

Immunohistochemical expression of MMP9, as a marker of local invasion in Hodgkin's and Non-Hodgkin's lymphoma of the head and neck region

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ABSTRACT

Background: Malignant lymphoma is the generic term given to tumors of the lymphoid system and specifically of lymphocytes and their precursor cells, While all lymphomas are malignant neoplasms, there is a wide spectrum of clinical behavior, with some following an indolent clinical course and others behaving in an aggressive manner (will causing death in a short time frame if left untreated). The metastatic process involves intravasation and extravasation of tumor cells, followed by reimplantation of tumor cells, formation of a new tumor stoma, Degradation of the extracellular matrix and components of the basement membrane by proteases facilitates the detachment of tumor cells, their crossing of tissue boundaries, and invasion into adjacent tissue compartments. The importance of tumor-associated proteases in invasion and metastasis has been demonstrated for a variety of solid malignant tumors. MMP family has been implicated in tumor cell invasion and metastasis.

Material and methods: This study included 67 formalin-fixed, paraffin-embedded histopathologically diagnosed lymphoma blocks (head and neck lesions), 24 Hodgkin's Lymphoma and 43 Non Hodgkin's Lymphoma. Immunohistochemical (IHC) evaluation of MMP-9 monoclonal antibodies, in relation to the clinicopathological parameters was assessed.

Results: The mean of expression of MMP-9 in NHL was higher (65.4 ± 18) than that in HL (56.7 ± 21.4) though it did not reach the level of statistical difference $P > (0.05)$, while the mean of expression of MMP-9 in relation to tumor grades was different as it had a value of (72 ± 14.1) in low grade tumors, (68.3 ± 15) in the intermediate grade tumor and (61.4 ± 20.7) in high grade tumors; consequently, this difference did not reached the level of statistical significance $P(\text{ANOVA}) > 0.05$. MMP 9 had no statistical significant correlation in regard to its mean in HL subtypes.

Conclusions: This study had shown that there was no significant correlation between age and mean of expression of MMP-9 in HL and NHL. There was no statistical significant difference in the mean of expression of MMP-9 between HL and NHL subtypes.

Keywords: MMP-9, local invasion, Hodgkin's lymphoma, Non Hodgkin's Lymphoma. (J Bagh Coll Dentistry 2015; 27(1):128-132).

INTRODUCTION

Malignant lymphomas can be divided into two major categories: HL and NHL. Lymphomas presenting in extranodal sites of the head and neck and these sites includes oral cavity, oropharynx, nasopharynx, paranasal sinuses, and larynx which are mainly NHLs of low or high grade, it may also present as cervical lymphadenopathy which is the most common head and neck presentation for both diseases ^(1,2).

The metastatic process involves intravasation and extravasation of tumor cells, followed by reimplantation of tumor cells, formation of a new tumor stoma, and neoangiogenesis to consolidate a secondary tumor at a distant site ⁽³⁾. Degradation of the extracellular matrix and components of the basement membrane by proteases facilitates the detachment of tumor cells, their crossing of tissue boundaries, and invasion into adjacent tissue compartments.

The importance of tumor-associated proteases in invasion and metastasis has been demonstrated for a variety of solid malignant tumors. MMP family has been implicated in tumor cell invasion and metastasis. As tumors vary considerably in their behavior; the degree of their differentiation and ability to invade and metastasize are not the same, however, not all cancers have equivalent ability to metastasize. In general the more likely anaplastic and larger is the primary neoplasm, the more likely is the metastatic spread, however, exceptions abound, i.e. extremely small cancers have been known to metastasize and conversely some large ugly lesions may not spread ^(4,5). Because of the obscure and variable biological behavior of head and neck Hodgkin lymphoma this study will concerned with tumor dynamics such as MMP- 9 as a biological marker in HL and NHL of the head and neck.

The aim of this study is to evaluate the expression of the biological marker of local invasion (MMP-9) in Hodgkin's and Non-Hodgkin's lymphoma via immunohistochemical technique.

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MATERIALS AND METHODS

This study included (67) formalin-fixed, paraffin-embedded histopathologically diagnosed lymphoma blocks (head and neck lesions). The diagnosis of each case was confirmed by the histological examination of the Hematoxylin and Eosin staining (H&E), examined by two experienced pathologists. Demographic and clinical data provided by the surgeon were obtained from the case sheets presented with the tumor specimens, including information concerning patient's age, gender, clinical presentation, site of tumor. Histological classification was determined according to the International Working Formula (IWF) criteria, where the lesions classified into Hodgkin's lymphoma (24cases) and Non-Hodgkin's lymphoma (43 cases).

Positive tissue controls included in this study were Breast carcinoma for MMP-9. The diagnosis of each case was confirmed by the use of CD15, CD30 for HL and CD20, bcl2 for NHL. Sections of 5µm thickness were mounted on glass slides for routine (H&E), from each block of the studied sample and the control group for histopathological re-examination. Other sections of 4µm thickness were mounted on positively charged microscopic slides (Biocare medical and Fischre brand) to obtain a greater tissue adherence. All of these collected specimens were subjected to immunohistochemical staining using specific monoclonal antibodies included in the study MMP- 9.

Abcam, Expose mouse and rabbit specific HRP/DAP detection IHC kit, Antigen Retrieval Solutions: - Citrate buffer PH 6.0., Mouse monoclonal [ab58803] to MMP9 Abcam, England.

Immunohistochemical staining is accomplished with antibodies that recognize the target protein. Only the number of cells showing cytoplasm and the cell membrane expression of

MMP9 was quantified by counting at least 1000 cells in five representative fields at 40X objective in each case, the intensity of staining was not considered for evaluation. Tumor cells with brown staining of the cell membrane predominately or of the cytoplasm and the cell membrane occasionally were considered positive for MMP-9

The percentage of MMP-9 positively stained cells was semi-quantitatively determined as follows (-) negative ≤5%, (+) low 6-25%, (++) moderate 26-50% and (+++) high 51-100%.

Statistical Analysis

An expert statistical advice was sought for. Statistical analyses were done using SPSS version 21 computer software (Statistical Package for Social Sciences) in association with Microsoft Excel 2010.

RESULTS

The mean of expression of MMP 9 in NHL was higher (65.4±18) (Fig 1, 2 and 3) than that in HL (56.7±21.4) (Fig 4 and 5) though it did not reach the level of statistical significant difference $P > 0.05$, this result is shown in Table 1

Regarding NHL the mean of expression of MMP 9 in low grade was 72±14.1 and in intermediate grade the mean was 68.3±15 while in high grade it had a mean of 61.4±20.7 and even when the mean of MMP -9 was different between the grades of NHL but it did not reach the level of statistical significance. These details are found in Table 2.

In HL cases the mean of expression of MMP 9 in mixed cellularity (65.9±19.4) was higher than that of nodular sclerosing (53.3±22.2). Obviously there was a difference in the mean of expression but it did not reveal any statistical significance. Table 3 gives the difference in mean of expression of MMP 9 between nodular sclerosing and mixed cellularity.

Table 1: Difference in mean of expression of MMP 9 between HL and NHL

MMP9	NHL compared to HL		P (t-test)
	HL	NHL	
Range	(17 - 93)	(23 - 97)	0.08 [NS]
Mean	56.7	65.4	
SD	21.4	18	
SE	4.36	2.75	
N	24	43	

Table 2: Difference in mean of expression of MMP 9 between tumor grades of NHL

MMP9	Tumor grade			P (ANOVA) trend
	Low grade	Intermediate grade	High grade	
Range	(49 - 92)	(38 - 97)	(23 - 97)	0.21 [NS]
Mean	72	68.3	61.4	
SD	14.1	15	20.7	
SE	5.77	3.75	4.52	
N	6	16	21	

Table 3: Difference in mean of expression of MMP 9 between nodular sclerosing and mixed cellularity

MMP9	Morphology		P
	Nodular sclerosing	Mixed cellularity	
Range	(17 - 93)	(37 - 87)	0.21 [NS]
Mean	53.3	65.9	
SD	22.2	19.4	
SE	5.55	7.33	
N	16	7	

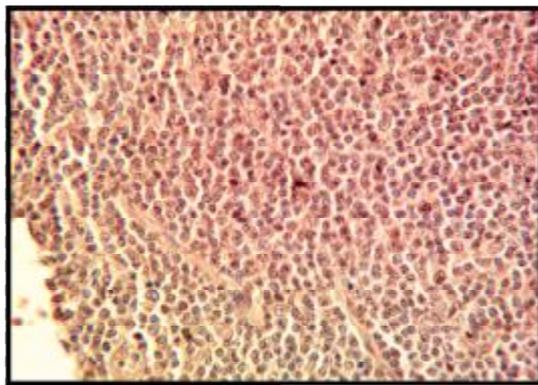


Fig 1: NHL DLBC MMP9 cytoplasmic expression (X400)

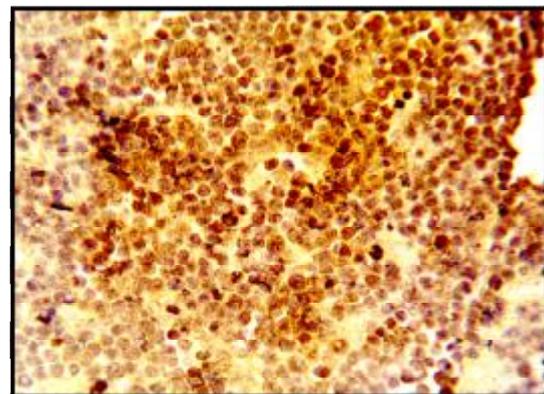


Fig 2: Burkitt's lymphoma MMP9 expression (X400)

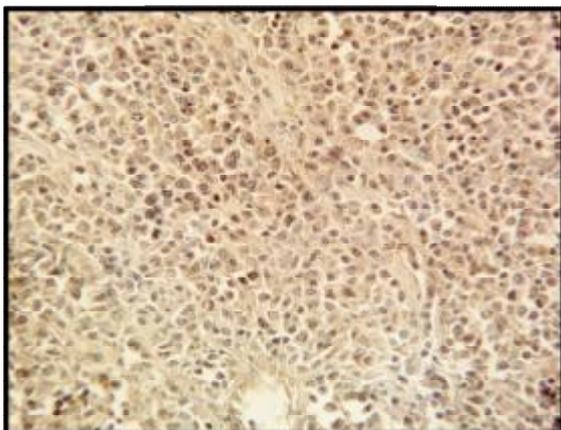


Fig 3: NHL Small cell type MMP9 cytoplasmic expression (X400)

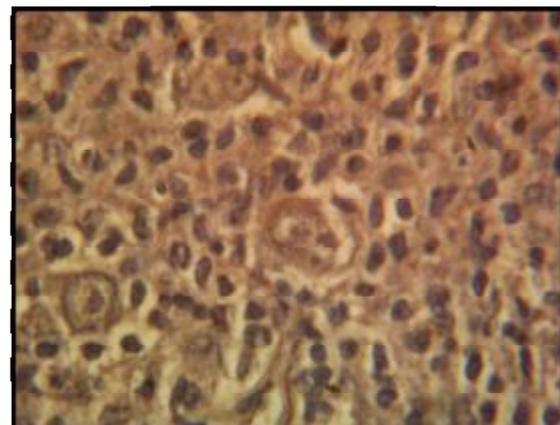


Fig 4: HL Mixed cellularity MMP9 expression in Hodgkin's cells. (X1000)

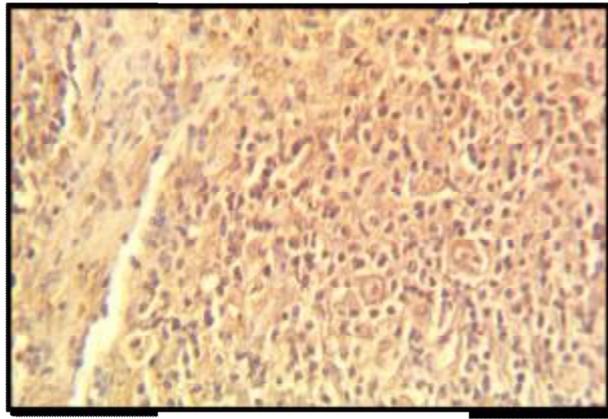


Fig 5: HL Mixed cellularity MMP9 expression in Hodgkin's cells. (X400)

DISCUSSION

Matrix metalloproteinase (MMP)-mediated degradation of the extracellular matrix is a key point in tumor development and expansion. A former study found elevated expression of MMP9 and MMP10 in endometrial tumor related endothelium.

Xue-lian du et al ⁽⁶⁾ stated that the metastatic process involves intravasation and extravasation of tumor cells, followed by reimplantation of tumor cells, formation of a new tumor stroma, and neoangiogenesis to consolidate a secondary tumor at a distant site. Degradation of the extracellular matrix and components of the basement membrane by proteases facilitates the detachment of tumor cells, their crossing of tissue boundaries, and invasion into adjacent tissue compartments. In recent years, the importance of tumor-associated proteases in invasion and metastasis has been demonstrated for a variety of solid malignant tumor.

One of the first observations that suggested a role for MMP-9 in tumor invasion relates to the fact that the release of MMP-9 is associated with the metastatic phenotype of transformed rat embryo cell 92,000 gelatinase release correlates with the metastatic phenotype in transformed rat embryo cells ^(7,8).

The increase in pro-MMP-9 in ovarian cancer and their corresponding metastases could be attributable to the infiltration of inflammatory cells that express a high amount of pro-MMP-9 and often surround malignant tumors that growth of liver metastasis from colon cancer was associated with elevated tumor tissues MMP-9, and treatment of ulinastatin significantly reduces MMP-9 expression ^(9,10).

MMP-9 may be particularly relevant to the progression of lymphomas. MMP-9 has been shown to be important for the in vitro degradation of extracellular matrix components by non-Hodgkin's lymphoma cells ⁽¹¹⁾. In vivo, MMP-9 is

a subset of high grade non-Hodgkin's lymphomas, and this correlates with a poor clinical outcome. The results of this study showed that MMP-9 is consistently expressed by the malignant Hodgkin and Reed-Sternberg cells of Hodgkin's disease ⁽¹²⁾.

This study had showed that the expression of MMP 9 in HL 56.7 ± 21.4 mean of expression and in NHL the mean was 65.4 ± 18 with positive staining in all cases and this is in accordance with previous studies in regard to its expression in all malignant tumors moreover its expression was higher in NHL cases than HL cases but it did not reached the level of statistical significance.

The infiltration of inflammatory cells that express a high amount of pro-MMP-9 and often surround malignant tumors ⁽¹⁰⁾. MMP-9 may be particularly relevant to the progression of lymphomas which has been shown to be important for the in vitro degradation of extracellular matrix components by non-Hodgkin's lymphoma cells; MMP-9 is also overexpressed in a subset of high grade non-Hodgkin's lymphomas, and this correlates with a poor clinical outcome. The results of previous study showed that MMP-9 is consistently expressed by the malignant Hodgkin and Reed-Sternberg cells of Hodgkin's disease ¹².

Regarding the grades of the tumors, MMP- 9 showed negative non-significant correlation with the tumor grades and this in agreement with other studies ^(13,14) whom concluded from their study that there is no correlation was found between MMP-9 expression and tumor invasion or histological grade; whereas other studies had reached to contradictory results as the levels of MMP 9 and MMP 2 are highly correlated with the histological grade of malignancy ^(15, 16). Moreover other study reached to a conclusion that MMP-9 has been showed a tendency to increase with increasing tumor grade ^(15, 17). The possible explanation for this difference in relation of MMP 9 with tumor grade might be attributed to

microenvironment component interaction and to small sample size of this study as compared with large studies.

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الخلاصة

خلفيه: اللغموما الخبيثه هو مصطلح عام يعطى الى الاورام التي تصيب الجهاز اللمفاوي وبصورة خاصة الخلايا اللمفاويه والخلايا المكونه لهل بينما كل اللغموما هي اورام خبيثه وهناك طيف واسع من التصرف العيادي حيث بعضها يتبع نمط عياديا بطيا والآخر يتصرف بطريقه عدوانيه مسببا الموت بفترة قصيره اذا ترك بدون علاج. عملية الانتشار تتضمن الدخول في الاوعيه الدمويه والخروج منها بالنسبه للخلايا السرطانيه متبوعه باستزراع الخلايا السرطانيه مكونه حشوه سرطانيه جديده. تحلل المصفوفه الخارجيه للخلايا ومكونات الغشاء القاعدي بواسطه محللات البروتين يسهل انفصال الخلايا السرطانيه وعبورها حدود الانسجه وهجومها الى الانسجه المجاوره المستقله اهميه محللات البروتين المصاحبه للاورام في عملية الغزو والانتشار قد تم استيضاحها لانواع من السرطانات الخبيثه الصلبه. عائلة MMP 9 قد تم تضمينها على خلايا الورم اثناء الغزو والانتشار.

المواد والطرق: سبعه وستين نسيج مثبت بالفورمالين ومطموره بشمع البرافين، مشخصه نسيجيا لمفوما من منطقه الراس والغرق اربعة وعشرون حالة هودجكن لمفوما وتلايه واربعون لاهوجكن لمفوما. تقنية المناعه الكيمياءيه النسيجه قد استعملت لتقييم تعبير MMP 9 كمضاد احادي النسل وربط ذلك مع المعايير العياديه المرضيه.

النتائج: معدل تعبير MMP 9 في هودجكن ولاهودجكن لمفوما كان اعلى 18 ± 65.4 من ما في هودجكن لمفوما، 21.4 ± 65 ورغم ذلك لم تصل الى مستوى الاختلاف الاحصائي $P > 0.05$ بينما معدل تعبير MMP 9 وعلاقته بدرجة الورم كان مختلف حيث كانت له قيمه 72 ± 14.1 في الورم واطيه الدرجه 15 ± 68.3 في الورم متوسط الدرجه و 20.7 ± 61.4 في الورم عالي الدرجه وبالنتيجه هذا الاختلاف لم يصل الى المستوى الاحصائي المهم $P > 0.05$ MMP 9. لم تكن له علاقه اخصائيه مهمه بالنسبه الى انواع هودجكن لمفوما.

الاستنتاجات: أظهرت هذه الدراره بأنه لم تكن هناك علاقه مهمه بين معدل تعبير MMP9 في هودجكن ولا هوجكن لمفوما . لم تكن هناك أختلافات أخصائيه مهمه بالنسبه الى معدل تعبير MMP 9 بين هودجكن ولاهودجكن وأنواعها الفرعيه.