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Expression of cyclooxygenase-2 in melanocytic skin lesions by immunohistochemistry

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Abstract

Introduction: cyclooxygenase-2 (COX-2) is involved in pathogenesis of various tumors and possibly malignant skin tumors including malignant melanoma; however, there is not enough information about COX-2 expression in benign melanocytic lesions. In the present study we compared the expression levels of COX-2 in malignant melanoma and benign melanocytic lesions.
Method: In this analytical study, 42 malignant melanoma from all 4 subtypes and 38 benign melanocytic lesions including dysplastic nevus, Spitz nevus and atypical nevus were evaluated for COX-2 expression using immunohistochemistry staining and intensity of cell staining (quantitatively and qualitatively).
Results: Malignant melanoma compared to benign melanocytic lesions had significantly higher levels of staining ($p<0.001$), and much more intense coloration ($p<0.001$) and higher overall staining score ($p<0.001$). Regarding the COX2 staining between malignant melanoma subtypes, all four subtypes mostly had staining intensity over 60%. Also in terms of quality, the most intense staining was in the ALM ,and LM and NM had moderate staining intensity. With increasing depth of involvement, Cox2 staining increases. Intensity of cell staining was also higher in cases with tumor depth of 2-4 mm.
Conclusion: The results indicates the effectiveness of COX2 in differentiating between malignant melanoma and benign melanocytic lesions. COX2 expression correlated with the depth of invasion. Although COX2 alone can not be used to differentiate melanoma from benign lesions, it can be helpful in combination with other methods,in determining the prognosis and future targeted therapies.

Keywords: Cycloxygenase-2, Malignant melanoma, Benign melanocytic lesion, Immunohistochemistry

Introduction

Melanoma is a malignancy arising from melanocytes.The overall rate of mortality caused by this disease has been increasingover recent decades. It is one of the most common malignancies in young adults and about 20 percent of these patients are suffering from metastatic disease, resulting in their death. Early detection and appropriate surgery of such lesions in the early stages leads to full recovery in 90% of patients (1).

Immunohistochemistry is used for diagnostic evaluation when thediagnosis of early melanoma is difficult. It is also applicable for metastatic tumors of unknown origin.A wide variety of antigens associated with melanoma including GP100 / HMB45, tyrosinase, and MART-MELAN have been used for the diagnosis (2). Today, surface receptors involved in cell adhesion and cell cycle regulating genes or gene products are being widely evaluated as biological markers (3).

Cyclooxygenase (Cox) are among the enzymes involved in the metabolism of arachidonic acid and in the production of prostaglandins.COX-2 is the majorCox isoenzymewhich reacts to UV rays and plays a role in UV-induced skin inflammation and apoptosis (4).Its main consequence is PGE2which is involved in the development of malignancies associated with the Cox2expression.In fact, PGE2 suppresses lymphocyte proliferation and activates the NK Cells. These factors are necessary for host resistance against tumor progression (4).

Cyclooxygenase -2 (COX-2) is an inducible enzyme which is involved in the production of prostaglandins during multiple inflammatory processes. It was also reported that COX-2 has a role in the pathogenesis

of cancer in various body organs includingcolorectal, gastric, breast, bladder and lungcarcinoma.

Similarly, several studies have revaled the COX-2 expression in malignant skin tumors such as squamous cell carcinoma, basal cell carcinoma, Bowen disease, actinic keratosis and malignant melanoma (9-4).More recently, COX-2 has been suggested as a marker for malignant melanoma (11, 10).

It is reported that COX-2 inhibitors or non-steroidal anti-inflammatory drugs (NSAIDS) are effective in the treatment of malignant melanoma (13 and 12), other skin malignancies (15 and 14), and other types of carcinomas (19-16).

With regard to the fact that various studies with contradictory results have been conducted in this regard around the world and given the importance of this issue, this study aims to examine the Cox-2 expression in malignant melanoma lesions for patients in the region and to compare the results with the Cox-2 expression in benign melanocytic lesions.

Materials and methods

Study design and sampling:

The participants in this cross-sectional analytic study consisted of 42 casesof malignant melanoma from four subtypes and 38 benign melanocytic lesions including dysplastic nevus, Spitz nevus and non-atypical moles taken from a pathology laboratory in Azerbaijan and Sinai Hospital Pathology Laboratory (2002-2012).

Using random numbers table, the samples were selected from the samplesavailable in the archivesof two above laboratorieswith definite diagnosis of benign and malignant melanocytic lesions and after reexamination of slides and confirmed diagnosis accuracy.

In order to determine the sample size, the results of a study conducted by Minami et al. (2011) were used.In this study, the average COX2 expressionsfor malignant and benign melanoma groups are22 ± 72 and 16 ± 53, respectively. Assuming α=.05 and β=80% and 5 unit differences between two groups, 36 samples were estimated for each group. Regarding the likelihood of sample loss and the study reliability, a total of 60 patients wasconsidered for this study.

Inclusion criteria of the study:

1. Consistency between the primary pathological diagnosis and reexamined diagnosis of samples.
2. The availability of patient records to enter personal information.

Exclusion criteria includingintervening variables:

1. A history of internal malignancy and other skin malignancies.

Procedure of the study:

A total of 42 paraffin blocks with a pathological diagnosis of melanomawere selected from the archives of the pathology sectors of thelisted laboratories (with equal size from each four subtypes) and tumor thickness and the level of involvement was determined based on Clark level after cutting and staining H & E andreconfirmation of the diagnosis by two pathologists.

A total of 38 benign melanocytic lesions including dysplastic nevus, Spitz nevus and non-atypical moles were also selected and reexamined through using the above-mentioned method.

Formalin-fixed paraffin blocks with3 microns thickness were again cut and transferred into a microwave through using EDTA buffer.After several steps of washing with buffer (TBS), the slides were covered with primary monoclonal COX2 antibody and placed in a moist chamber. Then,the tissue surfaces werecoated by secondary antibodies andperoxidase-labeled polymer. Finally, tracking was done and observed by using EnVision (DakoCytomation Protocol) and staining.

Immunohistochemistry staining wasquantitatively scored;six separate fields from each samplewere observed with a magnification of 60 and 100 cells were counted in each field. For example, in the case of malignant melanoma, two epidermal fields, 2 dermal field and 2 fieldsof the middle and deep dermis were selected.

In the case of non-atypical moles,only two epidermal and dermal fields were estimated and the overall average was calculated by examining different

regions. The criterion used for being positive was at least 10% of the tumor cells being stained.In fact, Cox-2 Immunohistochemistry staining was performed for all samplesand its intensity was quantitatively mentioned.

For positive cases, scoring was classified into score 1 (10%-30%), score 2 (31%-60%), and score 3 (61% and above) and the staining intensities of mild (+), moderate (++) and severe (+++)(in the tumor cellsand surrounding inflammatory cells) were recorded and they finally were determined asscores 2-6 (total of staining intensity and percentage).

Then, Cox-2 expression intensity differences were determined between benign and malignant groups, various subtypes of melanoma and based on the malignant melanoma depth.

Ethical considerations:

All information obtained from patients in this study was confidential.This study focuses on the patient's previous pathological sample and all ethical issues related to the use of documents and patient records were met. Given that there was no intervention in this studyand that diagnostic and therapeutic processes are not required for the patients; therefore, there was no need to obtain the written consent of the patients and their verbal consents were obtained only by calling them (using telephone numbers registered in their records).

Statistical Analysis:

All data were analyzed by using the SPSS software version 17.For statistical analysis, descriptive statistics (frequency, percentage) were used.To compare the quantitative data, the independent t-test was used for normally distributedcases. Furthermore, to compare the qualitative findings, Chi square test or Fisher's exact test were used if needed.In this study, $p \leq .05$ was considered significant.

Results

In this study, 80 patients (34 males and 46 females) with an average age of 49.37 ± 24.67 years and suffering from melanocytic skin lesions (24patients with malignant and 38 patients with benign melanocytic lesions) were examined.

Figure 4-1 shows the age distribution of patients in two groups. As it can be observed, patients with malignant melanoma were significantly of higher average age (p<.001).

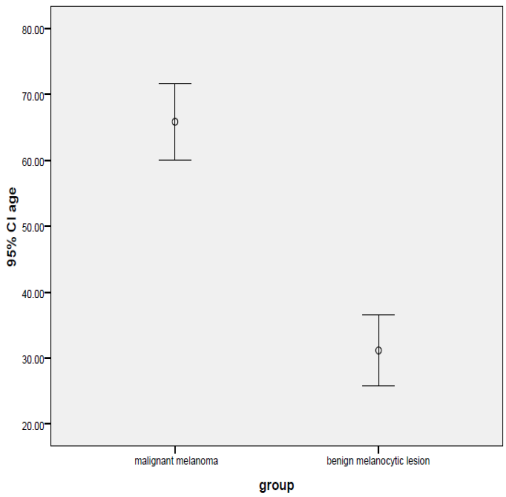

Figure 4-1: Distribution of mean age of patients in two groups.

There were 24 males (57.1%) and 18 females (42.9%) in the malignant melanoma group. Another group of patients with benign melanocytic lesions consisted of 10 males (26.3%) and 28 females (73.7 percent). It is observed that male patients had higher rates of malignant melanoma lesions (p= .005). Regarding the frequency, the subtypes of malignant melanoma groupwereNodular melanoma (NM) in 24 cases (57.1%), AcralLentiginous melanoma (ALM) in 8 cases (19%), Superficial Spreading melanoma (SSM) in 6 cases (14.3%), and LentigoMalignant melanoma (LMM) in 4 cases (9.5%), respectively.

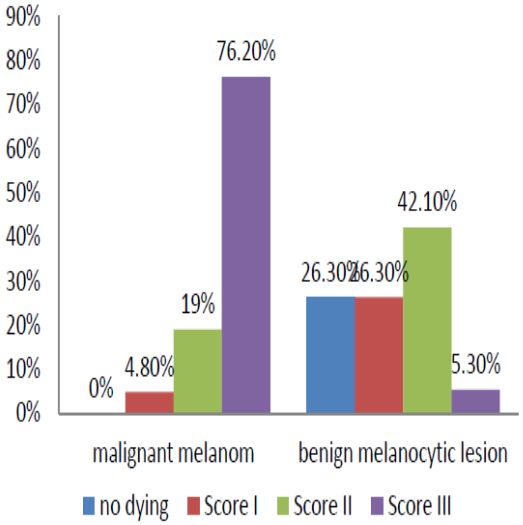

Figure 4-2: Staining percentage of the Cox2markers for two groups.

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Considering the level of involvement based on the Clark level for the malignant melanoma group, there were 6 cases (14.3 %) with level II (papillary dermis invasion), 8 cases (19%) with level III (invasion up to the reticular dermis surface), 28 cases (66.7%) with level IV (deep reticular dermis invasion).

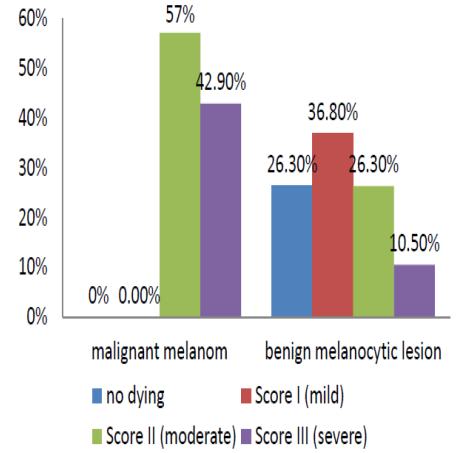

Figure 4-3: Cell staining for two groups.

Malignant melanoma tumor thicknessesdetermined by theBreslow method were 1-2 mm in 10 cases (23.8%), 2-4 mm in 16 cases (38.1%), and above 4 mm in 116 cases (38.1%). Figure 4-2 shows the stainingpercentage of theCOX-2 marker for two groups determined by theEnvision method.The malignant melanoma group had significantly higher percentage of staining; however, all samples without staining were in the benign melanocytic lesion group (p<.001).

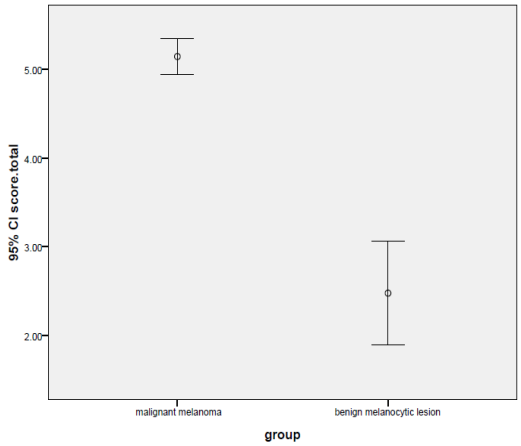

Figure 4-4: Total scores calculated in two study groups

Figure 4-3 also shows the stainingof cells for two groups.As it is shown, the malignant melanoma group all have moderate to severe cell staining; however, in another group of patients with benign melanocytic lesions, the cells are mainly of no staining or mild staining(p<.001).

Figure 4-4 also presents the total calculated scoresfor two study groups.As it can be seen, the malignant melanoma group had a significantly higher score resulting in higher staining intensity(p<.001).

Table 4.1 shows thestainingpercentage of the COX2marker as well as the cell staining intensity of malignant melanoma subtypes.As it can be seen, all subtypes of malignant melanoma are mainly ofstaining intensity above 60%. Regarding the cell staining, the most severe staining intensity was related to the ALM group; however, the moderate staining intensity was observed in the LM and NM groups.

Table 4-2 shows the staining percentage ofthe COX-2 markerand the cell staining intensity based on the depth of tumor invasion.As it is shown,increased depth of involvement enhances thestaining percentage of the COX-2 marker. The cell staining intensity was also higher in 4-2 mm-depth of invasion.

Discussion

COX-2 is inducible form of cyclooxygenasenzymes that associated withpathological states, including cancer.There is a substantial body ofevidence indicating that the COX-2 may be involved in cancers ofepithelial origin.it is observed that COX-2 is associated with invasion and prognosis in some tumors (23).Moreover, the potential role of COX-2 in epithelial and melanocytic skin cancers is not improbablesince the COX-2 is usually expressed in malignant melanoma (27 and 4) andsquamous cell carcinoma (29 and 28).

Kuźbicki et al. foundthat changes in the expression levels of the COX-2 is associated with the development and progression of human melanoma and the COX-2 proteincan be a prognostic factor and predictive marker in malignant melanoma (10). Goulet et al also stated that the COX-2 can have a functional role in melanoma metastases and treatment with the use of COX-2 inhibitors may be effective for the caseswith malignant melanoma (8).

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In the present study, we dealt with the COX-2 staining intensityin patients with malignant melanoma and benign melanocytic lesions. we evaluated expression of COX-2 in malignant melanoma specimens in different subtypes by immunohistochemical method.In this study, it was found that the staining intensitypercentage of the COX-2 andstaining intensity of cells in malignant melanoma was significantly higher than those in melanocytic lesions.In benign lesiongroup,there was no staining for the COX-2in 26.3% of cases.

In one of the first studies conducted on this issue, Denkert et al. observedthat benign moles such as normal epithelium were negative with respect to the COX-2expression (4). In a similar vein, Minami et al. claimed that there was a significant statistical difference between the malignant melanoma and benign melanocytic lesion groupswith regard tothe COX-2expression level.Staining intensity in cases of malignant melanoma, compared tothe benign lesion group,was greater (2).

Lee et al. also observed a marked increase in staining the COX-2 and PPARγin melanoma compared with benign lesions (27).Chwirot and Kuźbicki's study also indicated that the average expression of COX-2 in melanoma was significantly greater, in comparison with benign lesions.It was also found that the COX-2, with high sensitivity and specificity, provides the grounds fordistinguishing between melanomain the early stages andbenign melanocytic lesions (11).

The results of the above studies indicate that the mentioned marker can be used for the differential diagnosis of malignant melanoma and the diseaseprognosis.

In the present study, it was also observed that all subtypes of malignant melanoma are mainly of staining intensity above 60%. Regarding the cell staining, the most severe staining intensity was related to the ALM group; however, the moderate staining intensity was observed in the LM and NM groups. Furthermore, increased depth of involvement enhances the staining percentage of the COX-2 marker. The cell staining intensity was also higher in 4-2 mm-depth of invasion.

Similarly, Minami et al. also concluded that the staining intensity of dermal components of malignant melanoma is enhanced by increased tumor depth (2).

Meyer et al. also statedthat the COX-2 immunoreactivity significantly increase from nevi to primary MM and metastases,also COX-2 positivitywas correlated withshorter survival and the advancedClark level. The results of this implied that the COX-2 expression is associated with increased risk of tumor recurrence (30).

Beker etal. analysed the expression of COX-2 in 101 primary melanoma and 28 metastases and correlated their data withbreslow tumor thickness,clark levels,different melanoma subtypes,metastases and survival.They detected a strong COX-2 expression in 95% of all primary melanoma. A significant correlation between IHC staining intensity and tumor thickness was demonstrated,furthermore, a significant correlation was seen between staining intensity and survival. They concluded that COX-2 might be a novel prognostic marker and also a therapeutic marker(31)

Denkert et al.also concluded that the COX-2 expression was moderate to severe in 68% cases(4).On the contrary, in the present study, 100% of cases with malignant melanoma were ofmoderate to severestaining intensity.

However, Kuźbicki et al. claimed that the COX-2 expression level isindependent from the histopathological melanomastage and type. In general,these results indicate the fact that the changes in the COX-2 expression level are associated with the development and progression of human melanoma (10).

Conclusion

The results of the present study show the effectiveness of the COX-2 role in the identification of malignant melanoma cases from benign melanocytic lesions. The COX-2 staining intensity is also related to the depth of invasion.Although,the COX-2 cannot alone be used to differentiate melanoma, it can be helpfulincombination with other diagnostic methods for the disease prognosisand future therapies.

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Table 4-1: Staining percentage of COX-2 of the marker and the cell staining intensity of malignant melanoma subtypes

Variables		ALM	NM	LM	SSM
Staining percentage of COX-2	10-30% (I)	0	2 (8.3%)	0	0
	31-60% (II)	0	4 (16.7%)	2 (50%)	2 (33.3%)
	60%≤ (III)	8 (100%)	18 (75%)	2 (50%)	4 (66.7%)
Cell staining intensity	Moderate (II)	2 (25%)	16 (66.7%)	4 (100%)	2 (33.3%)
	Severe (III)	6 (75%)	8 (33.3%)	0	4 (66.7%)

Table 4-2: Staining percentage of the COX-2 markerand the cell staining intensity based on the depth of tumor invasion

Variables		1-2 mm	2-4 mm	4≤ mm
Staining percentage of COX-2	10-30% (I)	0	0	2 (12.5%)
	31-60% (II)	4 (40%)	4 (25%)	0
	60%≤ (III)	6 (60%)	12 (75%)	14 (87.5%)
Cell staining intensity	Moderate (II)	6 (60%)	8 (50%)	10 (62.5%)
	Severe (III)	4 (40%)	8 (50%)	6 (37.5%)

Conflicts of interest

Authors declare no conflict of interests.

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