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Expression of P27 and P53 Protein in Hodgkin's Lymphoma Patient Using Immunohistochemistry in Anbar Province

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Abstract

Hodgkin's lymphoma (HL) is the type of lymphoma, contains cells called Reed Sternberg cells. It is a common lymphoid malignancy that is diagnosed mostly in young adults. The present study was carried out on fifty Iraqi patients with Hodgkin's lymphoma. They were visited AL-Ramadi Teaching hospital during the period from January 2016 to March 2017. The patients included 43 males and 7 females. Fifty blocks of tissue biopsy from those patients were collected. The diagnosis was achieved according to histopathological findings. Evaluation of p53 and p27 proteins in these biopsies was examined in paraffin-embedded tissue sections. The control group was ten slides from healthy individuals with no HL. Immunohistochemistry study was carried out on the tissue biopsy samples of those patients according to Ramos-Vera (2005) procedure. The results revealed that p53 overexpression was in 22 patients (44%) with (Score 3), 15 patients (30%) (Score 2), and finally 13 patients (26%) with (Score 4) while (score 0 and score 1) were characterized by no expression. Regarding to p27 protein expression of Hodgkin's lymphoma patients, the results were 26 patients (52%) with (Score 3), 17 patients (34%) (Score 2), and finally 7 patients (14 %) with (Score 4) while absent in both (score 0 and score 1). The presence of p53 and p27 proteins in the patients' tissues with Hodgkin's lymphoma indicates that expression of these genes and they are playing a role in the development and occurrence of these tumors.

Keywords: Immunohistochemistry p53 and p27; Hodgkin's lymphoma

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Introduction

Hodgkin's lymphoma (HL) is the type of lymphoma, contains cells called Reed Sternberg cells. It is a common lymphoid malignancy that is diagnosed mostly in young adults [1]. It is one of two common types of cancers of the lymphatic system [2]. It is considered to be a potentially curable disease in most patients [3]. Hodgkin's lymphoma is seen more prevalent in males than females also it occurs in children and adult but the mode of treatment is differing between these groups [4].

The incidence of Hodgkin lymphoma varies according to the geographical area for instance the annual rate in Canada is 3 per 100.000 while in USA it accounts 7300 about 10% of population. Patients with immunodeficiency and those with AIDS have a great chance to get this type of cancer [5].

Nowadays, the scientists start to understand the molecular level of the HL disease to confirm the diagnosis and initiate the treatment. p53 gene encodes p53 protein is commonly exposed to nucleotides changes (mutations) such as addition, insertion or deletion of nucleotides that facilitate carcinogenesis. p53 plays a key role in the regulation of the cell cycle, DNA repair, senescence and angiogenesis. The protein

p27 is most important protein that regulates the cell cycle. The poor prognosis and treatment failure is related to Low levels of p27 [6,7]. Immunohistochemistry technique is a reliable and easy method for detection of both p53 and p27 proteins in the tissue depending upon using specific monoclonal antibodies to evaluate the Iraqi patients with Hodgkin's lymphoma to select the best protocol of treatment and exclude the non-effective ones.

Objective

To study the expression of the p53 and p27 markers in Hodgkin's lymphoma patients, by using Immunohistochemistry method of their tissue sections.

Tissue biopsy

Fifty blocks of tissue biopsy from fifty Iraqi patients with Hodgkin's lymphoma who visited AL-Ramadi Teaching hospital were collected. Evaluation of p53 and p27 proteins in their biopsies was examined in paraffin-embedded tissue sections.



Patients and Control Groups

Patients study group

Fifty patients with Hodgkin's lymphoma (males and females) were included in the present study. Their ages ranged from 13-79 years. The diagnosis is achieved by histopathologists in AL-Ramadi teaching hospital during the period from January 2016 to March 2017. The patients included 43 males and 7 females.

The Healthy Control Group

This group included ten normal slides of healthy individuals with no history of HL.

Method of Immunohistochemistry

Immunohistochemistry study was carried out on the tissue biopsy samples of the HL according to [8] procedure. The slides were deparaffinized, these were achieved by put the slides in xylene and alcohol with different concentrations from 100% to 50%, then the slides were incubated in D.W. for five minutes. The slides were incubated with retrieval solution its pH 9. The slides were cooled for 20 min at 25°C. The slides were put in staining racks and the cooled antigen retrieval solution was added to avoid the drying of the samples. Washing the slides were carried out. Blocking Reagent to block peroxidase enzyme was added for fifteen minutes. The slides were treated with H₂O₂ (hydrogen peroxide) before treated with horseradish peroxidase conjugate Antibodies. The slides were washed by buffer, and treated with antibody diluents for thirty minutes. Washing the slides were done. Incubation the slides with antibody was added to the slides, for 1 hr. reference slides were used as a positive control. Addition of Horseradish peroxide was carried out. then addition of Di aminobenzidine (DAB) was carried out for 15 min. The slides were washed twice vigorously. The counter stain was added Hematoxylin and Eosin (H&E) Solution, the slides were washed by tap water then the slides were dehydrated and mounted by specific media using LEICA CV5020 mounting system. The slides were left to dry and examined under microscope by the pathologist.

Results

Table 1 shows the grades of Hodgkin's lymphoma of the included patients. From this table it is obvious that the G1(Grade) constitutes the large number of samples 18 case (36%) of patients, followed by G3 which constitutes the second large group in number 16 case (32%) of patients, followed by G2 which represents the third group 11 case (22%) of samples, followed by G4 which accounts 5 cases (10%) that constitutes the smallest group of patients (Figure 1). Chi-Square showed a high significant difference ($P \leq 0.001$) among different grades of Hodgkin's lymphoma.

Staging

Table 2 shows the percentages of HL stages. It is obvious that the

Table 1: Percentages of the cancer grade in the Hodgkin's lymphoma patients.

Grade	Count	Percentage
G1	18	36 %
G2	11	22 %
G3	16	32 %
G4	5	10 %
Total	50	100 %
P value	≤ 0.001	

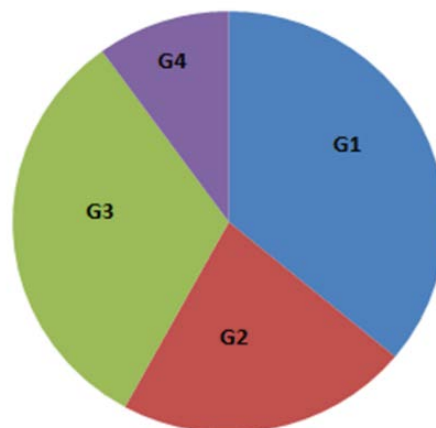


Figure 1: Grading of HL cases.

Table 2: Percentages of the cancer stage of Hodgkin's lymphoma patients.

Stage	Count	Percentage
T1	16	32 %
T2	8	16 %
T3	23	46 %
T4	3	6 %
Total	50	100 %
P value	0.038	

T3 (stage 3) constitutes the larger number 23cases (46%), followed by T1(stage 1) 16 case (32%), T2 which constitutes the third group in number 8 case (16%) and the T4 was the smallest group in number 3cases (6%)of samples.

Distribution of HL according to the age of patients

Table 3 shows the age frequency of HL patients. The patients were divided into six groups according to their ages.it is obvious that patients in the fifth decade of life (aged 41-50) years constituted the largest proportion [n=16, (32%)] of patients under study then followed by the group of patients in the fourth decade of life (aged 31-40) [n= 13, (26%)] years, followed by 2 groups of patients whom in the sixth decade of life (aged 51-60) years [n= 9, (18 %)] patients, and the patients in the third decade of life (aged 21 -30) years [n= 9, (18%)] patients then followed by patients in the second decade of life (equal or less than 20) years [n= 2, cases (4%)] (Figure 2). The last group of patients was in the seventh decade of life (age was above the 60) years [n= 1, (2%)] of patients.

Table 3: The age frequency of Hodgkin's lymphoma patients according to the age of intervals.

Age groups	Count	Percentage
≤ 20 years	2	4%
21-30 years	9	18%
31-40 years	13	26%
41-50 years	16	32%
51-60 years	9	18%
<60 years	1	2%
Total	50	100%
P value	≤ 0.001	

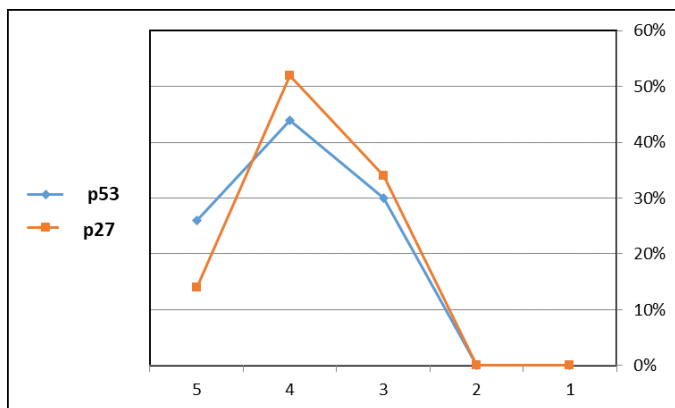


Figure 2: Shows the expression of both p53 and p27 in HL patients.

Results of immunohistochemistry

P53 expression

The immunohistochemistry study for the p53 expression in paraffin embedded tissues sections of fifty Hodgkin's lymphoma patients was determined semi-quantitatively by means of a visual grading system in as the following categories: 0 (0-4%), 1 (5-24%), 2 (25-49%), 3 (50-74%), or 4 (75-100%), from table 4 it is clear that the p53 protein was detected in all Hodgkin's lymphoma patients with different proportions, p53 overexpression was in 22 patients (44%) with (score3), 15 patients (30%) (Score 2), and finally 13 patients (26%) with (score 4) while (score 0 and score 1) were characterized by no expression.

p27 expression

Table 5 shows the p27 protein expression of Hodgkin's lymphoma patients. The results were 26 patients (52%) with (score3), 17 patients (34%) (Score 2), and finally 7 patients (14 %) with (score4) while absent in both (score 0 and score 1).

Discussion

In the present study it is clear that about 50% of HL patients were above 40 years. This age-specific incidence is related to high population density, low socioeconomic status, material deficiency and low living standards prevail [9]. These conditions facilitate and encourage the factors that responsible for disease occurrence. Other explanation may have related to immune suppression that appear in extremity of life of the patients.

Regarding immune histochemically analyses of cell cycle marker p53 of the tumor cells it is obvious that the expression of p53 gene was significantly different as compared with results obtained by control group (Table 4). Both various oncogenes and tumor suppressor genes play a role in cancer development and progression. The mutations of

Table 4: Frequency of p53 protein in Hodgkin's lymphoma patients and controls.

p53 score	Hodgkin's lymphoma		Control	
	Count	Percentage	Count	Percentage
Score 0	0	0%	8	80%
Score 1	0	0%	2	20%
Score 2	15	30%	0	0%
Score 3	22	44%	0	0%
Score 4	13	26%	0	0%
Total	50	100%	10	100%
P value				

Table 5: Frequency of p27 protein in Hodgkin's lymphoma patients and controls.

p27 Score	Hodgkin's lymphoma		Control	
	Count	Percentage	Count	Percentage
Score 0	0	0%	9	90%
Score 1	0	0%	1	10%
Score 2	17	34%	0	0%
Score 3	26	52%	0	0%
Score 4	7	14%	0	0%
Total	50	100%	10	100%
P value				

tumor suppressor genes play a role in development of tumors this is reflected the mode of treatment and mechanisms of drug resistance. Such important notes have led to the development of new method of evaluation of p53 which is easy, available and not time consuming called (IHC). Thus (IHC) regarded a novel method to detect of p53 protein in the studied tissues and it is a relatively simple and applicable method for fresh tissues [10].

Mutations of p53 gene in studied patients play a role in the development of these types of tumors. P53 expression is mediated by cellular DNA damage, low oxygen level, exposure to radiation, and mutagenic chemicals [11].

P53 gene is located on chromosome 17p. p53 is a protein that concentrated in the nuclei during interphase (especially S phase) of the cell cycle. The mutation of p53 in colon and Hodgkins lymphoma tumors are take place in specific location of the gene that has been conserved, so mutations, of p53 gene and overexpression of p53 protein prove the clear association of this protein and HL tumor progression. So these mutations lead to amino acids substitution that increase the stability of this protein and cause malignancy [7,12].

The loss of the normal p53 function regards as a key role in the tumorigenesis and carcinogenesis. This loss may occur directly to one copy of the p53 gene, then the remaining wild-type allele is deleted or the wild-type protein is inactivated or inhibited. So the cancerous cells contain a p53 mutation characterized by overexpression of the protein [13].

p53 proteins levels in cells is balanced by MDM2, which causes ubiquitination of this protein and finally degraded, the level of p53 is a result between the rate of degradation rather than the rate of synthesis. Full functionality of p53 as a transcription factor requires phosphorylation of serines and/or acetylation of lysines near the C-terminus. The modifications of structure of this protein are to enhance its DNA binding [14]. Table 5 shows the analysis of cell cycle marker p27 of the tumor cells compared with control; it is obvious that the expression of p27 gene was significantly different compared with those of control group.

p27 is an important inhibitor that regulates progression from G1 to S phase. The poor prognosis and treatment failure associated with p27 levels in tumor [15]. The level of p27/KIP1 expression in aggressive lymphomas is considered an important prognostic marker and monitoring of those patients. p27 KIP1 under-expression indicates wild-type p53 is inactive from central dogma point of view [16].

Conclusion

The presence of p53 and p27 in Hodgkin's lymphomas indicates that expression of the p53 and P27 genes may play a role in the development of these tumors. The over-expression of these genes play a role in occurrence of HL.



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