Immunohistochemical expression of MMP1 and TIMP1 as markers of migration in Hodgkin's and non-Hodgkin's lymphoma of the head and neck region (A comparative study)

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ABSTARCT

Background: Malignant lymphoma is a term that describes primary tumors of the lymphoreticular system, almost all of which arise from lymphocytes.MMP-1 is the most ubiquitously expressed interstitial collagenase, a subfamily of MMPs that cleaves stromal collagens. It is also called collagenase-1.TIMPs which inhibits MMP activity and thereby restrict extracellular matrix breakdown, TIMP-1 is a stromal factor that has a wide spectrum of functions in different tissues.
Material and Methods: This study was performed on (68) formalin-fixed, paraffin-embedded blocks, histopathologically diagnosed as lymphoma (head and neck lesions). Immunohistochemical staining of MMP1and TIMP1 was performed on each case of the study sample.
Results: The expression of MMP1was cytoplasmic, the study cases showed a 98.5% positive reaction to MMP1, score 3 was the most common and found in 60.3% of all cases.The expression of TIMP1was cytoplasmic, 92.6% of cases expressed positive reactions to TIMP1, score 1 was the most common and found in 57.4% of all cases.
Conclusion: This study showed for the first time the effect of MMP-1 in HL, which is considered to be as an invasive and migratory cell marker. A significant difference was found among the subtypes of NHL in relation to TIMP1, TIMP1inhibits the effect of MMP1 and as MMP1 is elevated the TIMP1 will be elevated too.
Keywords: Lymphoma, Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL), MMP1, TIMP1. (J Bagh Coll Dentistry 2014; 26(3):72-78).

INTRODUCTION

Malignant lymphoma is a term describes primary tumors of the lymphoreticular system, almost all of which arise from lymphocytes. They vary greatly in their behavior, and while most prove fatal if untreated, considerable advances have been made in their management. Most lymphomas arise in lymph nodes, but 30–40% develops in extranodal sites such as the stomach, though almost any organ may be primarily involved. They usually produce lymph node enlargement which may be localized or generalized, with widespread involvement of the lymphoreticular system at presentation. This latter tendency is a reflection of the normal recirculating behavior of lymphocytes. Ironically, more aggressive lymphomas may remain localized, at least for a time, and tend to spread to adjacent nodes.[1]

Lymphomas are subdivided into Hodgkin's lymphoma (HL) and Non-Hodgkin's lymphoma (NHL) and are more specifically classified into subtypes of HL or NHL. HL involve the lymph nodes predominantly and only approximately 5% arise in extranodal sites, whereas 30% of NHL present in extranodal sites.[2]

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Matrix metalloproteinases (MMPs), collectively called matrixins, are proteinases that participate in ECM degradation. Under normal physiological conditions, the activities of MMPs are precisely regulated at the level of transcription, activation of the precursor zymogens, interaction with specific ECM components, and inhibition by endogenous inhibitors. A loss of activity control may result in diseases such as arthritis, cancer, atherosclerosis, aneurysms, nephritis, tissue ulcers, and fibrosis. Tissue inhibitors of metalloproteinases (TIMPs) are specific inhibitors of matrixins that participate in controlling the local activities of MMPs in tissues.[3,4]

MMP-1 is the most ubiquitously expressed interstitial collagenase, a subfamily of MMPs that cleaves stromal collagens. It is also called collagenase-1, and has a prominent role in collagen degradation, specifically degrades type I collagen, which is a major component of the extracellular matrix, as well as other fibrillar collagens of types II, III, V, IX and X. The MMP-1 gene is localized on chromosome 11q22 and expressed in a wide variety of normal cells, such as stromal fibroblasts, macrophages, endothelial and epithelial cells, and in various tumor cells. It is constitutively expressed at low levels under normal physiological conditions; however, its expression may increase markedly in pathological conditions. Increased expression of MMP-1 has

been associated with a poor prognosis in several cancers.[5]

MMPs are counteracted by the tissue inhibitors of metalloproteinases (TIMPs), which inhibit MMP activity and thereby restrict ECM breakdown. The balance between MMPs and TIMPs plays an important role in maintaining the integrity of healthy tissues. A balance of MMPs and TIMPs is found in various pathologic conditions including malignant conditions. Tissue inhibitor of metalloproteinase 1 (TIMP-1) is a stromal factor that has a wide spectrum of functions in different tissues, and enhanced TIMP-1 expression is associated with poor clinical outcome in many cancer types also the TIMP-1 acts as a modulator of the survival and growth of germinal center B cells.[6]

This study was designed to evaluate and compare the immunohistochemical of MMP1 expression as marker for tumor cells migration, and TIMP1 expression as an inhibiter for MMP1, also to correlate the expression of MMP1 and TIMP1 in Hodgkin's and non-Hodgkin's lymphoma.

MATERIALS AND METHODS

This study was performed on (68) formalin-fixed, paraffin-embedded blocks, histopathologically diagnosed as lymphoma (head and neck lesions).The diagnosis of each case was confirmed by the histological examination of the hematoxylin and eosinstained sections by two experienced pathologists. Histological classification was determined according to the world health organization (WHO) for HL (25 cases) classification and the international formulation criteria for NHL (43 cases) classification. The diagnosis of HL was confirmed by using immunohistochemical staining with CD15 and CD30, and the diagnosis of NHL was confirmed by using immunohistochemical staining with CD3, CD20 and Bcl2.

Each case was stained by MMP1(Anti-MMP1 antibody [3B6] ab2461Abcom, England) and TIMP1(Anti-TIMP1 antibody [102D1] ab1827Abcom, England), for each antibody the following procedure is done, starting by deparaffinization of a 4µm thickness section of each block mounted on positively charged microscopic slides at 65◦ overnight, followed by dehydration, then application of hydrogen peroxidase block,Antigen retrieving was perform for MMP1 and TIMP only by applying the slides in hot citrate buffer solution ph 6.0 (95-99 ºC) for 10 minute, followed by protein block, then the application of primary antibody and incubation (2hr for MMP1 and 6hr for TIMP), then the rabbit anti-mouse antibody unconjugated application, followed by goat anti-rabbit HRP conjugate application, and finally the application of DAB plus chromogen then hematoxylin counterstain.

The expression for all markers was evaluated semi-quantitatively. It was obtained by counting the number of tumor cells in 5 fields (using 40X objective in most represented areas of sections) and calculates the percentage of tumor cells that labeled a brown cytoplasmic. Labeling index for each field was calculated using the following equation: (number of positive cells/ number of total cells); the mean value of labeling indices for the five fields was considered to be the label index for the case.

The scoring categories for each antibody;

1. MMP1 ; The tumor cells labelled by antibody display a brown cytoplasmic staining pattern, immunoreactivity was classified:, (1) 1%-10%, (2) 11%-25%, (3) 26%-50%, (4) >50%.7
2. TIMP1 ; The tumor cells labelled by antibody display a brown cytoplasmic staining pattern, immunoreactivity was classified: (0) <5%, (1) 6%-25%, (2) 26%-50%, (3) 51%-75%, (4) 76%-100%.8

Computerized statistical program (Statistical Package for Social Sciences, SPSS version 15) was used for the statistical analysis of data.

RESULTS

MMP1 expression is cytoplasmic. Sixty seven cases showed positive expression (98.5%), except one negative case. Tumor cells in the study sample mostly express MMP1 in "Score 3" in 41 cases (60.3%). No statistically significant correlation was found between the types of lymphoma related to the scoring category for MMP1 (Table1)

The expression of MMP1 in Hodgkin's lymphoma cases (Fig 1 and 2) according to its subtypes found in Table 2, all cases of HL were positive for MMP1 expression, the highest expression was found in "Score 3" were 17 cases (68%) have this score. No correlation different was found between the subtype of HL related to the score of MMP1.

97.7% was the percentage of MMP1 expression in NHL cases (Fig 3). The expression of MMP1 in Non- Hodgkin's lymphoma cases according to its subtypes found in Table 3, the highest expression was found in "Score 3" with 24 cases (55.8%), there was no significant different the subtype of NHL related to the score of MMP1.

The expression of TIMP1 is cytoplasmic, the positive cases were (92.6%) 63 cases of the

studied sample whereas 5 cases were negative (7.4%). Most of the studied cases were in “Score 1” (57.4%) affected 39 cases. No statistically significant correlation was found between the HL and NHL lymphomas related to the scoring categories of TIMP1 expression. (Table 4)

The expression of TIMP1 in Hodgkin's lymphoma cases (Fig 4 and 5) according to its subtypes found in table 5, were all cases of HL were positively expressed for TIMP1, the highest expression was found in “Score 1” with 17 cases (68%), there was no correlation between the subtype of HL regarding score of TIMP1 .

The percentage of positive expression of TIMP1 in Non- Hodgkin's lymphoma cases (Fig 6) where 88.3%, the destitution of TIMP1 expression according to its subtypes found in table 6, the highest expression was found in "Score 1” with 22 case (51.2%), there was a significant correlation different between the subtype of NHL in relation to the scoring categories, were P-value = 0.01714, were the percentage of positive expression of TIMP1 in low grade and intermediate grade was 100% in comparing with 76.1% in high grade subtype.

The correlation between percentages of expression of MMP1 and TIMP1in HL cases and NHL cases were analyzed using Spearman's rank correlation coefficient (Spearman's rho).In HL MMP1expression have not correlated with TIMP1expression while in NHL MMP1expression correlate significantly with the expression of TIMP1 at level 0.01.

Table 1: The numbers of lymphoma cases according to scoring categories for MMP1

Lymphoma types		MMP1					Total
		Negative	Score 1	Score 2	Score 3	Score4	
HL	No.	0	0	5	17	3	25
	%total	0%	0%	7.4%	25%	4.4	36.8%
	%type	0%	0%	20%	68%	12%	100%
NHL	No.	1	1	8	24	9	43
	%total	1.5%	1.5%	11.7%	35.3%	13.2	63.2%
	%type	2.3%	2.3%	18.7%	55.8%	20.9%	100%
Total	No.	1	1	13	41	12	68
	%	1.5%	1.5%	19.1%	60.3%	17.6%	100%

Chi-squared value 1.682, d.f. 3, Significance level P (2 sided) =0.6409.

Table 2: The expression of MMP1in HL study cases

Subtypes of HL		MMP1					Total
		Negative	Score 1	Score 2	Score 3	Score4	
Lymphocytic rich	No.	0	0	0	1	0	1
	%type	0%	0%	0%	4%	0%	4%
	%subtype	0%	0%	0%	100%	0%	100%
Mixed cellularity	No.	0	0	0	6	1	7
	%type	0%	0%	0%	24%	4%	28%
	%subtype	0%	0%	0%	85.7%	14.3%	100%
Nodular sclerosing	No.	0	0	5	10	2	17
	%type	0%	0%	20%	40%	8%	68%
	%subtype	0%	0%	29.4%	58,8%	11.8%	100%
Total	No.	0	0	5	17	3	25
	%	0%	0%	20%	68%	12%	100%

Chi-squared value 3.188, d.f. 4, Significance level P (2 sided) = 0.5268

Table 3: The expression of MMP1 in NHL study cases

Subtypes of NHL		MMP1					Total
		Negative	Score 1	Score 2	Score 3	Score4	
Low grade	No.	0	0	2	4	0	6
	%type	0%	0%	4.7%	9.3%	0%	14%
	%subtype	0%	0%	33.4%	66.6	0%	100%
Intermediate grade	No.	0	1	1	8	6	16
	%type	0%	2.3%	2.3%	18.6%	14%	37.2%
	%subtype	0%	6.3%	6.3%	50%	37.4%	100%
High grade	No.	1	0	5	12	3	21
	%type	2.3%	0%	11.5%	28%	7%	48.8%
	%subtype	4.8%	0%	23.8%	57.1%	14.3%	100%
Total	No.	1	1	8	24	9	43
	%	2.3%	2.3%	18.6%	55.8%	20.9%	100%

Chi-squared value 7.882, d.f. 6, Significance level P (2 sided) = 0.2468

Table 4: The numbers of lymphoma cases according to scoring categories for TIMP1

Lymphoma types		TIMP1					Total
		Negative	Score 0	Score 1	Score 2	Score 3	
HL	No.	0	0	17	5	3	25
	%total	0%	0%	25%	7.4%	4.4%	36.8%
	%HL	0%	0%	68%	20%	12%	100%
NHL	No.	5	0	22	14	2	43
	%total	11.6%	0%	32.3%	20.6%	2.9%	63.2%
	%NHL	11.6%	0%	51.2%	32.6%	4.6%	100%
Total	No.	5	0	39	19	5	68
	%	7.3%	0%	57.4%	28%	7.3%	100%

Chi-squared value 2.529, d.f. 2, Significance level P (2 sided) = 0.2823

Table 5: The expression of TIMP1in HL study cases

Subtypes of HL		TIMP1					Total
		Negative	Score 0	Score 1	Score 2	Score 3	
Lymphocytic rich	No.	0	0	1	0	0	1
	%HL	0%	0%	4%	0%	0%	4%
	%subtype	0%	0%	100%	0%	0%	100%
Mixed cellularity	No.	0	0	4	2	1	7
	%HL	0%	0%	16%	8%	4%	28%
	%subtype	0%	0%	57.2%	28.6%	14.2%	100%
Nodular sclerosing	No.	0	0	12	3	2	17
	%HL	0%	0%	48%	12%	8%	68%
	%subtype	0%	0%	70.6%	17.7%	11.7%	100%
Total	No.	0	0	17	5	3	25
	%	0%	0%	68%	20%	12%	100%

Chi-squared value 0.944, d.f. 4, Significance level P (2 sided) = 0.918

DISCUSSION

The percentage of expression of MMP-1 in the study sample cases was 98.5%, this high level of MMP-1 could be attributed to the fact that lymphocytes are normally found throughout the organs and connective tissues of the body as their role in defense mechanisms of the body and these mechanisms make lymphocytes have a normal migration and invasive ability and matrix metalloproteinase activity involved in the transmigration of lymphocytes from the vascular compartment to the surrounding tissues, the malignant lymphoid cells can migrate and invade through the lymph node capsule and, so the migration and invasion capacity of lymphoma cells and cell destruction of this neoplasm probably due to the participation of proteolytic enzymes, such as metalloproteases has been explored in lymphomas and reactive lymphocytes and peritumoral stroma.[9,10]

Table 6: The expression of TIMP1 in NHL study cases

Subtypes of NHL		TIMP1					Total
		Negative	Score 0	Score 1	Score 2	Score 3	
Low grade	No.	0	0	3	3	0	6
	%NHL	0%	0%	7%	7%	0%	14%
	%subtype	0%	0%	50%	50%	0%	100%
Intermediate grade	No.	0	0	5	10	1	16
	%NHL	0%	0%	11.6%	23.3%	2.3%	37.2%
	%subtype	0%	0%	31.2%	62.6%	6.2%	100%
High grade	No.	5	0	14	1	1	21
	%NHL	11.6%	0%	32.6%	2.3%	2.3%	48.8%
	%subtype	23.9%	0%	66.7%	4.7%	4.7%	100%
Total	No.	5%	0	22	14	2	43
	%	11.6%	0%	51.2%	32.6%	4.6%	100%

Chi-squared value 12.029, d.f. 4, Significance level P (2 sided) = 0.0171

A very limited number of studies have been carried out on the effect of MMP-1 on lymphoma in general and on the subtypes of lymphoma, regarding HL all cases give a positive expression (100%) with no statistical difference between subtypes of HL, "Score 3" were the positive expression range between 26% to 50% was the commonest score, no available articles have been found that study the MMP-1 in HL, but the destruction of lymph node architecture seen in HL and the relationship of Reed – Sternberg cells with the surrounding inflammatory cells suggest a role of MMP-1 in HL.

Meneses-García et al [10] showed that all their cases of extranodal T/NK cell lymphomas was positive for MMP-1; they concluded that, MMP-1 could be contributing to the degradation of collagen matrix and, participate in the degradation of the blood vessel wall, these is in agreement with the results of the present study.

The results of the present work showed that, 92.6% of all study cases were positive for TIMP1, this relatively high percentage of expression is expected because of the inhibiting action of TIMP1 on MMP1 so any increase in the expression of MMP1 lead to increase in expression of TIMP1in order to inhibit its action and establish a balance.

Oelmann et al [11] detected the TIMP-1 expression in the samples from 14 of the 15 patients with classic Hodgkin's lymphoma, and this in agreement with the results of the present work were 100% positive expression in all case of HL, also Oelmann et al [11] found no significant difference in TIMP-1 expression between histological subtypes of Hodgkin's lymphoma, and this result was in accordance with the result of the present study were there was no significant different between subtypes of HL.

Pennanen et al [12] studied the immunohistochemical TIMP-1 expression in 57 case of HL, and they found that 33% of their cases were positive for TIMP1, also they reported a statistically significant difference in the expression of the protein for TIMP-1 between the nodular sclerosis subtype and the other subtypes of Hodgkin's lymphoma and this disagree with the results of the present study were there was no significant difference between subtypes of HL, this difference in the results could be attributed to method that they used to evaluate the positivity of the marker were they perform the presence of positive staining in Reed–Sternberg cells and reactive stromal cells, while the present study examined the positivity of Reed-Sternberg cells only, furthermore there sample include Nodular lymphocyte predominant HL (22.8%) while this subtype was not present in the present study.

Regarding NHL, the percentage of positive expression was 88.3%, there was a significant difference between subtypes were low and intermediate grade show 100% positivity while high grade have 76.1% positive expression, Kossakowska et al [13] showed expression of TIMP-1 in 27 out of 28 case, Kossakowska et al [14] showed expression of TIMP-1 in 41out of 42 case, The results of these studies are relatively in agreement with the present results, were 38 out of 43 case show positive expression for TIMP-1. All these results conclude that elevated TIMP-1 expression is well correlated with tumor aggressiveness.

Kossakowska et al. [15] studied TIMP-1 expression in NHL, they show that TIMP-1 play a significant role in the pathogenesis of human NHL through acting as an anti-apoptotic and differentintion-promting factor, their study consisted of a wide range of different subtypes. They found that low-grade NHL express

relatively constant small amounts of TIMP-1, while high-grade tumors show more massive and variable expression of TIMP-1. Interestingly, in situ hybridization showed TIMP synthesis to be localized in stromal cells surrounding the tumor. Elevated TIMP-1 expression seemed to correlate with more extensive disease stage in high-grade NHL which was in disagreement with the results of the present study were high grade show the lowest expression rate (76.1% in comparing to 100% for intermediate and low grade subtypes) this deference could be related to the difference in the method that they used for TIMP-1 evaluation.

Citak et al [16] showed a 4% of positive expression of TIMP-1 in a sample consisted from 25 case of children non-Hodgkin's lymphoma, with no clear differences between staining patterns of each individual NHL subtype could be detected. They explain their low TIMP-1 expression level may be due to the high mitotic activity of childhood NHL.

The expressions of MMP1 and TIMP1 were significantly correlated in NHL cases this could be explain by the fact that MMP1enzyme degrade extracellular matrix proteins, and TIMPs suppress MMP activity critical for extracellular matrix turnover associated with both physiologic and pathologic tissue remodeling. TIMP concentrations generally far exceed the concentration of MMPs in tissue, thereby limiting their proteolytic activity to focal pericellular sites.[6]

In conclusion; this study for the first time showed the effect of MMP-1 in HL, which consider as an invasive and migratory cell marker, lymphomas show high migratory cells activates since all types of lymphomas could not be restricted to single lymph node and this was noted by the high expression rate of MMP-1 were it was positive in 100% for HL and 97.7% for NHL, the MMP-1 action is inhibited by TIMP-1 and this is confirmed by its correlation with TIMP1. A significant difference was found among the subtypes of NHL in relation to TIMP1 expression were high grade subtype showed the lowest expression in comparison with other subtypes of NHL. TIMP1inhibit the effect of MMP1 and as MMP1 is elevated the TIMP1 will be elevated too.

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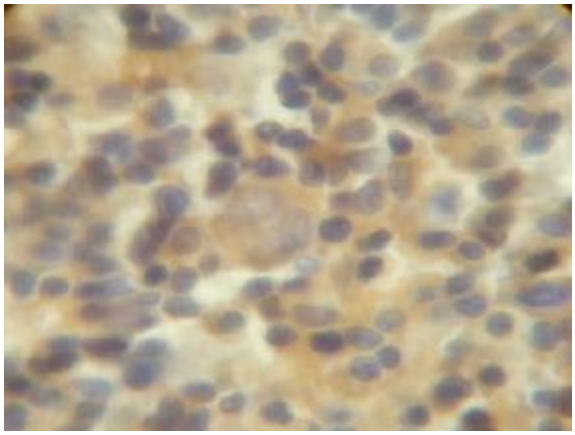

Figure 1: Cytoplasmic expression of MMP1 in nodular sclerosing HL case

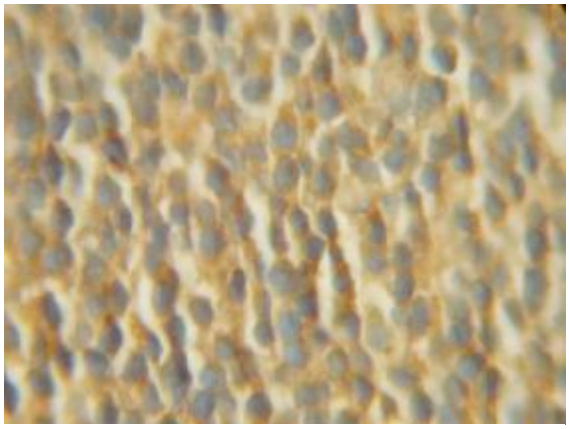

Figure 2: Cytoplasmic expression of MMP1 in low grade NHL case. (×100)

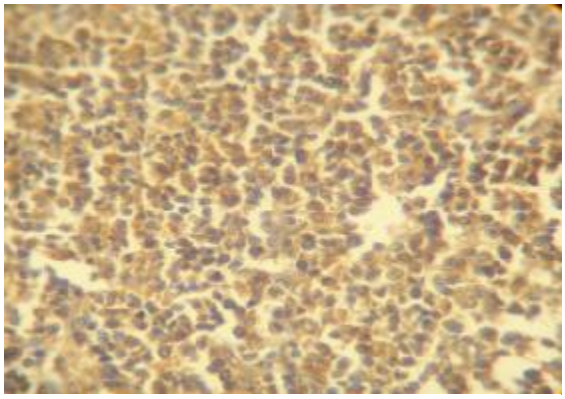

Figure 3: Cytoplasmic expression of MMP1 in low grade NHL case (×40)

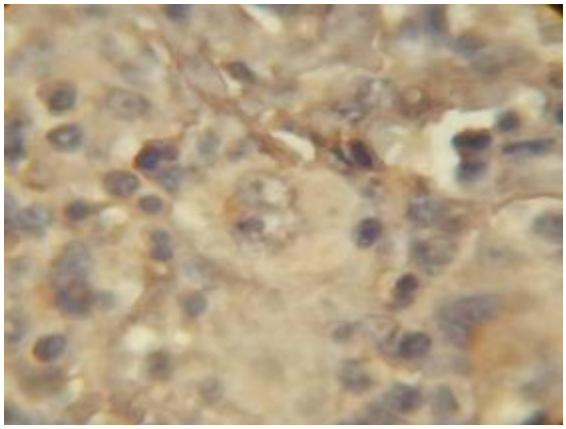

Figure 4: Cytoplasmic expression of TIMP1 in mixed cellularity HL (×100)

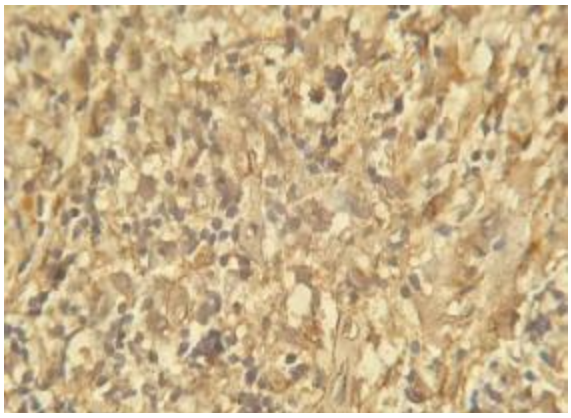

Figure 5: Cytoplasmic expression of Timp1 in nodular sclerosing HL case (×100)

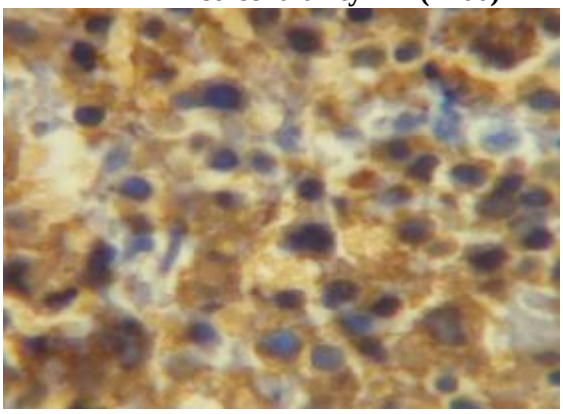

Figure 6: Cytoplasmic expression of TIMP1 in intermediate grade NHL (×100)