The*bcl2* t(14;18) Chromosomal Translocation in Iraqi Patients with Non-Hodgkin's Lymphoma

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Abstract

Non-Hodgkin lymphomas (NHLs) are hematologic malignancy with the highest prevalence worldwide. They are broadly classified as B-cell or T-cell lymphoma, depending on which type of lymphocyte becomes cancerous. Chromosomal translocations are the hallmark genetic aberration in NHL with specific translocations often selectively associated with particular NHL subtypes. An important example is the t(14;18) which leads to constitutive activation of the *bcl2* oncogene by the enhancers of the immunoglobulin heavy chain locus.One of the main objectives of this study to determine the frequency of *bcl2* t(14.18) chromosomal translocation in NHL Iraqi patients and also to determine the correlation of *bcl2* translocation with subtypes of NHL. A 46 formalin fixed paraffin embedded blocks were examined included 32 blocks from NHL patients, in addition to 10 blocks from reactive follicular hyperplasia, and 4 tonsils as a control group. Genomic DNA was extractedand amplified by polymerase chain reaction (PCR) by using oligonucleotide specific primers for major breakpoint region (MBR), and minor cluster region (MCR) of the *bcl-2* gene on chromosome 18 and conserved *JH* sequence on chromosome 14. The present result illustrate significantly higher frequency of *bcl2* t(14,18) at MBR in NHL and reactive follicular hyperplasia Iraqi patients.We concluded the *bcl2*t(14,18) chromosomal translocation is a hallmark in NHL Iraqi patients with no association with specific subtype.

الخلاصة

يعد اورام الغدد اللمفاوية اللاهودجيكن من الاورام الدموية الخبيثة الواسعة الانتشار في العالم . اعتمادا على نوع الخلية السرطانية يصنف المرض الى اورام الخلايا بي اوتي وتعد اورام بي اكثر انتشارا من خلايا تي. يعد انتقال مواقع الكروموسومات السمة المميزة لأورام خلايا بي اللمفاوية وترتبط هذه الانحرافات الوراثية احيانا مع امراض فرعية معينة من هذه الاورام وغالبا ما تعد كخطوة اولية في عملية التحول الخبيث ومن اهمها التناقل بين كروموسوم ١٤و١٨ والمؤدية الى تنشيط الجين الورمي *bcl2* المضاد لموت الخلايا المبرمج.من اهم اهداف هذه الدراسة هو تحديد نسبة انتشار تناقل كروموسومات (١٤و١٨) للجين الورميفيالمرضى االعراقيين المصابين باورام الغدد اللمفية اللاهوجيكن وعلاقته مع انواع اورام الغدد اللمفاوية. اذ اشتملت الدراسة على٤٦ خزعة نسيجية تضمنت ٣٢عينة اخذت من المرض المصابيين بالاورام اللمفاوية اللاهودجيكن بالاضافة الى ١٠ عينات من الاورام الحميدة و٤ عقد لمفاوية اخذت من مرضى مصابيين بالتهاب اللوزتين . تم استخلاص الحامض النووي نوع دنا (DNA) من هذه العينات والقيام بتضخيمه بواسطة تفاعل البلمرة المتسلسل وذلك للتحري عن وجود تناقل الكروموسومات (١٤و١٨) لجين *bcl2* باستخدام برايمرات خاصة لمواقع الكسر الاولية والثانوية مع برايمرات خاصة بجين الاميونكلوبيولين. اظهرت النتائج نسبة عالية لانتشار الانحراف الكروموسومي للجين الورمي في المرضى المصابين باورام الغدد اللمفاوية والاورام الحميدة في نقاط الكسر الرئيسية عند مقارنته مع مجموعة السيطرة .تم الاستنتاج بان الانحراف الكروموسومي للجين الورمي هو صفة عامة المرضى االعراقيين المصابين باورام الغدد اللمفية اللاهوجيكن ولا يعتبر علامة مميزة لنوع معين.

Introduction

Non-Hodgkin lymphomas (NHLs) are hematologic malignancy with the highest prevalence worldwide.They arise from the lymphoid system[1]. NHLs classified as B-cell or T-cell lymphoma, depending on which type of lymphocyte becomes cancerous, B-cell non Hodgkin

lymphoma (B NHL) is more common than T-cell lymphoma, [2]. They arise from an injury to the Deoxyribonucleic acid (DNA) of a lymphocyte progenitor. The damage in DNA is acquired rather than inherited [3]. Several subtypes are included within B cell NHL (B-NHL), diffuse large B cell lymphoma (DLBCL) is the most common type, while other types are follicular lymphoma (FL), small lymphocytic lymphoma (SLL), Burkitts lymphoma (BL) and other less- common and often rare sub-types of NHL exist [4].The exact cause of NHL remains unknown. However, researches have focused some factors that may contribute to the development of lymphoma, including genetic factors, impaired immune system, viruses such as, Epstein Barr Virus (EBV), bacterial causes (e.g. Helicobacter pylori), exposure to chemicals and heavy smoking [5].Chromosomal translocations have been observed in up to 90% of NHL cases [6]. These translocations, with or without additional genetic lesions, can precipitate the activation of oncogenes or inactivation of tumor suppressor genes [7]. An important example is t(14;18)(q32;q21) which leads to constitutive activation of the *bcl2* oncogene by the enhancers of the immunoglobulin heavy chain locus *(IgH)*. This chromosomal translocation is typically present in tumor cells of follicular lymphoma but can also be found in other types of NHLs [8]. The clustering of the breakpoints on chromosome 18 with or near the *bcl-2* oncogene facilitates the application of polymerase chain reaction (PCR) to amplify this *bcl-2*/*JH* breakpoint fragment [9].The objectives of this study are to determine the frequency of t(14.18) chromosomal translocation ofNHL Iraqi patients and also to determine the correlation of *bcl2* translocation with subtypes of NHL .

Subjects and Methods

The subjects included in this study were represented as formalin fixed paraffin embedded (FFPE) biopsy tissue blocks that were obtained from Iraqi patients and collected from the histopathology laboratories of Iraqi Hospitals and Private Labrotaries.Diagnosis of these tissue block were based on the obtained histopathological laboratory records of samples that had accompanied each tissue blocks in each laboratory . Also, a second histopathological reexamination of obtained tissue blocks was done by senior histopathologist. The collection samples of this study were carried out during the period from July 2012 until to May 2013. Study groups included 46 FFPE blocks;these samples were distributed as the following,32samples from patients of NHL, 10blocks from reactive follicular hyperplasia, and four tonsils used as a control group.The ages of NHL patients were ranged between 3-81 years with median age 55 years, and mean ± SD equal 45.718± 23.51 years. All NHL samples were taken before treatment and cases of NHL were classified according to the international working formulation (IWF) of the National Cancer Institute to 3 groups [10] as table (1) elucidate.

Table (1): Non Hodgkin lymphoma subtypes enrolled in this study

NHL subtypes	Number
High grades	7
Intermediate grade	17
Low grade	8

Formalinembedded blokes enrolled in this study sectioned by microtone, serial tissue sections were cut into 5-15 µm thickness from each tissue block.Genomic DNA was extracted from these sections by using Genomic DNAMinikit(InvitrogenAskutlanda) [11].*bcl2*t(14,18) gene was detected by using polymerase chain reaction . Amplification was performed in a programmable MultigeneThermal CyclerPCR (Labnet

International Inc.USA). primers sequences were revealed in table 2.

Table (2): Sequences of primer used for PCR amplification of *bcl2/JH* gene

Primer	Sequence	Size of Product	References
MBR JH	5'-TTAGA GAGTT GCTTT ACGTG-3' 5'-ACCTGAGGAG ACGGT GACCA GGGT-3'	**220bp**	**12-14**
MCR JH	5'-GACTC CTTTA CGTGC TGCTA CC-3' 5'-ACCTGAGGAG ACGGT GACCA GGGT-3'		

PCR conditions

Genomic DNA was amplified in a final volume of 20 µl (5µl Genomic DNA+ 3µlMBRor MCR primer + 3µl*JH*primer +5µlBioneer's master mix with Green Taq DNA polymerase + 4µl DDW) using the following conditions: Denaturation at 94 ^{o}C for 4 min. followed by 45 cycles of (denaturation at 94 ^{o}C for 45 seconds, annealing at 55^{o}C for 45seconds. and extension at 72 ^{o}C for 1 min. and a final extension was at 72 ^{o}C for 5 min. then hold at 4 ^{o}C for indefinite time. Then the amplification products were separated by electrophoresis through 1.5% agarose gel stained with ethidium bromide**.**

Statistical Analysis

The data were analyzed using SPSS statistical software (version 16). $P < 0.05$ was considered statistically significant. The distribution and comparison of each was made using the Chi-square test. Odds ratio (OR) with 95% confidence intervals (CI) were estimated for the effect of high risk translocation.

Results

The results of frequency *bcl2*t(14; 18) chromosomal translocation in studied groups were illustrated in tables (3 and 4). The result revealed that there were a surprising high degree of translocation rate at MBR region (96.9%, and 100%) in NHL and reactive follicular hyperplasia respectively. while low frequency have been showed at MCR 9.090% (3/32) among NHL, and (0%) in reactive follicular hyperplasia, study results also did not recorded any positive resultat MBR and MCR in tonsils (control group). The current results showedhighly significant differences ($p<0.00$) between patients and control group, The results of PCR for detection of t(14, 18) *bcl2* translocation in studied groups are reveal in figures (1, and2). The positive results detected in 220 bp for MBR, and MCR.On the other hand types of NHL were categorized into three groups according to WF classification low, intermediate, and high grades. We found that the percentages of MBR *bcl2* translocation was 100% for both low, and intermediate grades, while(85.7%) present in high grade lymphoma with no significant (0.634) differences between groups table (5) revealed that. In addition to that, the presence of *bcl2* translocation at MCR was respectively detected (28.6%, 0%, and 12.5%) in high, intermediate, and low grades. also no significant (0.628) variation observed among all types of NHL as in table (6).

Table (3): Frequency of *bcl2*t(14;18) chromosomal translocation at MBR region .

			MBR *bcl2* translocation		
			N	P	Total
Groups	NHL	Count	1	31	32
		% within group	3.1%	96.9%	100.0%
		% within MBR	20.0%	75.6%	69.5%
	RFH	Count	0	10	10
		% within group	.0%	100.0%	100.0%
		% within MBR	.0%	24.4%	21.0%
	Tonsils	Count	4	0	4
		% within group	100.0%	.0%	100.0%
		% within MBR	80.0%	.0%	8.5%
Total			5	41	46
		% within group	10.6%	89.4%	100.0%
		% within MBR	100.0%	100.0%	100.0%

P: positive**.** N: negative, MBR: major break point region, NHL: non Hodgkin lymphoma,RFH: reactive follicular hyperplasia

Table (4): Frequency of *bcl2*t(14;18) chromosomal translocation at MCR region.

			MCR*bcl2* translocation		
			N	P	Total
Groups	NHL	Count	29	3	32
		% within group	90.6%	9.4%	100.0%
		% within MCR	67.4%	100.0%	69.6%
	RFH	Count	10	0	10
		% within group	100.0%	.0%	100.0%
		% within MCR	23.3%	.0%	21.7%
	Tonsil	Count	4	0	4
		% within group	100.0%	.0%	100.0%
		% within MCR	9.3%	.0%	8.7%
Total		Count	43	3	46
		% within group	93.5%	6.5%	100.0%
		% within MCR	100.0%	100.0%	100.0%

MCR : minor cluster region

Table (5): Frequency of MBR *bcl2*t(14;18) chromosomal translocation in non-Hodgkin lymphoma subtypes

			MBR*bcl2* translocation		Total
			N	P	
Subtype	High	Count	1	6	7
		% within subtype	14.3%	85.7%	100.0%
		% within MBR	100.0%	19.4%	21.9%
	Intermediate	Count	0	17	17
		% within subtype	.0%	100.0%	100.0%
		% within MBR	.0%	54.8%	53.1%
	Low	Count	0	8	8
		% within subtype	.0%	100.0%	100.0%
		% within MBR	.0%	25.8%	25.0%
Total		Count	1	31	32
		% within subtype	3.1%	96.9%	100.0%
		% within MBR	100.0%	100.0%	100.0%

Table (6): Frequency of MCR*bcl2*t(14,18) chromosomal translocation in non-Hodgkin lymphoma subtypes

			MCR *bcl2* translocation		
			N	P	Total
Subtypes	High	Count	5	2	7
		% within subtypes	71.4%	28.6%	100.0%
		% within MCR	17.2%	66.7%	21.9%
	Intermediate.	Count	17	0	17
		% within subtypes	100.0%	.0%	100.0%
		% within MCR	58.6%	.0%	53.1%
	Low	Count	7	1	8
		% within subtypes	87.5%	12.5%	100.0%
		% within MCR	24.1%	33.3%	25.0%
Total		Count	29	3	32
		% within subtypes	90.6%	9.4%	100.0%
		% within MCR	100.0%	100.0%	100.0%

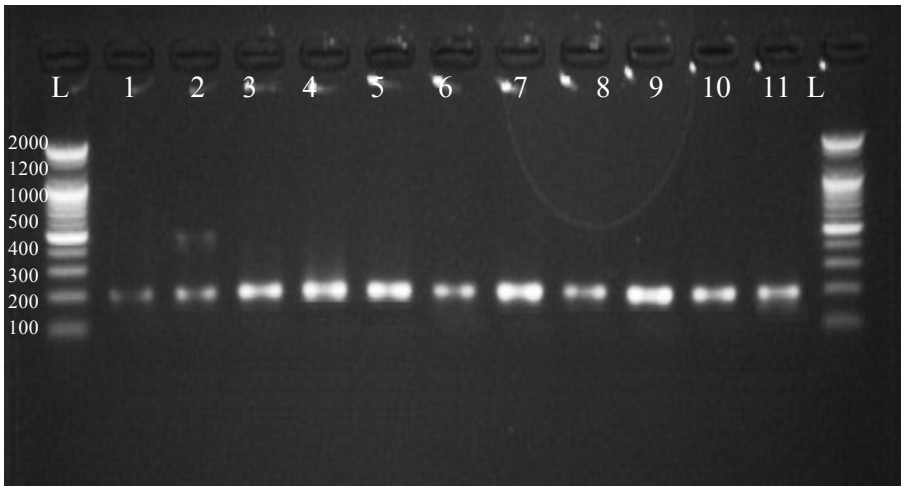

Figure (1): The PCR amplification products of the *bcl2* translocation at major breakpoint fragments on agarose gel (1.5%), 70 volt for 60 minute.
Lane (L): 100 bp DNA ladder
Lane (1-11):PCR positive products of NHL and RFH bands in size region 220 bp.

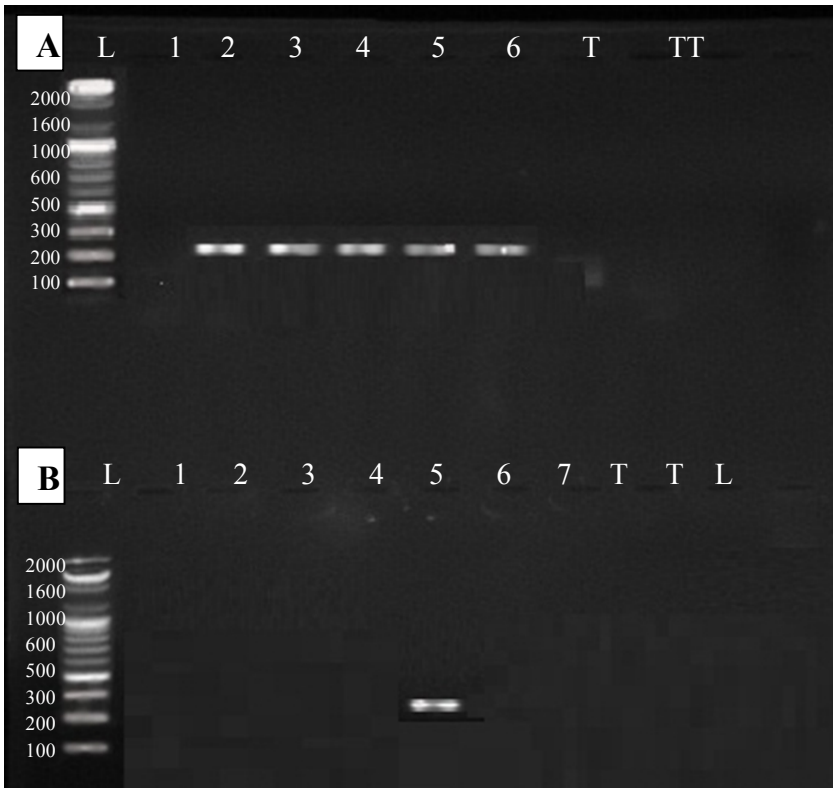

Figure (2): The PCR amplified products of *bcl2* translocation on agarose gel (1.5%) ,70 volt for 60 minutes. Bands in the size region 220 bp are indicative of the *bcl2*t(14;18) chromosomal translocation of NHL samples.

Lane L: (100)bp ladder, T:Tosile

A- Lane (2-6) shown positive result of NHL while negative result show in T lanes at major break point region.

B- Lane (5) only positive result of NHL at minor cluster region.

Discussion

In fact, chromosomal translocations have been observed in up to 90% of NHL cases [15]. These translocations, with or without additional genetic lesions, can precipitate the activation of oncogenes or inactivation of tumor suppressor genes. The most frequent translocation in human lymphoma is the t(14; 18) (q32; q21), which juxtaposes a putative oncogene *bcl2* from 18q21 with one of the six J segments of the immunoglobulin heavy chain locus on chromosome 14 [16].

Our results were found 96.8% positive result at MBR *bcl2* chromosomal translocation, and 9.4% at MCR region that consist with Xiang Zhi-fu*et al*. have been shown approximately 60% *bcl2* gene breakpoints occur within the 150 bp MBR atthe untranslated region in the third exon of *bcl2* gene. In comparison, 30% of breakpoints occur at MCR region [17]. Theclustering of breakpoints at MBR and MCR have made it possible to use PCR amplification to identify cellscontaining *bcl-2/JH* gene rearrangement, and this tumor-specific molecular marker can be used to follow the natural history of lymphoma and search for MRD [18]. The results that conducted from this study were full consistence with the results that shownthe *bcl2* t(14;18) translocation occurs in 57% to 90% of patients with follicular lymphoma (FL)) and in 9% to 30% of patients with DLBCL, 55- 60% of translocations occur at the MBR, whereas 10- 20% occur at the MCR [19-20]. On the other hand our results indicated high percentage (100%) of this translocation that was detected in reactive hyperplasia patients. In deed thet(14; 18) translocation can be detected in non-malignant

circulating lymphocytes of healthy subjects was fueled extensive debate on the role of the translocation in NHL and, at large aspect, on the biological steps underlying NHL development. Additional events, possibly involving exposures to carcinogenic agents, are required for t(14;18)-positive cells to develop a fully malignant phenotype [21],it has been suggested that t(14;18)-positive cells in healthy individuals are under control of immunological mechanisms [22]. This result high closely with the result of Schuler *et al* [23] which indicated this translocation show in 30–80% of healthy individuals, also highly-sensitive polymerase chain reaction techniques have allowed for the detection of a low number of t(14;18) copies in peripheral blood lymphocytes and other normal lymphatic tissues [24]. Furthermore, the high prevalence of the t (14; 18) translocation among healthy individuals would indicate that perhaps overexpression of the Bcl2 protein as a result of this transformation may not sufficient for malignant transformation [25]. Also, the frequency of translocation in healthy individuals increases with age and smoking that illustrated by Schuler *et al* [26], particularly in those who had longer smoking duration. While others dissimilarity with our results, the translocation was detected in very low level in peripheral blood, bone marrow, and lymphoid tissues of a high proportion of people who have no evidence of lymphoma [21].

The samples of current study only FFPE from cases study without knowing their history whether they have an associated other factors related to this aberrant. However, this finding needs further investigation for its potential impact on public health. In general, the differences in percentages of *bcl2*t(14, 18) translocation detected among the present as well as the previous studies could be attributed to the primer sensitivity as well as environmental factors, sample size, the quality and sensitivity of the techniques used in these studies.In this approach, the frequency of *t*(14;18) translocations determine also, according to NHL subtype .the current results were, high frequency of MBR *bcl2* t(14,18) translocation identified100% in intermediate and low and (85%) in high grades with no significant variation P>0.05, furthermore, our findings also revealed this translocation absence at MCR of intermediate ,while in high, and low grades viewed 11.8% and 12,5% respectively.in previous FISH-based study, the t(14;18) translocation were detected in 30-50% of the patients with intermediate NHL and in 87.7% of patients with low grade [27]. While the t(14;18) translocation was presented in seven (78%) of nine patients with intermediate NHL [28].This discrepancy may reflect the variation in t(14;18) in lymphomas due to biological differences among populations, differences in case selection and assay protocols may also contribute to variation among studies [29]. Variation in the prevalence of t(14;18) also, may reflect differences in diagnostic criteria among pathologists, who have been shown to be much less likely to agree on DLBCL versus follicular lymphoma diagnoses [30]. The high frequency of *bcl2* mutations in all NHL subtypes and non-random pattern that we and others [31] have observed supports the notion that these are noteworthy events in all types of NHL and not specific for any type.

Conclusion

The *bcl2* t (14, 18) chromosomal translocation is a hallmark of NHL Iraqi patients indicated in all subtypes of disease .The high frequencyof chromosomal translocation at MBR among NHL suggest that *bcl2* t (14, 18) has an essential role in pathogenesis of lymphoma.

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