Comparison of the effects of C-KIT, MMP-2, Ki-67, Bcl-2 and metallothionein expression on prognostic factors in skin and non-skin malignant melanomas

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Research Article

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Abstract

Objectives: We aimed the relationship between with selected prognostic factors of MM, 1) C-KIT (CD117), which related to MM pathogenesis, 2) Ki-67, which is the determinant of cell kinetics, 3) MMP-2, which degradation of the basement membrane and extracellular matrix required for cell migration, invasion and metastasis in melanogenesis, 4) bcl-2, which reduces the apoptotic response to cytotoxic chemotherapy and 5) MT's whose overexpression shows resistance to anticancer drugs and radiotherapy and thus poor prognosis.

Material and Methods: In this study, 30 skin and 15 non-skin MMM cases diagnosed between January 01, 2000 and June 01, 2011 in SCU-TF Medical Pathology Department were examined. C-Kit, MMP-2, Ki-67, bcl-2 and MT markers were applied to these cases.

Results: When comparing the skin and non-skin groups, the difference between Ki-67 and MMP-2 expressions was found to be statistically significant, but no significant difference was found in staining with c-Kit, MT and bcl-2. It was thought that high MMP-2, C-KIT, bcl-2 expressions and high Ki-67 index as well as loss of MT expression might be associated with poor prognosis. Despite the high level of KIT immunostaining in melanomas, this parameter does not appear to be a good predictor of the presence of molecular mutations. Mutations that activate KIT should be considered a rare event in this tumor.

Introduction

MM is one of the most important malignant tumors of the skin, with a high mortality rate and high invasion capacity. It can also arise from mucosal surfaces. These surfaces include oral and anogenital mucosa, sinonasal mucosa, esophagus, intestines, meninges, all layers of the eye and bladder [1-3].

Major prognostic factors of malignant melanoma are Breslow thickness, tumor size (largest tumor size and others), ulcer, histological type, Clark level in the skin, tumor infiltrating lymphocytes, lymphovascular invasion, perineural invasion, and regression. The first three of these (Breslow thickness, largest tumor size and ulcer) together with lymph node and distant organ metastases determine the pathological stage according to the TNM classification.

In the late 1980s, Holzmann B et al. proposed a model of melanoma in which benign melanocytes gradually evolved into melanoma cells with metastatic capability, in which every step was defined by the acquisition or loss of certain cellular markers that were easily detected by immunohistochemical analysis[4,5]. Of these, c-Kit (CD117) is a growth factor for melanocyte migration and proliferation. It shows different staining properties in various benign and malignant melanocytic lesions. While different rates of c-Kit expression were observed in some studies, the development of melanoma was found to be associated with loss of c-Kit expression in some studies [6,7].

Degradation of basement membranes and extracellular matrix is an essential step for melanoma cell migration, invasion, and metastasis formation in melanogenesis. Matrix metalloproteinases and their

tissue inhibitors play a crucial role in these complex multistep processes. Melanoma cells may express a number of matrix metalloproteinase family members (MMP-1, MMP-2, MMP-9, MMP-13, and MT1-MMP) as well as their tissue inhibitors (TIMP-1, TIMP-2, and TIMP-3)[8]. The combination of determining MMP-2, Ki67, and p53 immunoreactive proteins could be beneficial in the selection of high-risk melanoma patients for future adjuvant trials.Increased expression and functionally active of MMP-2 are associated with the progression of melanoma[9].

Ki-67, a determinant of proliferation kinetics, is not expressed in resting G0 cells of the cell cycle, but is expressed in G1, S, G2 and M phases. Therefore, the Ki-67 level is expected to be high in many aggressive MM cases[10-12].

Programmed cell death (apoptosis) has been implicated in tumor development and may affect the metastatic potential of tumor cells. The role of bcl-2, a proto-oncogene that inhibits apoptosis, has been studied in several malignancies, including cutaneous melanoma (CM). It is a family of pro- and anti-apoptotic proteins, regulates mitochondrial membrane permeability and proapoptotic mitochondrial pathway[13]. Dysregulation of Bcl-2 proteins appears of critical importance for melanoma cell survival and drug resistance, and among the fast increasing number of new targeted therapies, those, which affect Bcl-2 proteins, may especially apply for melanoma[14,15].

Metallothioneins (MT) are intracellular, low molecular weight, cysteine rich proteins. Its overexpression, which can be seen in many tumors, indicates resistance to anticancer drugs and radiotherapy and poor prognosis. A similar situation is also present in melanoma and non-melanoma skin tumors[16-18].

In our study, the relationship of these markers with selected prognostic factors (histological type, Breslow thickness, mitotic index, ulceration and localization) in cutaneous and mucosal malignant melanomas was investigated.

Materials And Methods

Ethics statement. Ethical approval for the study was obtained from Sivas Cumhuriyet University Scientific Research Assessment Board dated 04.12.2012 and numbered 2012-12/13. Institutional Review Boards of all participating institutions approved the study protocols and written informed consent was obtained from all study participants. Research methods of procedures were carried out in accordance with relevant guidelines and regulations.

30 CMM and 15 MMM cases diagnosed in SCU-TF-PD between 01 January 2000 and 01 June 2011 were included in the study. C-Kit, MMP-2, Ki-67, bcl-2 and MT markers were applied to these cases. The relationship of these markers with selected prognostic factors (histological type, Breslow thickness, mitotic index, ulceration and localization) was investigated.

All H&E sections of FFPE tissues were removed from the archive. These were re-examined and sections containing melanoma foci suitable for research were determined. FFPE blocks belonging to the selected

sections were detected. From these paraffin blocks, 3 µm thick sections were cut on slides covered with Poly-L-Lysine. Sections (Strept-) Avidin-Biotin-peroxidase (ABC-technique) with MMP-2 (Abcam, Mouse Monoclonal Antibody, clone 4D3, 1:20 dilution), bcl-2 (Novocastra, Mouse Monoclonal Antibody bcl-2 Oncoprotein , clone bcl-2/100 / D5, ready-to-use form), MT (Invitrogen, Mouse Monoclonal Antibody, clone E9, ready-to-use form), Ki-67 (Biocare, Rabbit Monoclonal Antibody, clone SP6, ready-to-use form), c -Kit (Biogenex, Mouse Monoclonal Antibody, clone T595, ready-to-use form) immunohistochemistry dyes were applied[19].

Evaluation

Histological evaluation was performed by re-examining all H&E stained preparations of the cases, NICON, OPTIPHOT, JAPAN under the light microscope.

Information on the localization of the tumor was obtained from the SCU-TF-PD report archive.

Histological type was specified as NM, SSM, ALM, LMM and other types.

Breslow thickness was classified as ≤ 1mm, 1.01–2.0 mm, 2.01–4.0 mm and> 4 mm under the same microscope.

The mitotic index was counted in the "hot area" where the mitotic activity was the highest, that is, in 4x400 area (in $mm^{2)}$ in our microscope and grouped as 0, (0.1–6) and 6.

It was stated that the ulcer was present or absent.

External control preparations were prepared for each IHC staining.

MMP-2 expression was in the form of cytoplasmic staining and it was evaluated as negative if there is less than 5% staining, weak positive if there is 5-20%, moderate positive if there is staining in 21-50% malignant cells and strong positive if there is> 50% staining[8].

Ki-67 expression was counted in the area of highest staining, given as a percentage. If staining <20%, it was considered as low expression, if ≥ 20% as overexpression[9].

Bcl-2 expression was also in the form of cytoplasmic staining, and staining less than 5% in tumor cells was accepted as 0 (negative), between 5-50% staining 1 (weak positive), and more than 50% staining 2 (strongly positive)[12].

MT expression is in the form of both cytoplasmic and nuclear staining. <10% staining in tumoral cells was considered negative, if there was more than 10% staining, it was accepted as overexpression. To prevent false positive results, 10% was taken as the cut-off value[14-16].

Cytoplasmic and membranous staining was sought for c-Kit expression. It was graded as 0 (negative) if there is no staining, if there is <10% (+), if there is 10-50% staining (++), if there is> 50% staining (+++) [6].

Results

The ages of the individuals in the CMM group varied between (3-89), with a mean (67.68 ± 16.17), and the mean age (49-88) of the individuals in the MMM group (69.84 ± 12.44). The difference between the two groups in terms of age was found to be insignificant (x^2: 0.41; p: 0.681; p> 0.05).

Again, 17 (57%) of the individuals in the CMM group are male, 13 (43%) are female; 7 (47%) of the individuals in the MMM group were male and 8 (53%) were female, and the difference between groups in terms of gender was found to be insignificant (x^2: 0.43; p: 0.508; p> 0.05).

Histological type: In CMMs, 22 cases (73.3%) were NM, 4 cases (13.3%) were SSM, 3 cases (10.0%) were LMM and 1 case (3.3%) was ALM. In MMM, 14 cases (93.3%) were in the NM type and 1 case (6.7%) was in the SSM type. When these groups were compared in terms of histological type, the difference between them was found to be insignificant (p> 0.05).

Breslow Thickness: 7 cases (23.3%) with Breslow thickness ≤ 1.0 mm in CMMs, 4 cases (13.3%) with 1.01-2.0 mm, 4 cases (13.3%) with 2.01-4.0 mm and 15 cases with> 4 mm (50%). In MMMs, there were 1 case (6.7%) each with ≤ 1.0 mm and 1.01-2.0 mm, 5 cases (33.3%) with 2.01-4.0 mm and 8 cases (53.3%) with> 4 mm. When CMM and MMMs were compared in terms of Breslow thickness, the difference between the groups was found to be statistically insignificant (p> 0.05).

Ulceration: While no ulcer was found in 13 cases (43.3%) in CMMs, ulcers were observed in 17 cases (56.7%). While there were no ulcers in 5 cases (33.3%) in MMMs, ulcers were observed in 10 cases (66.7%). When CMM and MMM were compared in terms of ulcer, the difference between the groups was found to be statistically insignificant (p> 0.05).

Mitosis: 13 cases (43.3%) without mitosis in CMMs, 15 cases (50%) with 0.1-6 mitosis and 2 cases (6.7%) with> 6 mitosis were detected. There were 4 cases (26.7%) without mitosis in MMMs, 9 cases (60%) with 0.1-6 mitosis, and 2 cases (13.3%) with> 6 mitosis. When CMM and MMMs were compared in terms of mitosis, the difference between the groups was found to be statistically insignificant (p> 0.05).

Localization: 11 (36.7%) of the CMMs were localized on the head and neck, 10 (33.3%) on the extremities (hands and feet), and 9 (30.0%) on the trunk. 4 (26.6%) of MMMs were conjunctiva, 4 (26.6%) were nasal passage, 2 (13.3%) were vagina, 2 (13.3%) were oral cavities, 1 each was intestine (%) 6.6), vulva (6.6%) and cervix (6.6%).

Ki-67 index: There were 19 cases (65.5%) with Ki-67 index <20% and 10 cases (34.5%) with ≥20% in CMMs. (In this group, Ki-67 stain could not be applied in one case due to technical reasons and it was excluded from evaluation.) In MMMs, there were 5 cases (33.3%) with Ki-67 index <20% and 10 cases (66.7%) with ≥20%. When CMM and MMM groups were compared in terms of Ki-67 index, the difference was found statistically significant (p <0.05) (see in Table 1).

MMP-2 Expression: 7 cases (24.1%) with <5% MMP-2 expression in their CMMs, 6 cases (20.7%) with 5-20%, 4 cases (13.8%) with 21-50% and 12 cases with ≥50% (% 41.4) was determined. (MMP-2 staining could not be applied in one case due to technical reasons and it was excluded from the evaluation.). There were no cases with MMP-2 expression rate <5% and 5-20% in MMMs. 7 cases (46.7%) with 21-50% staining and 8 cases (53.3%) with ≥50% staining were identified. When compared in terms of MMP-2 expression, the difference between groups was found to be statistically significant ($p < 0.05$) (see in Table 2).

bcl-2 Expression: 10 cases (33.3%) with <5% staining in their CMMs, 6 cases (20.0%) with 5-50% staining and 14 cases (46.7%) with ≥50% expression were determined. Among MMMs, 6 cases (40.0%) with <5% staining and 9 cases (60.0%) with 50% staining were determined. There were no cases showing 5-50% expression in MMMs. When CMM and MMMs were compared in terms of bcl-2 expression, the difference was found to be statistically insignificant ($p \geq 0.05$).

MT Expression: 23 cases (76.7%) with <10% staining in their CMMs, 7 cases (23.3%) with 10% staining; 9 cases (60.0%) with <10% staining in MMMs and 6 cases (40.0%) showing ≥%10 staining were detected. When compared in terms of MT expression, the difference between groups was found to be statistically insignificant ($p \geq 0.05$).

c-Kit Expression: No staining was detected in 8 (27.6%) of CMMs, <10% in 9 cases (31.0%), 10-50% in 7 cases (24%), and in 5 cases (17.2%) >%50 staining was seen at 50 percent. (Due to technical reasons, c-Kit staining could not be applied in one case and was excluded from the evaluation.) While no staining was detected in 3 (20.0%) of MMMs, <10% in 2 (13.3%) and 6 (40.0%) of them %10-50 and 4 (26.7%) of them > 50% staining was observed. When CMMs and MMMs were compared in terms of c-Kit expression, the difference was found to be statistically insignificant ($p \geq 0.05$).

Furthermore, when CMM and MMMs were compared in terms of Ki-67 expression according to their histological types, the difference between CMM cases was statistically significant ($p < 0.05$), and the difference between MMMs was found insignificant ($p > 0.05$). In the CMM group, in NM cases, 15 cases (.68.2%) with a rate of <20% were detected. In non-skin MM, in NM cases, 10 cases (71.4%) with ≥20% staining were observed (see in Table. 3).

When CMM and MMMs were compared in terms of MMP-2 expression according to their histological types, the difference was found to be statistically insignificant ($p \geq 0.05$). However, in 10 (45.5%) of the NM's in the CMM group, MMP-2 expression was detected at a rate of ≥50%. MMP-2 expression was ≥50% in 8 (57.1%) of the NM in the MMM group (see in Table 4).

When CMM and MMMs according to histological types were compared in terms of bcl-2 expression, the difference was found to be statistically insignificant ($p \geq 0.05$). However, bcl-2 expression was ≥50% in 10 (45.5%) of the NMs in the CMM group, while bcl-2 expression was ≥50% in 8 (57.1%) of the NMs in the MMM group (see in Table 5).

When CMM and MMMs according to histological types were compared in terms of MT expression, the difference was found to be statistically insignificant (p□0.05). However, while there were 17 cases (77.3%) with MT expression <10% in NM in the CMM group, 8 cases (57.1%) with MT expression <10% in NM in the MMM group were detected (see in Table 6).

When CMM and MMMs were compared in terms of c-Kit expression according to histological types, the difference was found to be statistically insignificant (p□0.05). On the other hand, 8 cases (36.4%) were detected with <10% staining in NM in the CMM group, and 10-50% was observed in 5 cases (35.7%) of the NM type in MMM (see in Table 7).

Discussion And Conclusion

The frequency of MM among all skin cancers is 3-5%. It is responsible for 75% of deaths due to skin cancers and 1-2% of deaths due to all cancers [1-3, 20]. It is stated that the incidence of whites in industrial countries has increased significantly in the last two decades, and nowadays its incidence has stabilized and even decreased. The incidence of melanomas is highest in Northern Australia. There are 42.89 new cases per 100,000 in women and 55.8 new cases in men every year. This rate is 8 in England, 24.4 in South Africa and 4.9 in Scotland. While the lifetime risk of melanoma development was 1 in 120 in the USA in 1987, this rate was reported as 1 in 75 in 2000 [21].

In a study conducted in Germany, the number of cases documented annually increased by 53.2% between 2002 (N = 4779) and 2011 (N = 7320). There was a statistically significant continuous positive trend in the proportion of stage UICC I cases diagnosed between 2002 and 2011 compared with a negative trend for stage UICC II. No trends were found for UICC III and IV stages, respectively [22].

Survival rates increase in patients with melanoma, as patients present earlier and with thinner melanoma. Five-year survival rates have been reported at 85% or more in women and 75% in men. Again, in another cohort analysis of 4791 patients diagnosed with primary CMM between 1976 and 2001 in southern Germany, primary CMM diagnosis during 1990-2001 was associated with a more favorable 10-year survival (88.6% versus 80.0%, P <.0001) compared with 1976-1989. Median tumor thickness at primary diagnosis was significantly lower in the second period (0.75 mm vs 1.07 mm, P <.0001). That is, tumor thickness has been found to be a dominant prognosis determinant [23].

In the study, the number of patients with ulcers (66%) and the number of cases with Breslow thickness □4 mm (53%) were higher in MMMs compared to CMMs. This situation may be associated with the worse prognosis in MMMs compared to skin melanomas.

In MMMs, there are many prognostic factors other than tumor thickness that have different importance. These are histological type, ulceration, mitotic index, presence of satellites, angiolymphatic invasion, advanced stage, occult metastases, local recurrence, Clark invasion depth, location, accompanying nevus,

lymphocytic infiltration, regression, nuclear volume, sex, vitiligo, age and can be counted as pregnancy [1-3, 24].

Also, risk factors for MM development include hair and skin color, the presence of multiple freckles, PUVA therapy, the presence of multiple large atypical nevi, genetic factors (CDK2A and CDK4 mutations), xeroderma pigmentosum, immunosuppression, chemicals, oil, and exposure to printer products. involves trauma (for subungual melanoma) and burns counted [1-3].

According to AJCC staging, three important histopathological criteria are Breslow thickness, ulcer and mitotic index (25). Age, gender, and anatomic location of the primary tumor that are not included in the AJCC system also affect the survival of primary MM cases (26,27). When these factors are combined with histopathological features, they show a better prognosis than AJCC system [24].

Since the late 1980s, immunohistochemistry markers such as Ki-67, c-Kit, MMPs, metallothionein, bcl-2, which contributed to the determination of treatment protocols, were added to these criteria (4-18). In this study, the number of cases with a high Ki-67 index (66%) was found to be higher in MMM cases compared to the CMM group. This situation may be associated with a worse prognosis in MMMs compared to skin melanomas.

c-Kit (CD117) is a tyrosine-kinase receptor typically found in GISTs and is also associated with the function and proliferation of melanocytes [28].

Gonzalez et al. showed that melanoma expresses c-kit, a gastrointestinal stromal tumor marker, but has not been extensively evaluated for protein kinase Cθ (PKCθ) or DOG1, and these stains have not been associated with prognostic factors. They immunostained 62 primary cutaneous and 15 metastatic melanomas for polyclonal c-kit (pc-kit), monoclonal c-kit (mc-kit), PKCθ, and DOG1, and correlated the results with prognostic parameters and survival. 34 (55%) of cutaneous melanomas were stained for pc-kit, 30 (48%) for mc-kit, 11 (18%) for PKCθ, and 2 (3%) for DOG1 [29].

Went et al. investigated C-KIT (CD117) expression in various tumors. For this purpose, the positivity of KIT in 28 of 28 GIST cases, 42 of 50 seminomas, 34 of 52 adenoid cystic carcinomas, 14 of 39 MM, 8 of 47 large cell carcinomas of the lung, as well as 47 other tumors. In addition, they investigated the KIT gene mutation in the same patient group. Among these cases, KIT gene mutation was found in 6 of 12 GIST cases analyzed and only 1 of 24 other tumors. Thus, it is important that they have shown that KIT gene mutation is rare in cases with c-Kit positivity due to IHC [30]. Ni et al. also reported that 7 out of 40 anorectal melanoma cases had a c-kit mutation, whereas 16 of 40 cases had CD117 expression, and 3 of these 16 cases also found c-kit mutations [31]. In the study of Alessandirini et al., C-KIT stains were documented in 48% of melanomas, 50% of PAMs and 24% of nevi. The mean score of KIT staining in the melanomas/PAMs group was significantly different from nevi (p=0.0076). No statistically significant differences were detected between either c-kit immunostaining score or pattern and each of the other

clinico-pathologic parameters considered. No KIT gene mutations were detected in melanomas and nevi. A silent mutation/polymorphism in KIT exon 13 was found in one PAM [32].

Liu et al. investigated c-Kit and Sox10 expressions in 28 patients with sinonasal mucosal melanoma, and determined c-Kit expression in 24 patients, therefore they emphasized that c-Kit expression may be useful in the regulation of treatment. However, they also stated that Sox10 is a sensitive determinant in SSMs [33]. In the study, c-Kit expression was detected in all conjunctival MM's. In addition, C-KIT expression was found in all but 3 of 11 nonconjunctival cases in the MMM group.

Weinlich et al. investigated the relationship between MT overexpression and prognostic factors in their second study, which included 1270 cases in 2006. While MT overexpression was determined in 310 cases (24.4%), MT was found negative in 960 cases (75.6%). The female / male ratio (51% / 49%) of the patients were close to each other, and the mean age at which lesions were excised was 54 years (7-95). During their follow-up, it was found that the disease progressed in 167 cases and death due to metastatic disease occurred in 110 cases. MT overexpression was detected in 117 of 167 cases and 80 of 110 cases in these groups. The proportion of MT positive melanomas increases with greater Clark invasion depth and Breslow thickness[17]. Accordingly, they also investigated whether MT overexpression was an independent prognostic factor or only a parameter correlated with increasing degree of invasion or tumor thickness. For this purpose, they found that MT overexpression was significant in a statistical model for disease progression ($p < 0.001$). In conclusion, MT overexpression is a strong, highly significant factor independent of tumor thickness, and determines and measures the risk of disease progression.

Weinlich et al. recently investigated the role of MT overexpression in sentinel lymph node biopsies in melanoma progression in 158 cases in 2007, and determined that metastases developed in 28 patients (17.7%), and 17 (10.7%) died from disseminated disease; demonstrated that the results support the validity of MT overexpression as a useful prognostic marker in patients with primary melanoma[18]. In the study, the number of cases with MT expression <10% was found to be higher in the skin and extradermal group than the cases with MT overexpression. However, most of the MT negative cases were histologically of NM type and Breslow thickness was higher. In addition, in the MMM group, in patients with low MT expression, ulcers were observed at a higher rate. These findings oft he study can be interpreted as indirectly, that the loss of MT expression indicates a serious prognosis, albeit a little.

Espindola and Corleta detected 74.3%, 85.7%, and 82.4% bcl-2 expression in lymph node, subcutaneous and visceral metastases, respectively, but after univariate and multivariate analyzes, positive bcl-2 expression and overall survival for the types of metastases evaluated were no correlation was found (13). In our series, bcl-2 expression among CMMs, 10 cases (33.3%) with <5% staining, 6 cases (20.0%) with 5-50% staining and 14 cases (46.7%) with ≥50% expression was found. Also, among MMMs, 6 cases (40.0%) with <5% staining and 9 cases (60.0%) with 50% staining were determined. There were no cases showing 5-50% expression in MMMs. When CMM and MMMs were compared in terms of bcl-2 expression, the difference was found to be statistically insignificant ($p \geq 0.05$).

Väisänen et al. in their CMM series of 157 cases, investigated the effect of MMP-2 expressions on Ki67 and p53 expression as well as survival, overexpression of the MMP-2 protein and high metastatic potential melanomas characterized by Ki67 and low MMP-2. They founded overexpression of the matrix metalloproteinase 2 protein in conjunction with overexpression of Ki67 characterized melanomas with high metastatic potential and was associated with declined survival with a 10-year disease-specific survival of 33% compared with 85% in the cases with low matrix metalloproteinase-2 and low Ki-67 levels ($p = .002$). Similarly, in cases with overexpression of matrix metalloproteinase-2 and a positive immunoreaction for p53, the 10-year disease-specific survival was only 42% compared with 80% in patients with matrix metalloproteinase-2 less than 20% and a negative immunostaining for p53 ($p < .001$). The presence of all 3 adverse prognostic factors was prognostically more significant than any marker alone with a 10-year survival of only 28%. This combination of determining matrix metalloproteinase 2, Ki67, and p53 immunoreactive proteins could be beneficial in the selection of high-risk melanoma patients for future adjuvant trials (9). In our series, 7 cases (24.1%) with <5% MMP-2 expression in their CMMs, 6 cases (20.7%) with 5-20%, 4 cases (13.8%) with 21-50% and 12 cases with 50% (% 41.4) was determined. There were no cases with MMP-2 expression rate <5% and 5-20% in MMMs. 7 cases (46.7%) with 21-50% staining and 8 cases (53.3%) with 50% staining were identified. When compared in terms of MMP-2 expression, the difference between two groups was found to be statistically significant ($p <0.05$).

As a result, when the CMM and MMM groups were compared, the difference between them was statistically significant in staining with Ki-67 and MMP-2, while staining with c-Kit, MT and bcl-2 was statistically insignificant. It was thought that high MMP-2, c-Kit, bcl-2 expressions and high Ki-67 index as well as MT expression loss were thought to be associated with poor prognosis when evaluated with associated histological prognostic factors. However, in order to precisely determine the prognosis of MMs and to determine more effective treatments, larger case series should be followed with molecular pathology studies.

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Tables

Table 1. Comparison of CMM and MMM in terms of Ki-67 index

Groups		Ki-67		Total
		<%20	≥%20	
CMM	N	19	10	29
	%	65.5	34.5	100.0
MMM	N	5	10	15
	%	33.3	66.7	100.0
Total	N	24	20	44
	%	54.5	45.5	100.0

X=4.13 , p=0.042, p<0.05

Table 2. Comparison of CMM and MMM in terms of MMP-2 expression

Groups		MMP-2				Total
		<% 5	% 5- 20	% 21- 50	>% 50	
CMM	N	7	6	4	12	29
	%	24.1	20.7	13.8	41.4	100.0
MMM	N	0	0	7	8	15
	%	0	0	46.7	53.3	100.0
Total	N	7	6	11	20	44
	%	15.9	13.6	25.0	45.5	100.0

X=11.3, p=0.010, p<0.05

Table 3. Comparison of CMM and MMMs according to their histological types in terms of Ki-67 expression

Groups				Ki-67		Total
				<% 20	≥% 20	
CMM	Histological Type	NM	N	15	7	22
			%	68.2	31.8	100.0
		SSM	N	0	3	3
			%	0	100.0	100.0
		ALM	N	1	0	1
			%	100.0	0	100.0
		LMM	N	3	0	3
			%	100.0	0	100.0
	Total		N	19	10	29
			%	65.5	34.5	100.0
MMM	Histological Type	NM	N	4	10	14
			%	28.6	71.4	100.0
		SSM	N	1	0	1
			%	100.0	0	100.0
	Total		N	5	10	15
			%	33.3	66.7	100.0

X□D=7.87, p=0.049, P<0.05

X□DD=2.14, p=0.143 , P>0.05

Table 4. Comparison of CMM and MMMs according to their histological types in terms of MMP-2 expression

Groups				MMP-2				Total
				<%5	%5- 20	%21-50	>%50	
CMM	Histological Type	NM	N	5	5	2	10	22
			%	22.7	22.7	9.1	45.5	100.0
		SSM	N	0	0	1	2	3
			%	0	0	33.3	66.7	100.0
		ALM	N	1	0	0	0	1
			%	100.0	0	0	0	100.0
		LMM	N	1	1	1	0	3
			%	33.3	33.3	33.3	0	100.0
	Total		N	7	6	4	12	29
			%	24.1	20.7	13.8	41.4	10.0
MMM	Histological Type	NMM	N			6	8	14
			%			42.9	57.1	100.0
		SSM	N			1	0	1
			%			100.0	0	100.0
	Total		N			7	8	15
			%			46.7	53.3	100.0

X□D=8.69, p=0.466, P>0.05

X□DD=1.22, p=0.268 , P>0.05

Table 5. Comparison of bcl-2 expression of CMM and MMM according to histological types

Groups				bcl-2			Total
				<%5	%5-50	>%50	
CMM	Histological Type	NM	N	9	3	10	22
			%	40.9	13.6	45.5	100.0
		SSM	N	1	2	1	4
			%	25.0	50.0	25.0	100.0
		ALM	N	0	0	1	1
			%	0	0	100.0	100.0
		LMM	N	0	1	2	3
			%	0	33.3	66.7	100.0
	Total		N	10	6	14	30
			%	33.3	20.0	46.7	100.0
MMM	Histological Type	NM	N	6	0	8	14
			%	42.9	0	57.1	100.0
		SSM	N	0	0	1	1
			%	0	0	100.0	100.0
	Total		N	6	0	9	15
			%	40.0	0	60.0	100.0

X□D=5.78, p=0.448, P>0.05

X□DD=0,71, p=0.398 , P>0.05

Table 6. Comparison of CMM and MMMs in terms of MT expression according to their histological types

Groups				MT		Total
				<%10	>%10	
CMM	Histological Type	NM	N	17	5	22
			%	77.3	22.7	100.0
		SSM	N	2	2	4
			%	50.0	50.0	100.0
		ALM	N	1	0	1
			%	100.0	0	100.0
		LMM	N	3	0	3
			%	100.0	0	10.0
	Total		N	23	7	30
			%	76.7	23.3	100.0
MMM	Histological Type	NM	N	8	6	14
			%	57.1	42.9	100.0
		SSM	N	1	0	1
			%	100.0	0	100.0
	Total		N	9	6	15
			%	60.0	40.0	100.0

X□D=2.81, p=0.422, P>0.05

X□DD=0.71, p=0.398 , P>0.05

Table 7. Comparison of CMM and MMM according to histological types in terms of c-Kit expression

Groups				c-Kit				Total
				0	< %10	% 10-50	>% 50	
CMM	Histological Type	NM	N	7	8	3	4	22
			%	31.8	36.4	13.6	18.2	100.0
		SSM	N	0	1	1	1	3
			%	0	33.3	33.3	33.3	100.0
		ALM	N	1	0	0	0	1
			%	10..0	0	0	0	100.0
		LMM	N	0	0	3	0	3
			%	0	0	100.0	0	100.0
	Total		N	8	9	7	5	29
			%	27.6	31.0	24.1	17.2	100.0
MMM	Histological Type	NMM	N	3	2	5	4	14
			%	21.4	14.3	35.7	28.6	100.0
		SSM	N	0	0	1	0	1
			%	0	0	100.0	0	100.0
	Total		N	3	2	6	4	15
			%	20.0	13.3	40.0	26.7	100.0

X□D=14.80, p=0.097, P>0.05

X□DD=1.60, p=0.658 , P>0.05