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Clonal Rearrangement of Immunoglobulin Heavy Chain Gene (IgH) and Detection of Bone Marrow Involvement in B- Cell Non-Hodgkin's Lymphoma

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ABSTRACT

Objective: The evaluation of staging or post-therapy bone marrow biopsies for involvement by B cell lymphoma has traditionally been based on morphologic findings. To improve sensi-tivity, morphologic examination is now augmented by ad-vances in molecular technology that detect clonal lym-phoid populations by immunoglobulin gene rearrange-ments and lymphoma associated chromosomal translo-cations.

The purpose of the present study is to correlate the sensitivity of morphologic and histochemical assessment of bone marrow lymphomatous involvement and PCR-based molecular studies in pa-tients with B-lineage lymphomas.

Patients and Methods: This study was conducted on 49 patients with histopathological diagnosis or clinical suspicion of NHL that were considered for bone marrow examination either for diagnosis, staging or post therapy re-evaluation. Bone marrow biopsies were evaluated for presence or absence of infiltration using conventional morphologic examination, and immunohistochemical staining using CD 79a and CD 20. Detection of clonal IgH gene rearrangement in paraffin embedded biopsy sections using PCR was performed.

Results: Of the 49 cases studied, 19 cases (39%) had evident morphological B.M. involvement, 11 cases (22%) showed minimal infiltration and 19 cases (39%) showed no marrow infiltration. Using immunohistochemical staining, 25 cases (51%) were positive for the monoclonal CD79a, while only 15 cases (30%) were positive for the monoclonal CD20. Using PCR technique, 35 cases showed clonal IgH rearrangement (71%), 31 cases using FRIIIA (88.6%), seven cases using FRII (20%) and three cases with both FRIIIA and FRII (9%).

Conclusion: In histopathologically diagnosed cases of B-NHL, presence of morphological evidence of bone marrow infiltration requires confirmation of a clonal B-cell nature where demonstration of clonally rearranged IgH gene may be considered a valuable test. Yet, in histopathologically diagnosed cases of B-NHL showing minimal non-conclusive or absent morphological evidence of infiltratrion, demonstration of clonal rearrangement of IgH using PCR represents a solid evidence of marrow involvement. In absence of a histopathological diagnosis of B type-NHL, presence of a clonal rearrangement of IgH, is not alone sufficient to document a B-cell lymphoma.

Key Words: *B-NHL - Minimal residua l - Disease - Clonal IgH rearrangement.*

INTRODUCTION

B cell lymphomas constitute about 85% of NHL. Most patients suffering from intermediate and high grade B cell NHL achieve a complete remission following conventional chemotherapy. However, 50% of these patients relapse within 2 years. The relapse is presumably caused by the persistence of a small number of lymphoma cells that are below the limit of detection of standard diagnostic procedures. Unlike more aggressive NHL histiotypes, indolent lymphomas (SLL, CLL , FCL, MCL) are disseminated disorders with frequent microscopic bone marrow and peripheral blood involvement [1].

The evaluation of staging or post-therapy bone marrow biopsies for involvement by B cell lymphoma has traditionally been based on morphologic findings. To improve sensi-tivity, morphologic examination is now augmented by ad-vances in molecular technology that detect clonal lym-phoid populations by immunoglobulin gene rearrange-ments and lymphoma associated chromosomal translo-cations. Development of the polymerase chain reaction (PCR) presumably detects clonal populations below the morphologic threshold. Although clone-

specific PCR methods are specific and sensitive to a level of one in 107 cells, these methods are too inef-ficient for routine testing because unique primers are required for each patient and may fail in some leukemias and lymphomas as a result of clonal evolution [2].

The development of consensus primers for the hyper variable region of the immunoglobulin heavy chain (IgH) permits routine use of gene rearrangement studies in the evaluation of lymphocytic disorders. These assays achieve excellent specificity, although they have demonstrated a lower sensitivity than the clone-specific primers. The specific sensitivity depends on the primer set, lymphoma subtype, degree of competitive amplifi-cation between monoclonal and polyclonal lymphocyte DNA, and tissue fixation [3]. With a multiple primer panel, the clonality detection rate in primary diagnostic tissue is approximately 80% overall, varying with the type of non-Hodgkin's lymphoma [2].

The purpose of the present study is to correlate the sensitivity of morphologic and histochemical assessment of bone marrow lymphomatous involvement with PCR-based molecular studies in pa-tients with B-lineage lymphomas.

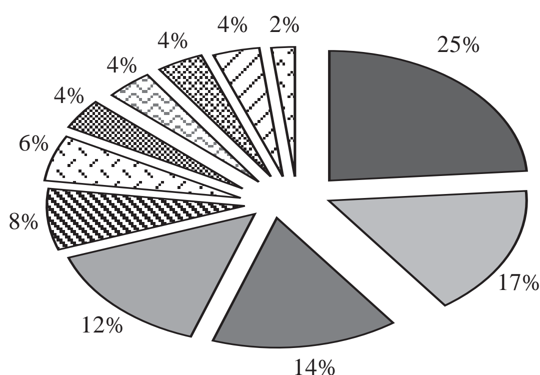
SUBJECTS AND METHODS

This study was conducted on a total of 49 patients who were admitted to Kasr Al-Aini hospitals with a histopathological diagnosis or clinical suspicion of lymphoma. Patients were candidates for bone marrow examination either for staging, re-evaluation, or primary diagnosis.

Patients comprised 32 males and 17 female with an age range of one year to seventy years.

All histopathological data were obtained for all patients

Patients were classified according to their pathology as follows: (Fig. 1).



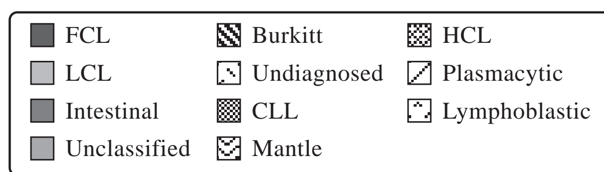


Fig. (1): Distribution of cases among various histopathologic types.

For each patient a unilateral or bilateral iliac crest bone marrow biopsy was obtained together with an aspirate for cytological evaluation of smears.

Paraffin embedded reactive and normal lymph node sections from archival blocks were used as a negative control for the molecular study.

Bone Marrow Evaluation:

Sample Processing:

1- *Bone marrow core biopsy:* Cores were fixed in a mercurial fixative (Zenker solution) for 24 hours, washed in running tap water, dehydrated in ethanol (70%, 95%, 100%), and embedded in paraffin. 3-5mm serial sections were cut on glass slides. For each patients 8 slides were prepared (2 on ordinary glass slides for routine examination, and four on positively charged slides for IHC. Prior to staining, samples were deparaffinized by immersing in two changes of xylene and rehydrated by dipping in decreasing concentrations of ethanol (100%, 95%, 70%) and finally in distilled water.

2- *Bone marrow aspirate smears:* Spread and stained with leishman -Giemsa.

Morphologic Examination:

Morphological examination of stained aspirate smears and routinely stained biopsy sections

(Geimsa and hematoxylin and eosin stains) was done for each case.

Immunohistochemical Staining Procedure:

Peroxidase block was done using 3% H_2O_2 in methanol. This was followed by heat retrieval using EDTA buffer pH 8.6 and pressure cooker heating for 1.5 min for CD79a antibody while CD20 required no antigen retrieval [4].

Protocol:

Dako Envision immunostaining Kit was used (DakoCytomation Denmark A/S, DK-2600 Glostrup, Denmark). The kit utilizes an immunoperoxidase staining technique. CD20: prediluted (Monoclonal Mouse Human B-cell, CLASS/SUBCLASS: lgG2a, kappa) and CD79a in a dilution of 1/10 (Dako, Monoclonal mouse Anti-Human CD2O, clone L26 and CD79a, B Cell Clone JCB117) were used as primary antibodies.

Sections of reactive lymph nodes were similarly treated and used as controls.

Molecular Studies:

Fixed Tissue DNA Extraction (DNAzol method)

DNA was directly extracted from fixed, paraf-fin - embedded trephine biopsies of all patients. Approximately 50 4-mm trephine sections were twice incubated with xylene at room temperature, centrifuged at 12000rpm, and decanted: After ethanol washing, evapo-ration, and proteinase K treatment, the supernatant was decanted and bone marrow biopsy pellet was used for DNA extraction using DNAzoL Reagent (Genomic DNA isolation Reagent).

Determination of DNA Concentration:

1- 5 µl of the DNA solution were diluted to about 50 times with 245 µl of water and mixed well by vortexing (this DNA is to be discarded and can therefore be treated in this way).

2- The absorbance (A) was read in a spectrophotometer (Pharmacia LKB-Ultraspec II, Pharmacia) at 260 nm against a water blank.

3- Calculations: A solution of DNA at a concentration of 50 pg/ml, gives an absorbance of 1.0 at 260 nm. Therefore the absorbance reading at 260 nm (A) multiplied by 50 gives the concentration, of the prepared diluted DNA, solution and multiplied by the dilution factor (50) gives the concentration of the original DNA solution.

The following equation was used: Concentration of DNA in pg/ml = absorbance reading at 260 x 2500.

Determination of the Purity of DNA Solution:

1- The absorbance reading at 260nm was taken (A260). The absorbance reading at 280nm was also taken (A280). The ratio of A260 to A280 should be in the range of 1.7 [5].

2- If it is less than 1.7, the DNA solution is contaminated with other proteins.

Analysis of Immunoglobulin Heavy Chain Genes Rearrangement By PCR [6]:

Polymerase Chain Reaction Methods:

Primers: (R & D Systems, Inc., Minneapolis, MN 55413, USA) (Table 1).

Table (1): Primers specifications.

VH-FRIIIA	5` ACACGGC(C/T) (G/C) TGTATTACTGT3
VH-FRII	5`GTCCTGCAGGC(C/T)(C/T)CCGG (A/G)AA(A/G)(A/G) GTCTGGAGTGG 3`
Anti sense VJH	5` GTGACCAGGGT (A/G/C/T) CCTTGGCCCCAG 3`
Anti sense JH	5` TGAGGAGACGGTGACC 3`

For each sample two amplifications were performed for each sense primer using a DNA ther-mocycler (Perkin Elmer Cetus 480, Norwalk, CT). For the first amplificaltion a standard 50µl reaction mixture was used that con-sisted of 2µl extracted DNA, 25µl master mix prediluted (Taq DNA polymerase, PCR buffer containing 3mM MgCl2, dNTP mix (Qiagen), 2µl VH-FRIIIA or VH-FRII, 2µl Anti sense JH and 19 ml distilled water. 30 PCR cycles were performed consisting of denaturation for 1 min at 94ºC annealing at 60ºC for 1 min, extension for 1 min at 73ºC.

For the second amplification a standard 50µl reaction mixture consisting of 2µl of amplified DNA from first amplification, 25µl master mix prediluted, 2µl VH-FRIIIA or VH-FRII, 2µl Anti sense VJH, 19 ml distilled water. 20 PCR cycles were performed consisting of denaturation for 1 min at 94ºC annealing at 60ºC for 1 min, extension for 1 min at 73ºC.

Amplified Material Analysis:

15μl of PCR reaction product together with a molecular weight marker were electrophoresed in a 6% polyacrylamide gel stained with ethidium bromide and photographed under ultraviolet light.

A PCR was considered positive when a sharp monoclonal band resided within the appropriate primer size range. The primer VH-FRIIIA gave the monoclonal band between 50 and 150 Kb while the VH-FRII primer gave the monoclonal band between 150 and 300 Kb.

RESULTS

Results of Bone Marrow Aspirate and Biopsy Examination:

Twenty eight cases (57%) showed no evidence of infiltration on aspirate examination, however biopsy examination revealed minimal infiltration in 9 of these cases (35%). In the remaining 21 cases (43%), examination of both bone marrow aspiration and biopsy revealed infiltration, thus bone marrow biopsy revealed infiltration in 61.2% of cases (30/49) (Fig. 2).

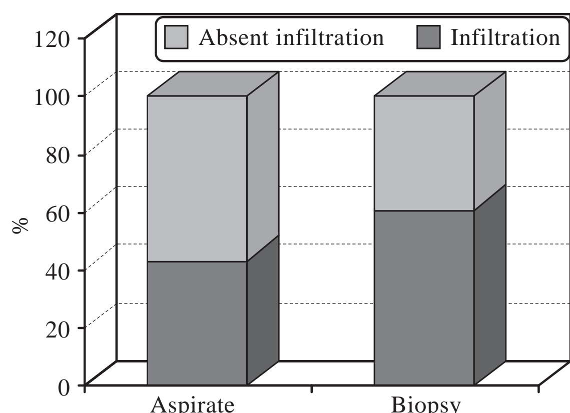


Fig. (2): Infiltration incidence according to aspirate and biopsy morphological examination.

According to morphological infiltration of bone marrow aspirate and biopsy, cases were classified into three groups as shown in the following table (2).

Table (2): Morphological classification of cases.

Group	No. of cases	%	Description
Group A	19	39	Marrow infilt. >10% of marrow cellularity
Group B	11	22	Minimally infilt. <10% (suspicious involve.)
Group C	19	39	No marrow infiltration

IHC staining & PCR results: (Table 3) (Figs. 3,4,5)

In all the studied cases (49):

Immunohisto-chemical staining with monoclonal CD79a showed positivity in twenty five cases (51%) (Fig. 3), while CD20 was positive in fifteen cases only (30%) (Fig. 4).

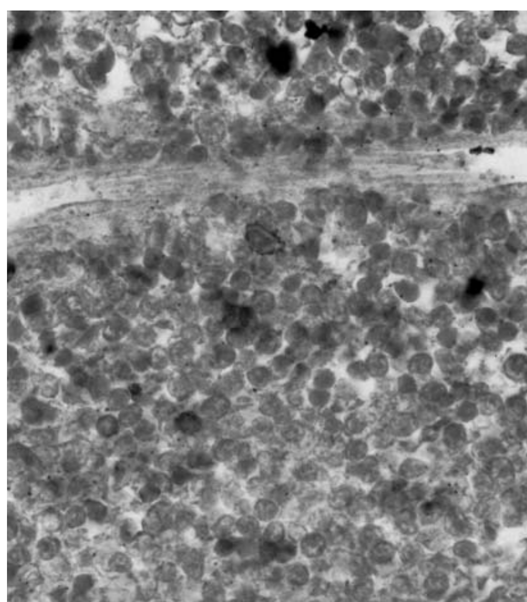


Fig. (3): CD20+ staining in an infiltrated case of FCL. Immunoperoxidase staining.

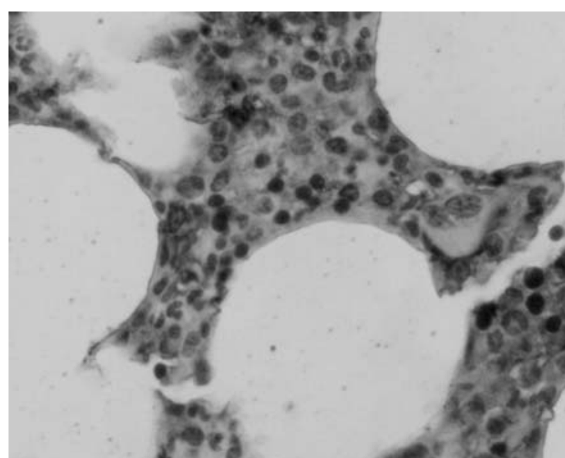


Fig. (4): CD79a showing few scattered positive cells. Immunoperoxidase staining.

Polymerase Chain Reaction:

PCR for immunoglobulin heavy chain gene rearrangement showed clonal rearrangement in thirty five cases (71%) (Fig. 5).

Table (3): IHC and PCR results of all the cases.

	CD20		CD79a		PCR	
+ve	15	31%	25	51%	35	71%
-ve	34	69%	24	49%	14	19%

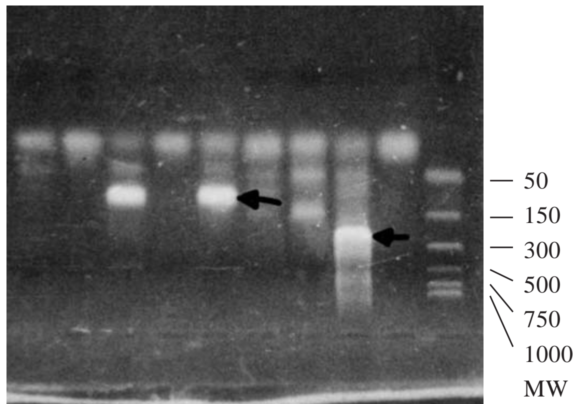


Fig. (5): Ethidium bromide stained agarose gel electrophoresis of PCR product obtained using the FRIIIA (solid arrow) & FRII (dotted arrow) consensus primers sets.

Distribution of PCR positivity among FRIIIA and FRII primers: (Fig. 6).

The majority of cases showing clonal gene rearrangement were detected by FRIIIA primer set alone [31/35 cases (88.8%)]. A clone was detected in 7/35 cases (20%) using FRII primer set alone, while three cases (9%) showed a clone using both FR IIIA and FRII primer sets.

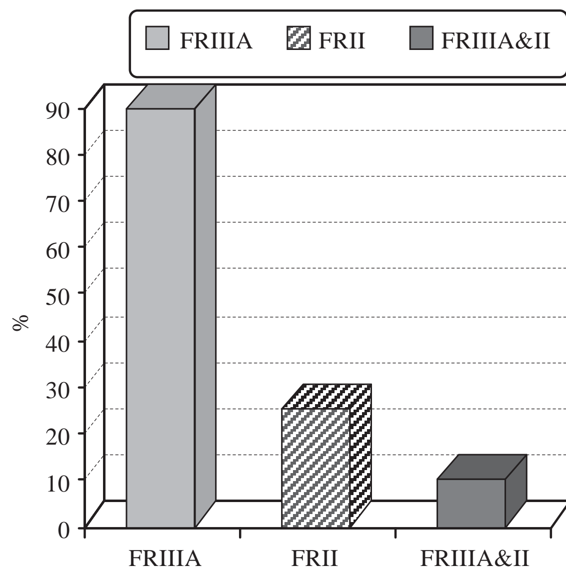


Fig. (6): Distribution of PCR positivity among FRIIIA and FRII.

Distribution of frame positivity among the FCL and LCL:

Distribution of frame positivity among the FCL and LCL revealed a clonal rearrangement of IgH gene in 67% of FCL cases (8/12) using both primer sets. FRIIIA primer set detected 6 of these cases (75%), FRII detected 3 cases (37.5%) while one case was detected using both primer sets (12%). Clonal rearrangement of the immunoglobulin heavy chain gene was detected in 75% of our LCL cases (6/8), including all 3 minimally infiltrated cases and 3/5 of the morphologically non-infiltrated cases. FRIIIA primer set detected all 6 (100%) cases while clonality was revealed in only one case using FRIIIA and FRII primer sets (Fig. 7).

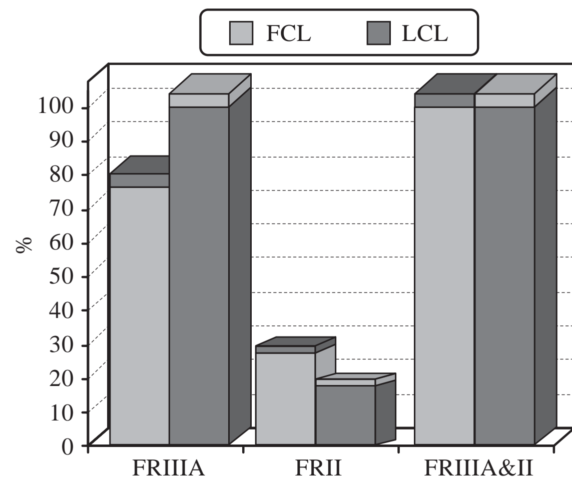


Fig. (7): Distribution of frame positivity among the FCL and LCL subgroups.

Distribution of CD 79a,CD20 and PCR results according to pathology (table 4a and b):

Pathologically Diagnosed Cases (40):

In the 24 cases with minimal or no morphological evidence of lymphomatous involvement (groups B & C), IHC staining using CD79a revealed an infiltrate in 5 cases while demonstration of clonal IgH rearrangement using PCR revealed an infiltrate in 14 cases. The combined use of IHC and PCR detected infiltration in a total of 16 cases in this group.

Thus the sensitivity of the IHC in detecting minimal marrow involvement in our cases; using CD79a was found to be 31.25% (5/16), while that of PCR was 87.5% (14/16).

Pathologically unconfirmed cases (9): Table (4-b).

Table (4-A): Distribution of CD79a, CD20 and PCR results among the different groups and pathological subtypes.

Pathology	No.	Group A: >10% infiltration				Group B: Minimally infiltrated <10%				Group C: Not infiltrated			
		No.	CD79a	CD20	PCR	No.	CD79a	CD20	PCR	No.	CD79a	CD20	PCR
FCL	12	7	7	7	7	1	0	0	1	4	1	0	0
LCL	8	–	–	–	–	3	1	0	3	5	1	0	3
Burkitt	4	2	2	2	2	1	0	0	1	1	1	0	0
SLL	2	2	2	2	2	–	–	–	–	–	–	–	–
MCL	2	2	2	2	2	–	–	–	–	–	–	–	–
LPL/L	2	1	1	0	1	–	–	–	–	1	0	0	1
HCL	2	1	1	1	0	1	0	0	1	–	–	–	–
L/L	1	1	1	0	1	–	–	–	–	–	–	–	–
Extra-nodal	7	–	–	–	–	1	0	0	1	6	1	0	3
Total no. of B-NHL cases	40	16	16	14	15	7	1	0	7	17	4	0	7
Percent	100	40%	100%	87.5%	93.8%	17.5%	14.3%	0%	100%	42.5%	23.5%	0%	41.2%

FCL = Follicular cell lymphoma. CLL = Chronic lymphocytic leukemia. LPL/L = Lympho-plasmacytic lymphoma/leukemia.
LCL = Large cell lymphoma. MCL = Mantle cell lymphoma. L/L = Lymphoblastic lymphoma/leukemia.

Table (4-B): Distribution of CD79a, CD20 and PCR results in pathologically unconfirmed cases.

Pathol.		No.	CD79a	CD20	PCR
Group A > 10% infilt.	Unclassified	3	2	1	3
	Undiagnosed	—	—	—	-
	Total no.	3	2	1	3
Group B < 10% infilt.	Unclassified	1	0	0	0
	Undiagnosed	3	0	0	3
	Total no.	4	0	0	3
Group C not infilt.	Unclassified	2	2	0	0
	Undiagnosed	—	—	—	—
	Total no.	2	2	0	0

DISCUSSION

The evaluation of staging or post therapy bone marrow biopsies for involvement by lymphoma has traditionally been based on morphologic findings. To improve sensi-tivity, bilateral and double bilateral trephine biopsies have been performed to reduce sampling error. Morphologic examination is augmented by advances in molecular technology that detect clonal lym-phoid populations by immunoglobulin gene rearrange-ments and lymphoma associated chromosomal translo-cations. In our study, Bone marrow biopsy infiltration was studied using different techniques in an attempt to establish a protocol for detecting and confirming lymphomatous involvement of the bone marrow in cases with B-NHL.

This study was conducted on 49 patients with histopathological diagnosis or clinical suspicion of NHL that were considered for bone marrow examination either for diagnosis, staging or re-evaluation post therapy. Nine of these cases had their bone marrow examined for a clinical suspicion of lymphoma without a documented pathological examination of another tissue. Bone marrow biopsies were evaluated for presence or absence of infiltration using

Conventional morphologic examination, and immunohistochemical staining using CD 79a and CD 20 which cover the whole spectrum of B lymphoid neopla [7]. Detection of clonal IgH heavy chain gene rearrangement, an early event in B-cell development, in paraffin embedded biopsy was performed to confirm the clonal nature of the infiltrate [8].

In this study consensus primers instead of clone-specific primers were used. Although clone-specific PCR methods are specific and sensitive to a level of one in l06 cells, these methods are too inef-ficient for routine testing because unique primers are required for each patient and may fail in some leukemia and lymphomas as a result of clonal evolution [2]. It has been reported by Abdel Rehim et al. [9] that the primers with the highest detection rate for the immunoglobulin heavy chain gene rearrangements are directed against the framework (FR) III region of the various VH genes. The addition of other framework regions, particularly

FRII primers, increases the detection rate of this test. We therefore included both primer sets in this study.

On morphological examination, twenty eight cases (57.2%) showed no evidence of infiltration on aspirate examination, however biopsy examination revealed minimal infiltration in 9 of these cases (35%). In the remaining 21 cases (42.8%), examination of both bone marrow aspiration and biopsy revealed infiltration. Thus bone marrow aspiration examination detected infiltration in 42.8% while bone marrow biopsy revealed infiltration in 61.2% of cases (30/49).

Following bone marrow biopsies examination, patients were classified into three groups; Group (A): Cases that showed marrow infiltration constituting more than 10% of marrow cellularity. This group included 19 cases (39%). Group (B): cases that were minimally infiltrated (suspicious) 11 cases (22%) Group (C): cases with no marrow infiltration (19 cases (39%). As regards infiltration incidence in the different histopathological subtypes, it was only feasible to analyse cases with FCL (12) and LCL (8), as we had only small number of cases in other pathologic categories. Among the follicular lymphoma group, seven cases (58%) were morphologically infiltrated, a single case was minimally infiltrated and four cases (33%) showed no evidence of infiltration. For large cell lymphoma, none of the 8 cases studied showed evident marrow involvement on morphological and/or immunohistochemical examination, while a minimal marrow involvement was detected in 3 cases on morphological examination, which is similar to the incidence observed by Kroft et al. (2001) **[10]**.

PCR amplification of IgH chain genes from DNA obtained from paraffin embedded biopsy sections detected a clonal rearrangement in a total of 35 cases. 88.6% of these cases were detected by FRIIIA primer set (31/35). A clone was detected in 7 cases (20%) using FRII primer set. Three cases (9%) showed a clone using both FR III and FRII primer sets. Thus the use of FRIIIA and FRII primer sets together detected all clonally rearranged IgH genes. In accordance with this finding, Coad et al. **[2]** detected PCR positivity in 37% of cases using single primer set while the percentage increased to 80% using multiple primer sets. Crotty et al. and **[11]**, Lehann et al. **[12]** detected a clone in 67.5% and in 66% of B-NHL cases respectively. Distribution of frame positivity among the FCL and LCL revealed a clonal rearrangement of IgH gene in 67% of FCL cases (8/12), with 75% of cases detected by FRIIIA primer set alone. FRII primer set detected the remaining 2 cases. In LCL cases Clonal rearrangement of the immunoglobulin heavy chain gene was detected in a total of 75% using FRIIIA primer set, FRII detected 1 case while both primer sets revealed clonality in one case. In a similar study a combination of FRII and FRIIIA primers detected 79 to 98% of B cell neoplasms depending on the type of disease **[13]**.

In group (A), CD79a was positive in all cases with a histopathological diagnosis of NHL while CD20 was positive in only 87.5% of these cases. A clonal rearrangement of the IgH was detected in 93.7% of histopathologically diagnosed cases. In a study by Kremer et al. **[14]** PCR was 100% positive in 12 patients with evident marrow involvement with B-NHL. Krober et al. **[15]** detected a clone in 77% of 529 patients in the same category. Thus the more the number of examined cases, the higher the possibility of evaluating the sensitivity of the technique. Failure to demonstrate a clonal IgH gene rearrangement in the case with HCL, in spite of an evident marrow involvement on morphological and immunohistochemical examination, may be due to the use of consensus primers which are not a perfect match to the sequence being amplified and result in less efficient amplification. In addition, somatic mutations of the immunoglobulin heavy chain gene of some mature B disorders might alter the sequence of the region amplified by the primers so that primer hybridization is sub optimal or does not occur **[16]**.

In group (B), PCR revealed a clonal rearrangement in 100% of cases, CD 79a detected only one case (14.28%), while CD 20 detected 0%. Failure to demonstrate cases with minimal infiltration by IHC staining may be attributed to the loss of lymphomatous tissue on serial sectioning due to their small size. Coad et al. 1997 had a lower percentage (25%) of detection of clonal rearrangement in minimally infiltrated NHL cases than in the present study, yet this could be attributed to the use of bone marrow aspiration rather than bone marrow biopsy, which decreases the amount of clonal tissue

available. In similar studies using PCR amplification, Kremer et al. [14], detected a clone in 20% of 5 cases with minimal bone marrow infiltration. Using laser micro-dissection technique, which greatly increased the amount of clonal tissue in the sample, they could detect clonality in 70% of their cases. The higher sensitivity in clonal detection in this group in the present study might also be attributed to the criteria used to define minimal infiltration. In our study cases showing small lymphomatous collections that do not meet the criteria of morphological classification as involvement were considered minimally infiltrated.

Seventeen cases were morphologically uninvolved with a lymphomatous infiltrate (group C). Immunohistochemical staining of these cases using CD 79a revealed scattered positive cells in 4 cases (23.5%), however only 2 of these cases showed a clonal rearrangement of the IgH gene. CD 20 failed to reveal any positivity. PCR revealed a clonal rearrangement in 41.2%of cases (7cases). Cases with interstitially scattered lymphomatous involvement are difficult to diagnose on morphological basis alone but require confirmation by IHC staining or demonstration of a clonal proliferation. Coad et al. [2] detected a clone in 13 cases (11%) out of 129 cases of B-NHL which were morphologically non-infiltrated. The lower incidence of infiltration detection in their study could be attributed to the use of bone marrow aspiration rather than bone marrow biopsy which decreases the amount of clonal tissue available. In the same study, DNA extracted from sections provided results more concordant with morphology, as PCR detected a clone in 10 out of 11 DNA specimens extracted from trephine biopsies with positive morphologic findings and negative PCR aspirates.

In the 24 cases with minimal or no morphological evidence of lymphomatous involvement, the sensitivity of the IHC staining in detecting minimal marrow involvement, in this study, using CD79a was found to be 31.25% (5/16), while that of PCR was 87.5% (14/16) Similarly, in a multicenter study, a PCR sensitivity of 83.5% in non follicular lymphomas was found [17]. Also, Uchiyama et al. [18] reported that PCR has been used with increasing frequency to detect clonal rearrangements of IgH in formaldehyde fixed, paraffin wax embedded tissues with a sensitivity ranging between 50 and 80%.

Nine cases were undiagnosed by histopathology, 6 were reported as unclassified and 3 had no accessible tissue for examination. Of the 6 unclassified cases, 3 belonged to group (A), two showed positive staining with CD 79a and one with CD20. All three cases showed a clonal IgH gene rearrangement. The remaining three cases, one belonged to group (B) and two to group (C), showed no staining with either CD79a or CD20 and no clonal rearrangement on PCR examination. The three cases with no accessible tissue but performed the biopsy based on clinical suspicion belonged to group B and all showed a clonal rearrangement of IgH gene. A follow-up of two of these 3 cases revealed a leukemic phase of a lymphoblastic lymphoma confirmed by immunohistochemistry, in one case. The other case was pathologically diagnosed as T cell lymphoma following surgical removal of a mediastinal lymph node. IgH gene aberrant rearrangements have been detected in 16% of T-cell neoplasms [19]. Follow-up of the third case was not feasible.

From the above results, we suggest the following scheme for studying and interpreting clonality in B-NHL, involving the bone marrow:

I- In histopatholgically diagnosed cases of B-NHL

• A positive PCR bone marrow result in a case of known B-NHL is a confirmatory test for the presence of a clonal involvement of the bone marrow in presence of evident morphological and/or immunohistochemical infiltration.

• A positive PCR bone marrow result in a case of known B-NHL is mandatory and an indispensable proof for the presence of a clonal involvement of the bone marrow in presence of minimal morphological and/or immunohistochemical evidence or absence of either.

II- In histopathologically non-diagnosed cases of B-NHL a clonal IgH gene rearrangement, in absence of morphological and immunohistochemical evidence of infiltration, though confirming the presence of a clonal disorder, yet is non-conclusive for diagnosing a B type-NHL in the bone marrow. Further confirmation by demonstration of Ig light chain gene rearrangement is recommended.

Thus, in morphologically minimally-infiltrated or non-infiltrated bone marrows of

histopathologically documented cases of B-NHL; positive IHC and/or PCR findings are strong suggestive evidence of involvement.

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