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RESEARCH PAPER

Nuclear Co-Localization of Expressional Products of CDK4&CDK6 and Human T Cell Lymphtropic Virus Type-1- Genes: An *in Situ* Hybridization and Immunohistochemical Study of Hodgkin's Lymphoma Tissues from a Group of Iraqi Patients

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Abstract

Background Human T-cell leukemia/lymphoma virus type 1 (HTLV-1) infects 15-20 millions individuals worldwide. This on co retrovirus can be transmitted through 3 ways: horizontally, vertically (mother to child) and via blood transfusion. HTLV-1 causes 2 major diseases: Adult T-cell leukemia/lymphoma (ATLL) and tropical spastic par paresis/HTLV-1-associated myelopathy [1]. Although tumor cells exhibit rather infrequent mutations of cdk genes with the exception of G1 kinesis Cdk4 and Cdk6 amplification, over expression or hyper activation of basic cell cycle regulators is a general feature of human tumors [2]. Objective: To analyze the impact of concordant expression of cdk4, cdk6 and HTLV-1 infection on a group of tissues with Hodgkin's lymphoma (HL). Patients and methods Eighty formalin-fixed, paraffin-embedded lymph node tissues were enrolled in this study; (40) biopsies from Hodgkin's lymphoma (HL), and (40) lymph nodes with (unremarkable pathological changes) as apparently healthy controls. Detection of HTLV-1was done by ultra-sensitive version of *in situ* hybridization method whereas immunohisto chemistry detection system was used to demonstrate the expression of cdk4and cdk6 gene expression Results: The *HBZ* gene of HTLV-1positive – CISH reaction was detected in (22.5%: 9 out of 40 cases) of Hodgkin lymphoma tissues.NoHTLV-1 positive –CISH reaction was detected in healthy lymph nodes tissues of the control group. The differences between the percentages of HTLV-1 detection in HL tissues and control groups were statistically highly significant (P value = < 0.05). The positive cdk4&cdk6-IHC reactions were detected in 35% (14 out of 40 cases) and 57.5% (23 out of 40 cases) of Hodgkin lymphoma cases, respectively. A strong positive correlation was found between the detection, scores and intensity of cdk6 and cdk4markers. Conclusions: Significant expressions of both cdk6 and cdk4markersas well as HTLV-1 genes in Hodgkin's lymphoma could indicate for their possible roles both in lymph node pathogenesis and carcinogenesis.

Keywords: *HTLV-1,Hodgkin's lymphoma (HL), CDK6, CDK4, ISH, IHC.*

Introduction

Hodgkin's disease is a malignant neoplasm of the hematopoietic-lymphoid system, meaning the usual criteria for malignancy, including the potential to spread to many sites and the production of large tumor masses containing neoplastic cells Reed–Sternberg (RS) cells. Hodgkin's lymphoma (HL) is recognized by the presence of special cells called the Reed-Sternberg cells where it can constitute only 12.5% of all lymphomas. The name of Hodgkin's lymphoma is often refers to just the cancerous ones rather than all such tumors [3].

Human T-cell lymph tropic virus type 1 or human T-lymph tropic virus type 1 (HTLV-I), is from the Retroviridae family, Or thoretrovirinae subfamily, Deltaretro virus genus, Species Simian T-lymph tropic virus and the Serotypes HTLV-1[4].Human T-cell lymph tropic virus type-1 is the causative agent of an aggressive Adult T-cell Leukemia/Lymphoma, or ATL [5]. Of the 15-20 million people world-wide who are infected with HTLV-1, approximately 1 to 5% will develop ATL after a prolonged latency period of 20-30 years. Although the detailed pathogenesis is uncertain, exposure during infancy through breast feeding carries an increased risk in the development of ATL [6].

The pathogenesis is not completely understood, but HTLV-1 encoded Tax appears to be required for the initial events and perhaps HBZ for subsequent maintenance that ultimately result in ATL [7].

Cdk4-Cyclin D or Cdk6-Cyclin D and later also Cdk2-Cyclin E complexes sequentially phosphorylate retinoblastoma proteins (Rb) on different serine and threonine residues. Resulting Rb protein inactivation is required for the transcriptional activation of genes in G1/S phase. In G1 phase endogenous inhibitors of monomeric Cdk4 and Cdk6 like INK4 and inhibitors of Cdk2/Cdk4/Cdk6-Cyclin complexes like Cip and Kip proteins exert important influence on Cdk catalytic activity [8].

Cyclin-dependent kinase 4 (cdk4) and its functional homologue cdk6 act as master integrators in the G1 phase, coupling with the cell cycle mitogenic and antimitogenic signals as well as with their oncogenic perversions in cancer cells [9]. It is generally considered that mitogens activate cdk4/cdk6 by inducing D-type cyclins to concentrations that allow an inhibitory threshold imposed by INK4 cdk4/cdk6-inhibitory proteins to be overcome [10].

Lu*et al*., [11] were reported that cdk4 deficiency markedly accelerated lymphoma development and cdk4 may increase the risk for the development and/or progression of lymphoma. While, Brito-Babapulle*et al*., [12] was confirmed that cdk6 expression is dysregulated even when the breakpoint on 7q21-22 is located 66kb upstream from the coding region. Interestingly the precise assignment of the lymphoma type this study was done to unravel the rate as well as impact of either HTLV-1 or cdk4 &cdk6 in a group of Iraqi patients with Hodgkin's lymphoma.

Materials and Methods

The study was designed as a retrospective control cases one. It has recruited 80 selected formalin fixed, paraffin embedded lymph node tissue blocks were obtained, among them (40) tissue biopsies from Hodgkin's lymphoma with different grades as well as (40) lymph nodes with (unremarkable pathological changes) as apparently healthy controls. The diagnosis of these tissue blocks were based on their accompanied records. A consultant pathologist reexamined all these cases to further confirmation of their diagnosis. One section was mounted on ordinary glass slide and stained with hematoxyline and eosin, while other slides were mounted on charged slide to be used for ISH as well as IHC.

The detection of HTLV-1 by CISH kit (Zyto Vision Gmb H. Fischkai, Bremer haven Germany) was performed on 4µm - paraffin embedded tissue sections.The sequence of oligonucleotides for HBZ- HTLV-1 used in this study was 5¯-CCA TCA ATC CCC AAC TCC TG-3¯ (nucleotide positions 645–664) .The synthesized DNA probe was made to order by Bio-Synthesis (Lewisville, TX, USA).

For the *In Situ* Hybridization procedure, the slides were de-paraffinized and then treated by the standard methods of rehydration according to the details of processes for performing ISH reaction and then the probe was applied according to the instructions of the manufacturing company (Zyto Vision GmbH. Fischkai, Bremerhaven. Germany). Then application of pepsin solution to the tissue section, immersion of slides in distilled water, air drying the sections, denaturation of the slides on hot plate, adding the 20 µl of c DNA probe After that probe and target DNA were denaturized by placing in pre-warmed oven at 75°C for 8-10 minutes, slides were transferred to a pre-warmed humid hybridization chamber and incubated at 37°C for overnight.

At the next day, slides were soaked in pre-warmed protein block at 37°C and remain in the buffer for 3 minutes. Then application of AP-Strep avid in to the slides and incubate for 30 min at 37°C in a humidity chamber. Then washed in wash buffer TBS and then twice times for 1 min in distilled water and application of 5-bromo3-chloro 3- indoly/ phosphate/ nitro blue tertrazolium substrate-chromogen solution NBT/BCIP and incubated for 40 min at 37°C in humidity chamber.

Slides were incubated at 37°C for 30 minutes or until color development was developed completed. Color development was monitored by viewing the slides under the microscope. A dark blue colored precipitate forms in positive cells. Then the slides were counter stained by immersion in Nuclear Fast Red

stain for 30 seconds, then washing process was followed by immersion the slides for 1 minute in distilled water. After that sections were dehydrated by ethyl alcohol, cleared by xylene, then mounted with permanent mounting medium (DPX) Then final evaluation by light microscope.

Immunohisto Chemistry Detection System

(Abcam. England) was used to demonstrate the cdk4 and cdk6 gene expression (protein) in cells using a specific monoclonal antibodies, i.e. primary antibody for that specific epitope which binds to nuclear targeted protein .The bound primary antibody is then detected by secondary antibody which contains specific label peroxides labeled polymer conjugated to goat anti mouse immunoglobulin) . The substrate is DAB in chromogen solution produced a positive reaction resulting in a brown- color precipitate at the antigen site in these tissues. Chi-square test was used to detect the significance between variables of our study. All the statistical analysis was done by SPSS program (Version-19) &P value was considered significant when $p < 0.05$.

Results

Distribution of Patients with Hodgkin Lymphoma According to Their Age

The archival specimens collected in this study were related to Hodgkin lymphoma patients whom ages were ranged from three years to eighty years. The mean age of the Hodgkin lymphoma (33.15± 20.492years), while the mean age of apparently healthy control (AHC) was (38.70± 22.519) years. However, there was no significant difference between HL and AHC in age distribution Table (1).

Table 1: Distribution of Study Groups According to the Mean and Range of Their Age (Years)

Studied groups	No	Mean Age / Year	Std. Deviation	Std. Error	Range		ANOVA test (P-value)
					Min.	Max.	
A.H. Control	40	38.70	22.519	3.561	3	80	P^2=0.125 NS)
HL	40	33.15	20.492	3.240	3	80	
Total	80						

In Hodgkin lymphoma, the most affected age stratum less than 20 years and 21 - 40 years were constituting (32.5%:13) for each group followed by the age stratum of 41 - 60years (22.5%:9) and lowest affected group of Hodgkin lymphoma was the age stratum of 61-80 years which constituting (12.5%: 5).The statistical analysis shows non-significant differences (P>0.05) among age strata distribution of those studied groups as shown in the Table (2).

Table 2: Statistical analysis for the distribution of age strata according to the histo pathological diagnosis of studied groups

Age groups /Year		Studied groups		Pearson Chi-Square (P-value)
		A.H. Control	HL	
≤ 20	N	10	13	P=0.348 Non sign. (P>0.05)
	%	25.0%	32.5%	
21 – 40	N	12	13	
	%	30.0%	32.5%	
41 – 60	N	10	9	
	%	25.0%	22.5%	
61 – 80	N	8	5	
	%	20.0%	12.5%	
Total	N	40	40	
	%	100.0%	100.0%	

***** Non-Significant differences using Pearson Chi- square test at **P>**0.05 level.

Distribution of the Patients with Hodgkin According to Their Gender

In this study, it was found that 23 (57.5 %) of Hodgkin lymphoma were males, while the rest 17 cases (42.5%) were females. The male/female ratios of the patients with Hodgkin lymphoma was 1.35:1.The statistical analysis showed non- significant difference (P>0.05) between lymphoma patients and control groups according to gender Table (3).

Table 3: Distribution of study groups according to their gender

Gender		Studied groups		Pearson Chi-Square (P-value)
		A.H. Control	HL	
Male	No.	23	23	P=1.00 Non sign. (P>0.05)
	%	57.5%	57.5%	
Female	No.	17	17	
	%	42.5%	42.5%	
Total	No.	40	40	
	%	100.0%	100.0%	

* Non-Significant differences using Pearson Chi- square test at P>0.05 level.

Grading of Lymphoma Group Cases

In this study, the highest percentage and number of Hodgkin lymphoma patients was seen in grade I (42.5%:17) followed by grade II (35.0%:14) and the lowest was in grade III (22.5%:9) Table (4).

Table 4: Statistical analysis for the distribution of lymphoma group according to their grade

Grade		Studied groups	Pearson Chi-Square (P-value)
		HL	
I	No.	17	P=0.899 Non sign. (P>0.05)
	%	42.5%	
II	No.	14	
	%	35.0%	
III	No.	9	
	%	22.5%	
Total	No.	40	
	%	100.0%	

*Non-Significant differences using Pearson Chi- square test at P>0.05 level.

HTLV-1Associated with Apparently Healthy Lymph Node Control Tissues Using Wide Spectrum DNA-CISH Detection

In this study, the apparently healthy lymph node control tissues were tested by using wide spectrum **HTLV-1-**DNA-CISH detection. All cases were negative; therefore they are excluded from the statistical analysis.

Results of HTLV-1in Patients with Hodgkin Lymphoma

In this study the results showed as in Table (5) that the HTLV-1associated with Hodgkin lymphoma was highly significant at 1 percent level (P<0.01).

Table 5: Statistical Analysis for HTLV-1 Associated with Hodgkin Lymphoma Using CISH Technique

HTLV-1	HL (no.=40)	%	P-value
Negative	31	77.5%	ZtestP=0.001 Highly sign. (P<0.01)
Positive	9	22.5%	

Positive HTLV-1 DNA- CISH Signal Scoring

The highest percentage of HTLV-1 score signaling (15%:6) was found in the high score (score III), whereas (5 %:2) and (2.5%:1) were found within moderate (score II) and low (score I) scores, respectively. There was highly significant difference among them at 1 percent level **(P<0.01)** as shown in Table (6) & Fig.(1).

Table 6: Distribution of HTLV-1Signal Scoring Associated with Hodgkin Lymphoma by Using CISH Technique

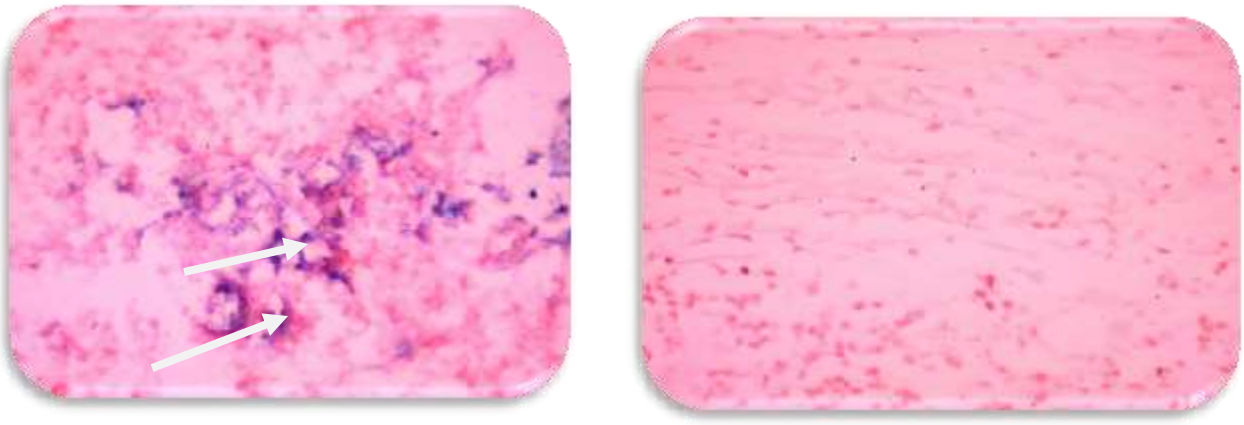
HTLV-1signal scoring		Hodgkin lymphoma(no.=40)		P-value
		No.	%	
Negative		31	77.5	X^2test P=0.00 Highly sign. (P<0.01)
Positive		9	22.5	
Scoring	I	1	2.5	
	II	2	5	
	III	6	15	

Signal Intensity Results of HTLV-1-CISH Testing

The percentage of HTLV-1-infected cells that were evaluated for the intensity of HTLV-1DNA-CISH reactions was shown in Table 7 & Fig. (1). among fourty of Hodgkin lymphoma 22.5% (9 out of 40 cases) showed positive reactions to HTLV-1-CISH test. Strong signal intensity was found in 12.5% (5cases), moderate signal intensity in 7.5% (3cases) and weak signal intensity in 2.5% (1 case). The statistically analysis shows extremely significant differences (P<0.01).

Table 7: Distribution of HTLV-1Signal Scoring Associated with Hodgkin Lymphoma by Using CISH Technique

HTLV-1signal intensity		Hodgkin lymphoma(no.=40)		P-value
		No.	%	
Negative		31	77.5	χ^2test P=0.00 Highly sign. (P<0.01)
Positive		9	22.5	
Intensity	I	1	2.5	
	II	3	7.5	
	III	5	12.5	



A B

Figure1: In Situ Hybridization(ISH) for Generic HTLV-1 Detection Infiltrative Lymphoma Cancers Using Dig oxigenin-Labeled HTLV-1 Probes ;Stained with (Blue)and Counter Stained by Nuclear Fast Red (Red).A)-HL with Positive HTLV-1 -CISH Reaction with High Score and Strong Signal Intensity (40X).B)-HL with Negative HTLV-1–CISH Reactions. (40X)

The Results of CDK4-IHC Score Signal in Hodgkin Lymphoma

Table (8) display the positive result of cdk4-IHC detection where was35 % (14out of 40 cases) from Hodgkin lymphoma group showed positive signals including 22.5 % (9 out of 14 cases) in the low score (score I) followed by 12.5 % (5 out of 14 cases) in the moderate score (score II) Fig.(2).Statistically, highly significant differences between negative, low and moderate scoring cases at 1 percent level (P<0.01).

Table 8: The percentage of CDK4-IHC score signaling in Hodgkin Lymphoma

CDK4-IHC signal scoring		Hodgkin lymphoma(n=40)		P-value
		No.	%	
Negative		36	65	χ^2test P=0.00 Highly sign. (P<0.01)
Positive		14	35	
Scoring	I	9	22.5	
	II	5	12.5	
	III	0.00	0.00	

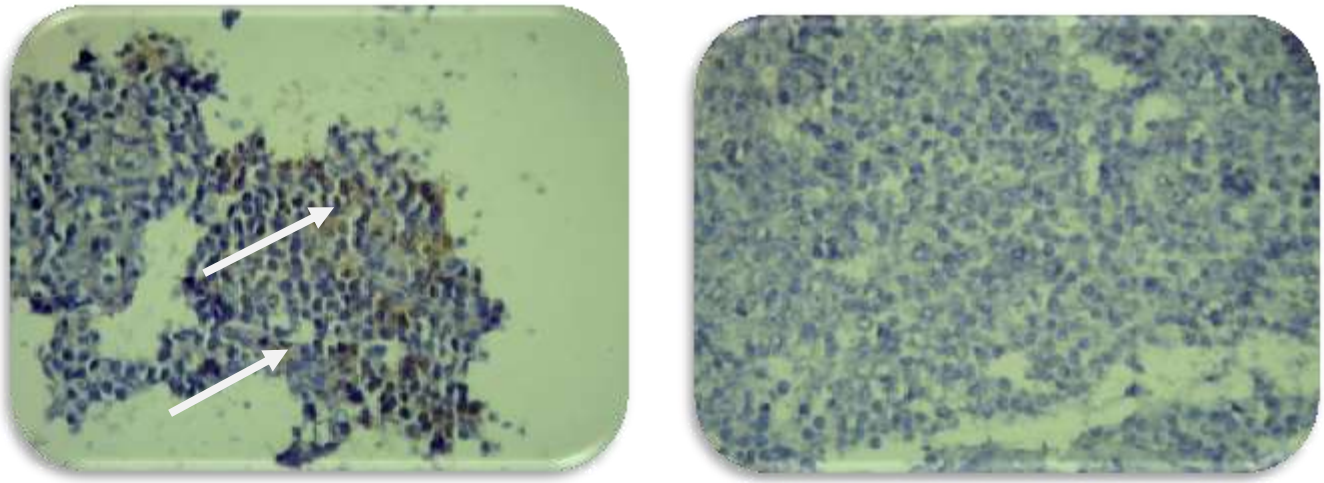
The Results of CDK4-IHC Intensity Signal in Hodgkin Lymphoma

Table (9) presents the positive result of cdk-4-IHC detection where as 35 % of Hodgkin lymphomaexhibits positive signals including 17.5 % (7 out of 14 cases) in the moderate intensity(II), followed by 15% (6 out of 14 cases) in the weak intensity (I) ,and 2.5% (1 out of 14cases) in the strong intensity (III)Fig.(2).Statistically, highly significant differences between negative, weak, moderate

and strong intensity cases at 1 percent level **(P<0.01)** in Hodgkin lymphoma group.

Table 9: The Percentage of CDK4-IHC Intensity Signaling in Hodgkin Lymphoma

CDK4-IHC signal intensity		Hodgkin lymphoma(n=40)		P-value
		No.	%	
Negative		36	65	χ^2test P=0.00 Highly sign. (P<0.01)
Positive		14	35	
Intensity	I	6	15.0	
	II	7	17.5	
	III	1	2.5	



A B

Figure 2: Infiltrative Lymphoma Cancers Showing the Results of Immunohistochemistry Staining of CDK4 Protein Using Biotinylated Anti- CDK4 Protein- Antibodies, Stained by DAB-Chromogen (Brown) and Counter Stained by Mayer·s Hematoxylin (Blue). A) - HL with Positive CDK4 -IHC Reaction (40X). B) - HL with Negative CDK4 -IHC Reactions. (40X)

Results of CDK6- IHC Signal Scoring

In the Hodgkin lymphoma group,47.5%(19out of 23 cases)have low score (score I) ,whereas,7.5% (3out of 23cases) and 2.5% (1 out of 23 cases)were found to have scores of moderate score (score II) and high score (score III) , respectively Fig.(3).Statistically, highly significant differences were found among negative, low, moderate and high scoring casesat 1 percent level (P<0.01) Table (10).

Table 10: The CDK6- IHC Score Signaling Assay Tests Results of Hodgkin Lymphoma Patients

CDK6-IHC signal scoring		Hodgkin lymphoma (no.=40)		P-value
		No.	%	
Negative		17	42.5	χ^2 test P=0.00 Highly sign. (P<0.01)
Positive		23	57.5	
Scoring	I	19	47.5	
	II	3	7.5	
	III	1	2.5	

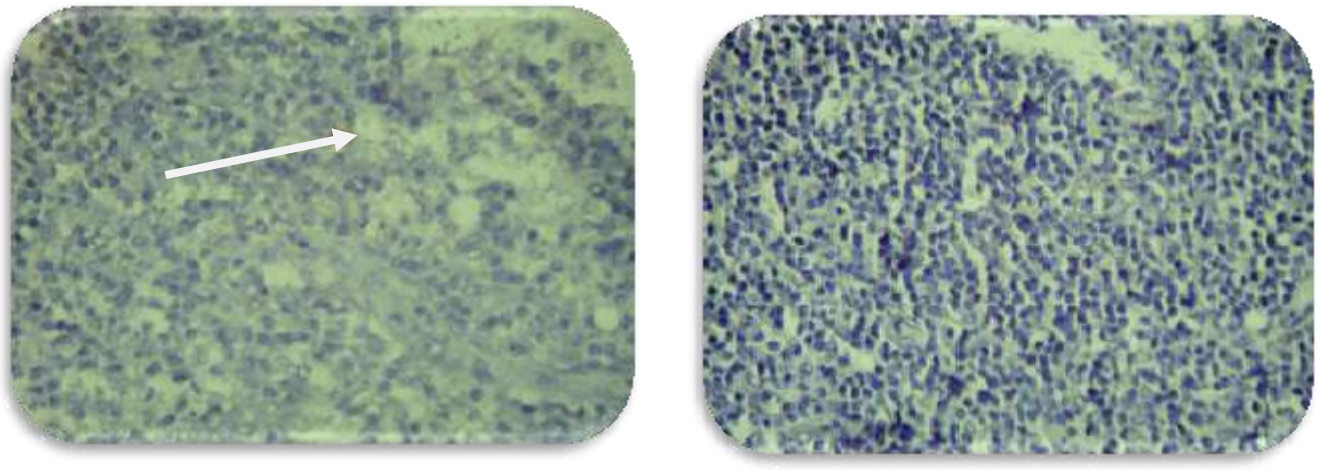
The Results of CDK6-IHC Intensity Signal in Hodgkin Lymphoma

Table (11) presents the positive result of cdk-6-IHC detection where as 57.5 % of Hodgkin lymphoma exhibits positive signals including 52.2 % (12 out of 23 cases) in the moderate intensity(II), followed by 34.8% (8out of 23 cases) in the weak intensity (I) ,and 13.0% (3 out of 23cases) in the strong intensity (III).

Statistically, significant differences between negative, weak and moderate and strong intensity cases at 5 percent level **(P<0.05)** in Hodgkin lymphoma group.

Table 11: The CDK6- IHC Intensity Signaling Assay Tests Results of Hodgkin Lymphoma Patients

CDK6-IHC signal intensity		Hodgkin lymphoma (no=40)		P-value
		No.	%	
Negative		17	42.5	χ^2test P=0.014 Sign. (P<0.05)
Positive		23	57.5	
Intensity	I	8	20.0	
	II	12	30.0	
	III	3	7.5	



A **B**

Figure 3: Infiltrative Lymphoma Cancers Showing the Results of Immunohistochemistry Staining of CDK6 Protein Using Biotinylated Anti- CDK6 Protein- Antibodies, Stained by DAB-Chromogen (Brown) and Counter Stained by Mayer·s Hematoxylin (Blue). A) - HL with Positive CDK6 -IHC Reaction (40X). B) - HL with Negative CDK6 -IHC Reactions. (40X)

Correlations among of Studied Markers (Wide Spectrum HTLV-1, CDK4, CDK6, Bcl2) in Patients with Hodgkin Lymphoma HTLV-1

There is a strong positive relationship (with highly significant correlation) between HTLV-1 and cdk4 markers in Hodgkin lymphoma.{r = 0.483, P = 0.002, (p <0.01)}. However, there are no significant correlations among HTLV-1 and other markers Table (12).

CDK6

There is a strong positive relationship (with highly significant correlation) between CDK6 and Bcl-2 markers in Hodgkin lymphoma.{r = 0. 413, P = 0.008, (p <0.01)}.Similarly, there is a strong positive relationship (with highly significant correlation) between cdk6 and cdk4 markers in Hodgkin lymphoma.{r = 0. 419, P = 007, (p <0.01)} Table (12).

Table 12: Spearman's rho Statistical Testing to Evaluate Studied Molecular Markers in Relation with HTLV-1 Infections in Hodgkin Lymphoma

Assay (HL)						
Spearman's rho		Age groups /Year	Grade	HTLV-1	CDK6	CDK4
Grade	r	-.125				
	P-value	.442				
HTLV-1	r	.030	.133			
	P-value	.855	.412			
CDK6	r	.190	.028	.221		
	P-value	.241	.863	.171		
CDK4	r	.152	-.295	.483**	.419**	
	P-value	.350	.065	.002	.007	

***Correlation is significant (P<0.05). **Correlation is highly significant (P<0.01).**

Discussion

In Hodgkin lymphoma, the mean age of patients was (33.15± 20.492years) and the most affected age stratum is less than 20 years and between 21 - 40 years followed by the age stratum of 41 - 60years Table (1).

From the present results, it was noticed that malignant tumors have also increased with the proceeding of age of patients and our results are broadly agreed with the results obtained by SEER,[13],they revealed that the most common age of diagnosis in HL is between 20 and 40 years old.

The present study is contracted with study conducted by Adedayo *et al*., [14], who found that the mean age of clinical presentation was 56 years for lymphoma and were in the age range of 9–89 years. The present results are consistent with those reported world-wide where Hodgkin lymphoma was usually affecting the adults in the third decade and a second peak after the age of 50[15]. Jakovic *et al*., [16] who reavlead that the range of HL patients was 16–68 years and the mean age was 35.43 ± 13.5 years. The most affected age was older than 45 years (25%).

In this study, it was found that 23 (57.5 %) of Hodgkin lymphoma were males, while the rest 17 cases (42.5%) were females. The results of the current study are in concurrence with the results of most other studies. Hussein *et al*., [17] was found that HL is more common in male (70%) than female (30%). Dorak and Karpuzoglu in 2012 [18] found in Hodgkin lymphoma, the gender ratio reverses toward adolescence.

Besides immune surveillance, genome surveillance mechanisms also differ in efficiency between males and females. Other obvious differences include hormonal ones and the number of X chromosomes. Also the difference might be due to the peculiar characteristics of the referral centers, smaller case numbers, or geographic distribution. However, still there is need to generate more data regarding variation in gender predominance in our population for better studies.

The highest percentage and number of Hodgkin lymphoma patients was seen in grade I (42.5%:17) followed by grade II (35.0%:14) and the lowest was in grade III (22.5%:9). The present results could mark for the occurrences of low grade malignant lymphomas in Iraqi patients at earlier age than that expected worldwide. This discrepancy could be attributed or as the result of small sampling in the present study, as compared to other abroad studies. These results also call for more research works into the reasons for the prevalence of these low grade lymphomas in our country.

Since these and the present result are fronted by the results in western countries, who had documented a majority of lymphomas in these countries of low-grade typed (54.5%) [19]. One reason for this difference could be due to the low mean age of the population in Iran & Iraq compared to the western countries. Inadequate screening of the patients might have also contributed to the differences. In addition, the patients often present themselves to the medical care system at much later stages of the diseases where the low grade lymphomas have evolved into secondary type of high grade once.

A definitive connection has been demonstrated between HTLV-1infections and cancer of the lymphoma and leukemia because of the multiple analogues between lymphoma and leukemia carcinogenesis it would seem evident that HTLV-1 is an important risk factor also for lymphoma .The concept of the relationship between HTLV-1 and lymphoma is based on the identification of HTLV-1 genome sequences in lymph nodes tissues and immortalization of T cells by HTLV-1 [20].

The positive results of HTLV-1-CISH detection, where 22.5% (9 of total 40) showed positive signals, while, 77.5% negative signals which represented 31 out of 40 cases in this group. The negative results are probably related to the absence of HTLV-1-DNA in these biopsies or could be related to its presence in the cells at different regions of that tissue. The presence of different type of HTLV-1 other than these type used in this study is another possibility.

The HTLV-1positive cases of Hodgkin lymphoma in present study may predominantly constitute with the results obtained by O'connor*et al*., [21] .The ratio of positive cases of HTLV-1 in HL patients were 25%. Also our results are broadly agreed with Adedayo and Shehu, [22] revealed that 50% of HL cases were positive with HTLV-1.

Moreover, Monavar*et al.* [23] conducted a study using enzyme-linked immunosorbent assay (ELISA) for anti-HTLV-1 and they found 33.3% of HL patients were positive. The differences between these studies and our study may be due to the peculiar

characteristics of the referral centers, smaller case numbers, or geographic distribution and this may be described to genetic and environmental etiologic factors involved in these studies.

In other hands, a study conducted by Mortreux *et al*., [24] revealed that only 5% of HL lymphoma patients were infected with HTLV-1.

The deregulation of the *CDK4* gene at the genetic and protein levels suggest a functional role for these genes in the transformation process and could potentially provide targets for prognostic tests or therapeutic interventions[25]. The positive of current result of cdk4-IHC detection was 35 % (14out of 40 cases) from Hodgkin lymphoma group showed positive signals.

Marzec *et al*., [25] strongly expressed cdk4 in all Mantle Cell Lymphomas (MCL) cell lines and patient samples but failed to express detectable cdk6. Interestingly, in this regard, cdk4 not only interacts with cyclin D1 but is markedly over expressed in MCL. Moreover, Hernandez *et al*., [26] was detected cdk4 in 4 of 19 (21%) highly proliferative blastoid variants and was associated with mRNA and protein over expression occurs in some mantle cell lymphomas (MCL).

The present results showed that 57.5% of cdk6protein expression was positive in Hodgkin lymphoma cases. Our results are in agreement with the report of Kollmann *et al*. [27]. Who revealed that cdk6 is frequently expressed at high levels in human and murine lymphoma and has been proposed to be a driving force for these diseases.

The correlations analysis of this study showed that there is a strong positive relationship (with highly significant correlation) between HTLV-1 and cdk4markers in Hodgkin lymphoma{r= 0.483, P = 0.002, ($p < 0.01$)}. The results of our study are in agreement with other reports revealed by many researchers. Schmitt *et al.,* [28] reported that, in the presence of Tax, Cdk4 was activated. The suppression of Tax synthesis, however, resulted in a significant reduction of the Cdk4 activities but did not influence the expression of Cdk4, cognate D-type cyclin proteins. They suggested that Tax induces Cdk4 activity in primary human T lymphocytes; this Cdk activation is likely to account for the mitogenic Tax effect and for the abnormal T-cell proliferation of HTLV-1-infected lymphocytes.

The results of the correlation analysis in this study are in concurrence with the result of Bockstaele *et al.* [9], who, reported that the biochemical and genetic characterization of D-type cyclins, their cyclin D-dependent kinases (cdk4 and cdk6), and the polypeptide CDK4/6 inhibitor $p16^{INK4}$ over two decades ago revealed how mammalian cells regulate entry into the DNA synthetic phase of the cell division cycle in a retinoblastoma protein (RB)-dependent manner.

The deregulation of cdk4 and cdk6 kinase activities associated with D-cyclins resulting in Rb hyper phosphorylation is associated with a loss of control between mitogenic stimuli and cell cycle regulation, which leads to uncontrolled cell proliferation. Cdk 4 hyperactivity has been well documented in a wide variety of cancers, and in particular in melanoma, lung cancer and lymphoma [2, 29].

The significant detection of HTLV-1 along with *cdk4* and *cdk6* genes expression production Hodgkin lymphoma patients are supporting the hypothesis of an etiologic roles for that virus along with mutated and /or defected *cdk4* and*cdk6* genes in Hodgkin lymphoma development.

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