ROLE OF ZEBRA PROTEIN OF EPSTEIN-BARR VIRUS (EBV)AND ITS IMPACT ON P53 EXPRESSION IN HODGKIN'S AND NON HODGKIN'S LYMPHOMA PATIENTS

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ABSTRACT

Hodgkin's lymphoma (HL) is uncommon malignant tumor of the lymphatic system, some were shown to contain clonal, Epstein-Barr virus (EBV). Non Hodgkins lymphoma (NHL) are associated with EBV, ZEBRA protein plays a dominant role in the switch from latent cycle to productive infection of the virus. To access the expression of ZEBRA protein in Hodgkin's and non Hodgkin's lymphoma patients and its impact on P53 expression. A total of 28 of patients newly diagnosed to have HL and NHL investigated for EBV ZEBRA protein and p53 expression by immunohistochemistry technique. p53 was expressed in 80.9% and 100% of males and females respectively. In this study (86.7%) of HL and 88.6%of NHL cases were p53 positive. 87.5% of mixed cellularity were p53 positive. While 83.3% intermediate grade were p53 positive .No significant difference found between p53 expression and gender, type of lymphoma but there is significant difference with histopathological type. In regard to ZEBRA protein ,it is found that all the males and females with HL and NHL cases under study showed ZEBRA positivity.Also the 16 cases of the mixed cellularity type and all the 12 cases of intermediate grade are ZEBRA positive. No significant difference found between ZEBRA expression and gender, but there is significant difference with type of lymphoma and the histopathological type. This study shows that there is interaction between ZEBRA and p53 which depend on the relative amounts of those two proteins as confirmed previously and the expression of ZEBRA protein in lymphoma reflects the immunocomprimized state of patients and confirmed that EBV contribute to the development of malignancy.

Key words : Hodgkin's lymphoma, Non Hodgkin's lymphoma, p53 tumor suppressor, ZEBRA protein.

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دور بروتين ZEBRA للفايروس ابشتاين بار EBV وتأثيره على تعبير بروتين السيطرة p53 في مرضى الهوجكن واللاهوجكن ليمفوما

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الخلاصة

تعتبر الأورام اللمفية لامراض الهوجكن واللاهوجكن ليمفوما من الأورام الخبيثة والتى تترافق مع وجود الفايروس ابشتاين بار، إن البروتين ZEBRA يلعب دوراً مهماً داخل الخلايا المصابة اثناء خروجه من الطور الكامن الى المتكاثر. هدفت الدراسة الى قياس نسبة التعبير الخلوي للبروتين ZEBRA فى خلايا الأورام تحت الدراسة و تأثيره على تعبير بروتين السيطرة p53، شملت هذه الدراسة ثمإنية وعشرون مريضا من مرضى الهوجكن واللاهوجكن ليمفوما بإستعمال طريقة التصبيغ المناعى الخلوي لفحص نسبة التعبير للبروتين ZEBRA وp53. كانت نسبة التعبير الايجابي لبروتين السيطرة p53 هي80.9% ,100% فى الذكور والإناث على التعاقب. ووُجد أن نسبة التعبير لنفس البروتين هي 86.6% في مرضى الهوجكن و84.7% في مرضى اللاهوجكن ليمفوما. كإنت نسبة التعبير للبروتين p53 تصل الى87.5% فى Mixed cellularity و 83.3 % في Intermediate grade ولم يكن هناك فرق معنوي بين تعبير بروتين p53 و نوع الجنس و نوع الليمفوما ولكن هناك فرق معنوي مع نوع النسيج المرضي. كانت نسبة التعبير للبروتين ZEBRA موجبة في الذكور و الإناث وفي نوع الليمفوما ونوع النسيج المرضي. ولم يكن هناك فرق معنوي بين تعبير بروتين ZEBRA و نوع الجنس ولكن هناك فرق معنوي مع نوع الليمفوما ونوع النسيج المرضي. بينت نتائج هذه الدراسة وجود علاقة وتداخل بين البروتين ZEBRA وكذلك بروتين السيطرة المناعية P53، كما إن التعبير الموجب للبروتينZEBRA في مرضى الليمفوما يؤكد علاقة هذا الفايروس مع هذه الأورام.

INTRODUCTION

Hodgkin's lymphoma is uncommon malignant tumor of the lymphatic system where Reed Sternberg cells (R-S) replace the normal structure. About 40% of Hodgkin's tumors were shown to contain clonal Epstein-Barr virus (EBV), Highest rates in young adults and children were found in developing countries, this is in contrast to the west where EBV association with Hodgkin's Disease **(** HD) is strongest in young children and the elderly (1). Several types of non B-cell, Non Hodgkin's lymphoma **(**NHL) are associated with EBV(2). In Iraqi study by Ridah (3), stated that 62.5% HD cases, 30% NHL cases were EBV positive. Epstein-Barr virus (EBV)efficiently infects human B lymphocytes and establishes latent infection, in which the entire EBV genome is maintained as an episome and restricted numbers of EBV genes are expressed, (4). Recently (International Agency for Research on Cancer) IARC confirmed the classification of EBV as a Group 1carcinogen and concluded that there is sufficient evidence for a causative role of EBV in nasopharyngeal cancer, endemic Burkitt's lymphoma, immune suppression-related NHL, extranodal NK/T-cell lymphoma (nasal type) and a subset of Hodgkin's lymphoma **(**HL) (5). The p53 tumor suppressor serves as a checkpoint for DNA and cellular damage in many cell types (6). Altered p53activation that results from hereditary conditions or that exists in mice lacking p53 expression is associated with increased malignancy (7). Oncogenic viruses often encode proteins that are associated with altered p53 function and altered cellular apoptosis (8). The p53 tumor suppressor interacts with proteins expressed by common human viruses, including EBV(9). One of the EBV encoded protein, Z, plays a dominant role in the switch from latent cycle to productive infection, and is transcribed from the immediate early genes, Z has also been referred to as ZEBRA, Zta, and the BZLF1 protein. It controls the viral lytic cycle and. activates the transcription of viral genes required for replication. ZEBRA also interacts with components of the viral replication machinery(10). Transcription factors are expressed following induction of the lytic cycle and the EBV genome is often detected in T cell tumors (11,12). Inactivation of the p53 gene was mentioned as a possible pathway through which EBV can abrogate apoptosis and lead to sustained genetic damage (13). Also over expression of p53 strongly the modulate tumor response to chemo and radiotherapy and has significant impact on survival in some EBV – associated neoplasm (14). Hence in this study try to explore any relationship between Zebra protein and p53 in HL and NHL patients.

MATERIALS AND METHODS

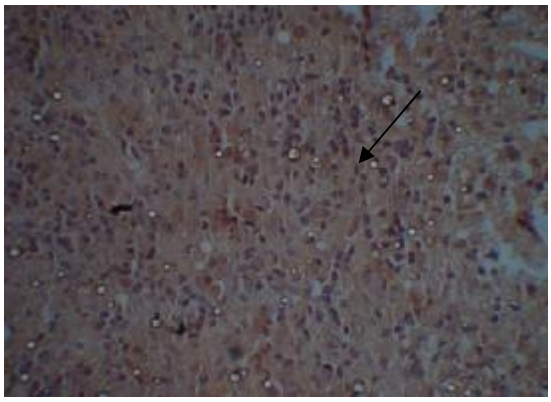
A total of 28 of patients newly diagnosed by biopsies to have HL and NHL at Baghdad medical city and private laboratories were involved in this study. Four to five micron section were prepared from paraffin embedded lymphoma tissues, one section was stained with haematoxylin and eosin for histopathololgic review. Two sections had been prepared to be stained immunohistochemically with p53 and Zebra monoclonal antibody using primary monoclonal mouse anti-human p53 protein (clone Do7from Dako, Carpinteria calif.) and primary monoclonal mouse anti human ZEBRA antibody (Dako, Carpinteria ,Calif) method used according to Dako cytomation immunohistochemistry detection kit ,USA and examined for Zebra and p53 protein expression. To determine the signal specificity negative control slides prepared by omission of the primary antibody were included. The Positive control tissue used was a specimen from nasopharyngeal carcinoma tissue, which was known to be almost 100% EBV positive and run with each batch. All the slides were examined by the light microscope at 40x, for the analysis of all the cases, a random selection of the fields was used. Positive Zebra and p53 results gave nuclear dark brownish color. The results of Zebra and p53 positivity in each specimen were analyzed according to Sophia *et al*.(15). The positive results of each case were analyzed according to the following: Scoring was done at 40×objective as follows: Negative (Score0): None of the cells revealed positivity, Weak or mild: Staining(5%-<10%) positive of tumor cells (score+1), Moderate: Staining (<25%)(score+2),Strong: Staining (<25% -<50%) (score+3), Highly strong (over 50%) (score +4).

STATISTICAL ANALYSIS

Chi – square test was used for tables with frequencies and percentages. Values were considered statistically significant when $p< 0.05$.

RESULTS AND DISSCUSSION

Immunohistologic staining revealed nuclear localization of ZEBRA and p53 figure(1).



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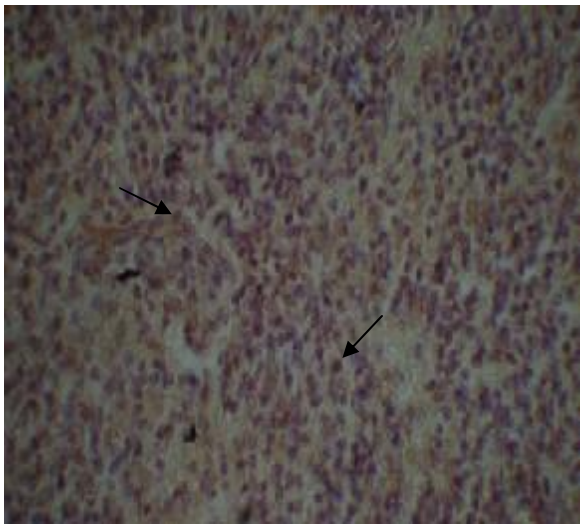

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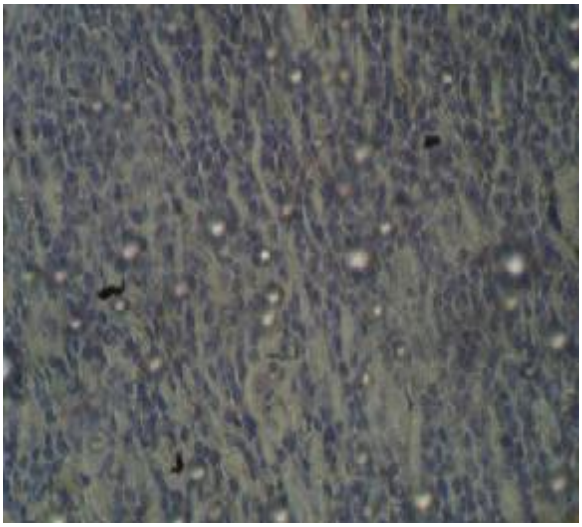

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Figure(1): Immunostaining of Hodgkin's lymphoma for *p53*, ZEBRA Protein
A - P53 immunostaining of nucleus at the tip of the arrow, B- Nuclear ZEBRA protein immunostaining, D-Negative control. Original magnification x 400.

Among the 28 patients enrolled in this study 15(53.57%) was diagnosed to have HL. While 13(46.42 %) having NHL table(1), 80.9% of males were p53 positive while, all the females shows p53 positivity.

Table (1) : Association between p53 over expression and clinicopathological features of patients.

		p53 Score											Chi-square	df	Sig.
		Negative		Score 1		Score 2		Score 3		Score 4					
		Count	%	Count	%	Count	%	Count	%	Count	% to	(positive%)			
gender	Male	4	19.0	4	19.0	10	47.6	1	4.8	2	9.5	(80.9)	6.413	4	0.17
	Female	0	0.0	3	42.9	2	28.6	2	28.6	0	0.0	(100)			
	Total	4	14.3	7	25.0	12	42.9	3	10.7	2	7.1				
type	HL	2	13.3	1	6.7	8	53.3	2	13.3	2	13.3	(86.6)	7.132	4	0.129
	NHL	2	15.4	6	46.2	4	30.8	1	7.7	0	0.0	(84.7)			
	Total	4	14.3	7	25.0	12	42.9	3	10.7	2	7.1				
Histo-pathological type	Mixed cellularity	2	12.5	1	6.3	10	62.5	1	6.3	2	12.5	(87.5)	10.889	4	0.028*
	Inter-mediate grade	2	16.7	6	50.0	2	16.7	2	16.7	0	0.0	(83.3)			
	Total	4	14.3	7	25.0	12	42.9	3	10.7	2	7.1				

(*)significant($p \leq 0.05$).

In regard to the type of lymphoma out of 15 cases of HL, (86.6%) were p53 positive and the rest(13.3%) were p53 negative, 53.3% were within score2. In the consideration of NHL cases, 84.7% were p53 positive while the rest were p53 negative, 46.2% were within score 1. Histopathological type revealed that all HL cases have mixed cellularity while all the NHL patients, showed intermediate grade. About 87.5% of the mixed cellularity showed p53 positivity and 12.5%were p53 negative, 62.5% were within score2. In intermediate grade, 83.3% showed p53 positivity and 16.7% were p53 negative, 50.0% were within score1. Immunohistochemistry results for ZEBRA protein expressed (100%)positive reactivity for all cases studied. It was found that 53.3% of HL cases were within score 3 and 53.8% of NHL cases were within score1. While mixed cellularity have 50% within score 3 and intermediate grade have 35.7% within score 2 table (2).

Epstein–Barr virus (EBV) is an a ubiquitous human gamma herpes virus that is associated with various malignancies, including, HL and NHL(16).

EBV encodes series of products interacting with or exhibiting homology to a wide variety of antiapoptotic molecules, cytokines, and signal transducers, hence promoting EBV infection, immortalization, and transformation(17).One of the expressed proteins of EBV is the ZEBRA (BZLF1) which is expressed as an immediate-early protein during transformation of primary B lymphocytes by EBV viral proteins, is thought to play an important role in virus-induced malignancy by deregulation of wild-type p53 function(11,18).

This study showing that there was no significant correlation between p53 and patient gender, this may be due to the small number for statistical significance, this is in agreement with Abeer *etal.* (19). In the present study 86.6% of the HL cases were p53 positive, this results was in agreement with Gupta *et al.*(20) and Kanavaros *et al*. (21). Barisik *et al.*(22) found p53 expression in 46% of HL cases .Also in our study 84.7% of NHL cases were p53positive. while Ann *et al.*(23) recorded that p53 positivity in 23/69(33.3%) of NHL patient. Another study by Abeer *et al*. (24)stated that p53 was expressed in 62 % of NHL cases. The present study demonstrated that p53 expression is frequent in all specimens with a significant impact ($p<0.05$) on clinical outcome indicated by histopathologic type of patients while Wang and Taylor (12)evaluated the association of p53 with the clinical stage and detected p53 gene in 14 patients among the 62 Classical HL cases.

Table (2): Association between ZEBRA over expression and clinicopathological features of patients.

		ZEBRA Score											
		Score 1		Score 2		Score 3		Score 4		Total	Chi-square	df	sig
		Count	%	Count	%	Count	%	Count	%	Count(positive %			
gender	Male	4	19.0	9	42.9	6	28.6	2	9.5	21(100	4.533	3	0.209
	Female	4	57.1	1	14.3	2	28.6	0	0.0	7(100)			
	Total	8	28.6	10	35.7	8	28.6	2	7.1	28(100)			
type	HL	1	6.7	6	40.0	8	53.3	0	0.0	15(100)	14.833	3	0.002*
	NHL	7	53.8	4	30.8	0	0.0	2	15.45	13(100)			
	Total	8	28.6	10	35.7	8	28.6	2	7.1	28(100)			
Histo-pathological type	Mixed cellularity	0	0.0	6	37.5	8	50.0	2	12.5	16(100)	18.200	3	0.001**
	intermediate grade	8	66.7	4	33.3	0	0.0	0	0.0	12(100)			
	Total	8	28.6	10	35.7	8	28.6	2	7.1	28(100)			

(*)significant($p \leq 0.05$)
(**)highly significant ($p \leq 0.05$)

However Barisiki *et al.*(22) stated that no significant relationship was found between the clinical stages, histological subtypes in HL patients with p53 expression and no association was determined with histological subtypes, they explained that when activation or altered regulation of pro apoptotic proteins, such as p53, by viral gene products may result in selection for cells lacking functional p53 or p53-regulated gene products (25). Our results indicate that mixed cellularity expressed high p53 positivity reaching 87.5%, this is in accordance with Gupta *et al.*(20) who stated that *p53* expressed in 57% mixed cellularity

of HL while in Hungary mixed cell type is most frequent with 65% expression and EBV was highest in this type by PCR (60%), this may be explained by the socioeconomic differences of the population living under different economic conditions. In Intermediate grade of NHL 83.3% of cases expressed p53 This is in accordance with Hussein (26) who shows gradual upregulation of p53, with the transition from low to intermediate to high grade NHL lymphoma. They explained this up-regulation may be due to the presence of p53 gene mutations that stabilize the mutant p53 protein, and increase its half-life leading to its accumulation in the cells. There was a significant association between ZEBRA and type of lymphoma and histopathological type ($p<0.05$) and this can be explained by the association of EBV with HL and NHL. These results are in association with Barisiki ***et al***. (22)and Theresa ***et al***. (27), and not come in association with Beck ***et al***.(28) who detected weak expression of ZEBRA protein in 13 of the 25 (52%) Hodgkin lymphoma cases and in 6 of the 18 LMP1 positive non-Hodgkin lymphoma samples. Our results which indicated the presence of ZEBRA protein of EBV in HL and NHL could be explained by Theresa ***et al***.(27)who stated that EBV could promote tumor maintenance through a variety of mechanisms, including the same pathways it uses to persist in healthy persons by increasing host cell proliferations, decreasing apoptosis and eluding the immune system in part by down regulating HLA- mediating presentation of viral peptides.

The over expression of ZEBRA together with P53 expression were in agreement with Dreyfus (29)who stated that Stable expression of ZEBRA was associated with the activation of p53-dependent transcription and increased p53 dependent apoptotic cell death. The Epstein-Barr virus (EBV) ZEBRA protein binds to p53 in vitro and has been associated with the altered transcription of p53-regulated genes in B lymphocytes and epithelial cells. Also our results were in accordance with previous studies who demonstrated that EBV infection and LMP1expression lead to loss of function of p53gene even in absence of mutations either through enhanced degradation of the cellular protein or increased half life by viral protein p53 interaction (30). We conclude that there is interaction between ZEBRA and p53 which depend on the relative amounts of those two proteins as confirmed previously- and the expression of ZEBRA protein in lymphoma reflect the immunocomprimized state of patients and this protein contribute to the development of malignancy.

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