion

Trunk was the most common tumor location followed by lower extremity. The most frequent subtypes were nodular melanoma and Acral melanoma. Females are more commonly affected than males 2:1. A relationship was found between stages and Clark level, especially stage III-IV tumors with IV-V Clark level, and correlated with high mortality rates.

Limitation

However, due to the small number of cases future clinical studies with large sample sizes, randomization, grouping, and long-term monitoring periods are required in order to conclude these findings more precisely.

Conflict of interest

We have no conflict of interest to disclose that the process and results of this study are not affected by the relevant equipment, materials, and pharmaceutical companies.

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