Immunohistochemical expression of P53, as a marker of apoptosis in Hodgkin's and Non Hodgkin's lymphoma of the head and neck region

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ABSTARCT

Background: Malignant lymphomas represent about 5% of all malignancy of the head and neck region which can involve lymph nodes as well as soft tissue and bone of the maxillofacial region. Apoptosis is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system. Inappropriate apoptosis is a factor in many human conditions including neurodegenerative diseases, ischemic damage, autoimmune disorders and many types of cancer. Expression of p53 Proteins in Hodgkin's and Non Hodgkin's lymphomas suggested that it can help in monitoring of patients and the markers may aid in controlling the progression of lymphoma and detect the degree of aggressiveness of the disease to give suitable treatment and management of patients.

Material and methods: Sixty seven formalin-fixed, paraffin-embedded tissue, histopathologically diagnosed lymphoma blocks (head and neck lesions) as (24) Hodgkin's Lymphoma and (43) Non Hodgkin's Lymphoma. Immunohistochemical (IHC) technique was used for the evaluation of P53 monoclonal antibodies expression and correlated with the clinicopathological parameters.

Results: The mean of expression of P53 in relation to tumor grades was different as it had a value of (51.2±13.5) in low grade tumors, (55.7±20) in the intermediate grade tumor and (45±17.6) in high grade tumors; consequently, this difference did not reached the level of statistical significance P(ANOVA) >0.05.

Conclusions: This study had shown that there was no significant correlation between the mean of expression of P53 in HL and NHL.

Key words: P53, Apoptosis, Hodgkin's lymphoma, Non Hodgkin's Lymphoma. (J Bagh Coll Dentistry 2014; 26(4):129-132).

الخلاصه

خلفيه:اللمفوما الخبيثه تمثل حوالي 5% من كل الامراض لمنطقة الرأس والعنق والتي يمكن ان تنطوي على العقد اللمفاويه وكذلك الانسجه الرخوه ومنطقة الوجه والفكين. موت الخلايا المبرمج يعتبر عنصر حيوي لكثير من العمليات المتنوعه من ضمنها عملية التحول الطبيعي للخلايا والتطور المناسب وعمل الجهاز المناعي. موت الخلايا المبرمج الغير مناسب (اما بصوره قليله أو كثيره) هو عامل في العديد من الحالات البشريه من ضمنها أمراض التحلل العصبي، التلف نتيجه فقر الدم الموضعي ،حالات أضطراب المناعه الذاتيه وعدة أنواع من السرطان. التعبير عن بروتين P53 في هودجكن ولاهودجكن لمفوما مستعملين طريقة النسيجي المناعي الكيميائي ، اقترحت بأن الدراسات المناعيه النسيجيه الكيميائيه لعامل P53 وظهوره في أورام الانسجه يمكن أن يساعد في رصد المرضى والدراسات وكذلك المعلمات ممكن أن تساعد في ضبط تقدم اللمفوما ونتحقق من درجة عدوانية المرض لاعطاء العلاج الملائم وأدارة المرض.

المواد والطرق: سبعه وستين نسيج مثبت بالفورمالين ومطموره بشمع البرافين، مشخصه نسيجيا من منطقة الراس والعنق.اربعه وعشرون حالة هودجكن لمفوما وثلاثه وأربعون لاهوجكن لمفوما. تقنية المناعه الكيميائيه النسيجه قد استعملت لتقييم تعبير P53 كمضاد احادي النسل وربط ذلك مع المعايير العياديه المرضيه.

النتائج: معدل تعبير P53 وعلاقته بدرجه الورم كانت مختلفه كما كانت لها قيمه 51.2±13.5 في الورم واطيء الدرجه و 55.7±20 في الورم متوسط الدرجه و 45±17.6 في الورم عالي الدرجه: وكنتيجة الاختلاف لم يصل الى المستوى الاحصائي المهم P>0.05 .

الاستنتاج: هذه الدراسه قد أظهرت بأنه لم تكن هناك علاقه مهمه بين معدل تعبير P53 في هودجكن ولاهوجكن.

INTRODUCTION

Malignant lymphomas can be divided into two major categories: HL which is almost exclusively a nodal disease 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 presents as cervical lymphadenopathy which is the most common head and neck presentation for both diseases. The process of programmed cell death, or apoptosis, is generally characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms; also apoptosis is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death. Inappropriate apoptosis is a factor in many human conditions including neurodegenerative diseases, ischemic damage, autoimmune disorders and many types of cancer. The capacity of p53 for multiple biological functions can be attributed to its ability to act as a sequence- specific transcription factor to regulate expression of over one hundred different targets, and thus to modulate various cellular processes including apoptosis, cell cycle arrest and DNA repair (1).

Expression of p53 Proteins in Hodgkin's lymphomas and Non Hodgkin's lymphoma patients using immunohistochemistry (2), suggested that these studies can help in monitoring of patients at risk, and the markers

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may aid in controlling the progression of lymphoma and detect the degree of aggressiveness of the disease to give suitable treatment and management of patients [3].

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 and confirmed 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 all cases classified into Hodgkin's lymphoma (24 cases) and Non-Hodgkin's lymphoma (43cases). Positive tissue control included in this study was: Breast carcinoma .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 10 sections of 4µm thickness were mounted on positively charged microscopic slides to obtain a greater tissue adherence. All of these collected specimens were subjected to immunohistochemical staining using different and specific monoclonal antibodies included in the study.

Abcam, Expose mouse and rabbit specific HRP/DAP detection IHC kit, Antigen Retrieval Solutions: - Citrate buffer PH 6.0., Anti-P53 Mouse monoclonal[PAb] to P53, 1:250-1:500, overnight, Abcam, England. Immunohistochemical staining is accomplished with antibodies that recognize the target protein. Only the number of cells showing nuclear expression of P53 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. All fine to coarse brown granular nuclear precipitate were regarded as positive. The percentage of P53-positively stained cells was semi-quantitatively determined as follows (-) negative ≤5%, (+) low 6-25%, (++) moderate 26-50% and (+++) high 51-100% [2,3].

Statistical Analysis

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

RESULTS

There was no statistical significant difference in the mean of expression of P53 between HL (Fig 1) and NHL (Fig 2) as shown in Table 1

The mean of expression of P53 in relation to tumor grades was different as it had a value of (51.2±13.5) in low grade tumors, (55.7±20) in the intermediate grade tumor and (45±17.6) in high grade tumors; consequently, this difference did not reached the level of statistical significance P(ANOVA) >0.05as shown in table 2:

The mean of expression of P53 in nodular sclerosing HL (48.1±18.4) was higher than that of mixed cellularity (46.3±26.4). Obviously there was a difference in the mean of expression but it did not revealed any statistical significance (Table 3).

Table 1: Difference in the mean of expression of P53 between HL and NHL

	NHL compared to HL		P (t-test)
	HL	NHL	
P53			0.6[NS]
Range	(18 - 81)	(13 - 88)	
Mean	47.3	49.8	
SD	20.1	18.4	
SE	4.1	2.8	
N	24	43	

Table 2: Difference in the mean of expression of P53 in relation to the tumor grades

	Tumor grade			P (ANOVA) trend
	Low grade	Intermediate grade	High grade	
P53				0.46[NS]
Range	27 - 65	24 - 85	13 - 88	
Mean	51.2	55.7	45	
SD	13.5	20	17.6	
SE	5.53	5.01	3.83	
N	6	16	21	
	r=-0.254 P=0.1[NS]			

Table 3: Difference in the mean of expression of P53 in relation to HL subtypes

	Morphology		
	nodular sclerosing	mixed cellularity	P
P53			0.85[NS]
Range	(20 - 80)	(18 - 81)	
Mean	48.1	46.3	
SD	18.4	26.4	
SE	4.59	9.98	
N	16	7	

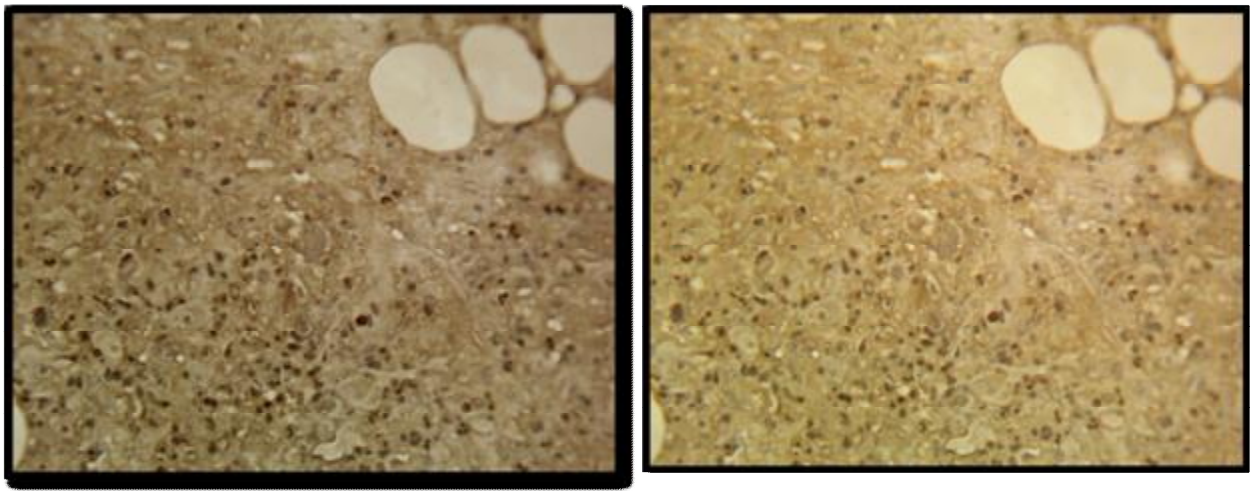

Fig. 1: HL Mixed cellularity P53 brown staining of nucleus of tumor cells (X 400)

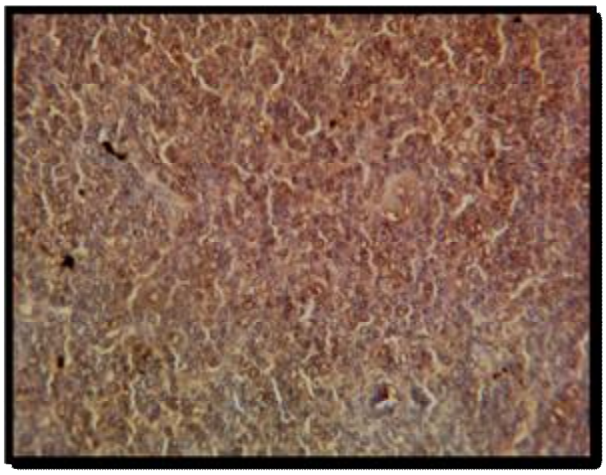

Fig. 2: NHL DLBC P53 brown staining of nucleus of tumor cells. (X400)

DISCUSSION

In non-Hodgkin's lymphomas, apoptosis plays an important role together with proliferative activity in counter-balancing tumor volume [1,4]. There has been accumulating evidence that Hodgkin and Reed-Sternberg (H/RS) cells, the presumed neoplastic-cell population in HL are characterized by a profound disturbance of the cell cycle and apoptosis regulation [5]. the mean of expression of P53was different among the tumor grade with increasing toward intermediate grade but the difference did not reach the level of statistical difference; a result that merely the same what Mishra and Crasta [6] conducted to the expression of apoptotic markers is higher in high-grade OSCs, which also have a higher proliferative activity compared with those in low-grade OSCs a conclusion that of Ozer et al. [7]. There is growing evidence that p53 also exerts its effects on multiple aspects of tumor formation, including suppression of metastasis and, inhibition of new blood vessel development (angiogenesis) [8]. This study had shown that there

is a difference in mean of expression of P53 in HL47.3±20.1 which lower as compared to the mean of its expression in NHL 49.8± 18.4 and even when this difference did not reached to level of statistical significance but it could explain the difference in behavior between the more aggressive tumor (NHL) as compared to (HL).

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