Academic Journal of Cancer Research 2 (1): 51-60, 2009
ISSN 1995-8943

Association of Specific Structural and Numerical Chromosome Abnormalities in Lymphoma Cell Lines: The Extent of Genetic Instability

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Abstract: To carry out karyotopic analysis of lymphoma cells and on their *in-vitro* passages to ascertain the extent of genetic instability with an aim to understand the genetic etiology of cancer in general. Human malignant cell lines obtained from ECACC were incubated and cultured. Cytogenetic slides were prepared as per standard protocol. G-banding was carried out description of chromosomal rearrangements followed the norms of ISCN. Five lymphoma cell lines (Daudi, Raji, MC116, HS602, Namalwa) were analyzed to determine the extent of structural and numerical variation using Giemsa band analysis. All cell lines displayed abnormal chromosomes. The aneusomics observed were, in general, consistent with the aneusomics determined by previous analyses with some added complexicities. All cell lines displayed a range of aneusomies involving trisomies of chromosome number 7 and 18, while some chromosomes were not involved in aberrations at all. MC116 and Namalwa cell lines were prone to most of the structural abnormalities. Except Namalwa, which was found to be consistently hypodipliod, others were hyperdiploid. High levels of karyotype heterogeneity and marker were found. Analysis showed a wide range of numerical changes affecting multiple chromosomes in lymphoma cell lines. The data suggest that chromosomal instability (CIB) is responsible for extensive aneuploidy associated with these tumors. Cytogenetic characterizations of the five human lymphoma cell lines were attempted. The study revealed new information since their deposition with ATCC. The karyotypic differences in cells from different clones and the degree of heterogeneity in the cell population observed is an indication of the involvement of different chromosome regions, with the possible implication at the level of gene expression and the structure of genes itself. The study is expected to help identify characteristic chromosome abnormalities in human lymphoma cell lines.

Key words: Lymphoma · Daudi · HS 602 · MC 116 · Namalwa · Raji

INTRODUCTION

The study of chromosomal anomalies in cancer has become a very exciting field of investigation since the discovery of concordance between chromosomes and certain cancer diseases such as Burkitt's lymphoma, chronic myeloid leukemia and retinoblastoma. The investigations of chromosomes in cancers have revealed that in most cases, cells show chromosomal aberrations involving numerical and structural changes and the defects are consistent in some of these [1-3]. The changes can readily be classified as primary and secondary changes [4]. The primary changes describe the monoclonal origin of tumors and any change that confers proliferative advantage on the cell is retained and conserved in the daughter cells, there by characterizing entire cell populations with them. Clonal evolution occurs as a result of additional changes arising from tumor instability and therefore, related to tumor progression and generally characterized as secondary changes. There can be additional random changes as well, unrelated to tumor initiation or progression making the scenario even more complex [5-7].

In comparison, the hematological neoplasm have relatively few chromosomal changes allowing the identification of primary or specific chromosomal alterations easier, while, a vast majority of solid malignancies demonstrate complex karyotypic changes obscuring primary lesions in the process [8-10]. Some neoplasm contain no known chromosome abnormality

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indicating the possibility of neoplastic condition being associated with submicroscopic rather than gross primary chromosomal changes and have their likely origin in point mutations [11]. The presence of normal cells in neoplasm, as argued by Levan and Mitelman, may represent normal stromal elements [12]. The cancer cytogenetic involving karyotypic alterations apparently show that these aberrations are unevenly distributed throughout the cell genome. Other studies on tumors have shown that different chromosomes, regions and bands are preferentially involved [13]; as a result, considerable numbers of new studies on karyotypic changes in tumors are added every year. Some of them are discussed in subsequent text. Chromosomal changes in hematological malignancies though, make the concept of a genetic etiology of cancer more clear in comparison [14].

Though, fairly established, chromosomal abnormalities have been the subject of intense investigation with an object to correlate, if not all, at least some. In a similar effort, Kolomietz *et al.* [14] were even able to demonstrate the virus-induced tumors, damaging the chromosomes following infection. However, a close relationship between chromosome changes and oncogene activation is exemplified in Burkitt's lymphoma where, the activation of the *MYC* oncogene is activated by specific translocation between specific chromosomes [15-16]. In addition, amplification of oncogenes has also been reported in some chromosomes [17]. These studies emphasize the accurate chromosomal analysis. The chromosome rearrangements comprising inversion, insertion, deletion and translocation have drawn attention to chromosomal hypothesis of oncogenesis [3]. In fact, activation of a chromosomal segment, known for its role in embryogenesis is important and if suppressed during cellular differentiation, can result in a number of chromosome rearrangements [2]. It is established fact that, the chromosomal abnormalities in cancer reflect a multistep development of the malignant phenotype [2, 13]. The original abnormalities may involve submicroscopic changes leading to premalignant or moderately malignant conditions. In either case, the cytogenetic analysis of cancer cells and their cell lines has not only become mandatory in the quest to understand and subsequently, control the cancer, but also needed to be monitored consistently.

Since, the nature of chromosomal changes appears to be dependent on primary abnormality and on the type of neoplasm, a study of the karyotypic analysis of lymphoma cells is essential and hence attempted in direct preparation from cancer cells and extended to their serial *in-vitro* passages. The study is of prime importance in demonstrating the karyotype stability under new growth environment. The results presented here are, a part of detailed study on molecular cytogenetic analysis of lymphoma cell lines, emphasizing the usefulness of multicolor FISH and CGH in conjunction. And yet the molecular cytogenetic techniques only serve as a useful addition to routine banding analysis, so the presented venture is more classical. In this study we employed Giemsa banding to measure the range of chromosomal aberrations.

MATERIALS AND METHODS

Cell Lines and Passages: Human malignant lymphoma cell lines Daudi, HS602, MC116, Namalwa and Raji were obtained from the European collection of Cell Culture (ECACC). The HS602 cell line was obtained from the American Type Cell Culture (ACTCC). All were incubated at 37°C in the presence of 5% CO_2 in air with humidified conditions. Sterility tests for mycoplasma, bacteria and fungi were employed and remained negative throughout the observation.

Cell Culture and Cytogenetic Preparation: All cell lines were cultured in a medium recommended by ATCC (Gibco BRL, Gaithersburg, MD, USA). Primary tumor specimens were finely minced and/or supplemented with 15% fetal calf serum (Gibco, BRL), 2 m mol L-Glutamine (Gibco, BRL) and antibiotics. Cytogenetic slides were prepared as described by Dracopoli [18]. Briefly, short term cultured cells (<5 days) were treated with 0.1 μg/mL Colcemid (Gibco, BRL) for two hours, hypotonically treated with 0.075M KCL, fixed in 3:1, methanol: acetic acid and then prepared on to slides. The cells were aged for 3-5 days prior to Giemsa banding.

Giemsa-banding (G-banding): Slides were left for two to seven days at room temperature or incubated at 80°C for 3-4 hrs, before applying G-banding. Immersion lens oil was removed from the slides by rinsing in ethanol. Destaining was performed by rinsing slides with fixative and washing with Sorenson's buffer and trypsin was spread over for 35-54 seconds at room temperature. Slides were washed with Sorenson's buffer and stained in freshly prepared Leishman's stain for 1-2 m and washed with Gurr's buffer, applied fiber free postlip paper to remove extra buffer. The description of chromosomal rearrangements followed the norms laid down by International System for Human Cytogenetic

Nomenclature (ISCN). The compound light microscope (Olympus BH2) equipped with high quality optics was used to obtain information from metaphase chromosomes.

RESULTS

A very sedate growth rate was observed in the cells of HS602 cell line in comparison to MC116 cell lines (Fig 1).

The profile of numerical and structural changes of various cell lines of lymphomas has been summarized in Table 1. Individually, chromosome X, Y, 4, 9, 12, 17 and 19 appeared to be less susceptible to numerical and structural changes in most of cell lines whereas, MC116 and Namalwa were prone to structural abnormalities of both types for most of the chromosomes, followed by Raji and Daudi fairly stable in comparison.

The range of counts and model number of chromosomes in five cell lines are shown in Table 2, Figure 2. None of the strain showed less than 42 or more than 52 chromosomes, beside, none of the cell lines registered 43 chromosomes. Highest number of cells (95%) were observed with total chromosomes 47 in Daudi, Raji, HS602, in that order; followed by second highest (72%) in MC116 with chromosomes number 50. The cell lines tend to register chromosome between 47 and 50 and the tendency of having chromosomes less than 42 was not observed. The modal chromosome number was found to be hyperdiploid in Daudi, Raji, MC116, HS602, while Namalwa consistently appeared hypodiploid. The breaks up of numerical changes for various cell lines are summarized in Table 3.

Few cytogenetic features detected in Daudi cell line are of note. All cells shared a common karyotypic theme represented by a translocation between chromosome 8 and 14 with classical breakpoints (q24; q32). In a minority of cells, additional material on the short arm (p15) of chromosome 11 was observed. In HS602, a small marker was observed in 60% of the cells along with an additional material on the q29 of chromosome 3 in majority of cells (85%). In Raji cell lines, all cells shared a common karyotypic theme, i.e. a translocation between chromosomes 8 and 14 with the classical breakpoints (q24; q32) with an additional material on the q35 of chromosome 4 and the p23 of chromosome 8. Besides, additional material on the p25 and q27 of chromosome 6 was further seen in 12% and 13% of the cells respectively.

Karyotypic heterogeneity was profound in MC116. The most common chromosomal aberrations were duplication in the long arm (q12q31) of chromosome 1, a derivative (10) t(10;11)(q25, q13), t(8;14)(q24;q32) and up to four unidentified markers. Other abnormalities included

Table 1: showing the presence (+) or absence (-) of numerical (N) or structural (S) abnormalities in various lymphoma cell lines

		chromosome number																							
Strains		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	x	y
Daudi	N	_	_	_	_	_	_	+	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
	S	_	_	_	_	_	_	_	+	_	_	+	_	_	+	_	_	_	_	_	_	_	_	_	_
HS 602	N	_	_	+	_	_	_	_	_	_	+	_	+	_	_	_	_	_	_	_	+	+	_	_	_
	S	_	_	+	_	_	+	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
MC 116	N	_	+	_	_	_	_	+	_	+	+	+	_	_	+	+	_	_	+	_	_	+	+	_	_
	S	+	+	_	_	_	+	+	+	_	+	+	_	_	+	+	_	_	_	_	_	_	+	_	_
Namalwa	N	+	+	+	_	+	+	+	_	_	+	+	+	+	+	_	_	+	+	+	+	+	+	_	_
	S	+	+	+	_	+	+	_	+	_	+	_	_	+	_	+	_	+	+	_	_	+	_	_	+
Raji	N	_	_	_	_	+	_	+	_	_	+	_	_	+	+	_	_	_	+	_	+	_	+	_	_
	S	_	_	_	+	_	+	_	+	_	_	_	_	_	+	_	_	_	_	+	_	_	_	_	_

Table 2: The break up of numerical analysis in various lymphoma cell lines

			Distribution of chromosome number in percent											
Cell lines	# of cells observed	Chromosome varied from	Model #	42	43	44	45	46	47	48	49	50	51	52
Daudi	150	46-48	47	-	-	-	-	2	95	3	-	-	-	-
HS 602	65	42-47	47	4	-	-	38	-	58	-	-	-	-	-
Raji	220	44-48	47	-	-	10	18	2	60	10	-	-	-	-
MC 116	250	48-52	50	-	-	-	-	-	-	3	10	77	3	7
Namalwa	250	42-54	45	8	-	21	41	23	-	-	-	-	-	7

Table 3: Analysis of Aneusomies in lymphoma cell lines

	Percentage of cells with			
Cell lines	Trisomies	%	Monosomies	%
Daudi	Chro.7	100	-	
HS 602	-		Chro.3	40
			Chro.1, 10, 12, 21	5
Raji	Chro.7	33	Chro.5, 10	20
	Chro.18	9	Chro.13, 14, 22	11
	Chro.20	55		
	Chro.22	16		
MC 116	Chro.7	83	Chro.2, 9, 10	10
	Chro.14	10	Chro.14	67
	Chro.15	79	Chro.22	6
	Chro.18	22		
	Chro.21	20		
Namalwa	Chro.7	100	Chro.3, 21	100
	Chro.18	42	Chro.14	14
	Chro.20	37	Chro.10	39
	Chro.6, 12, 13, 22	6	Chro.13, 17	32
			Chro.5	18
			Chro.6	21
			Chro.11, 19	6

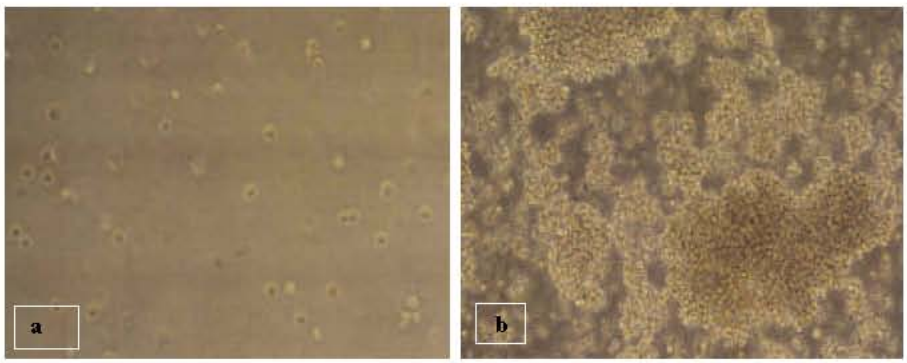

Fig. 1: Suspension cultures of HS602 (a) and MC116 (b) human lymphoma cell lines comparing the growth rate of cells. MC116 shows the growth of cells at brisk pace than HS602

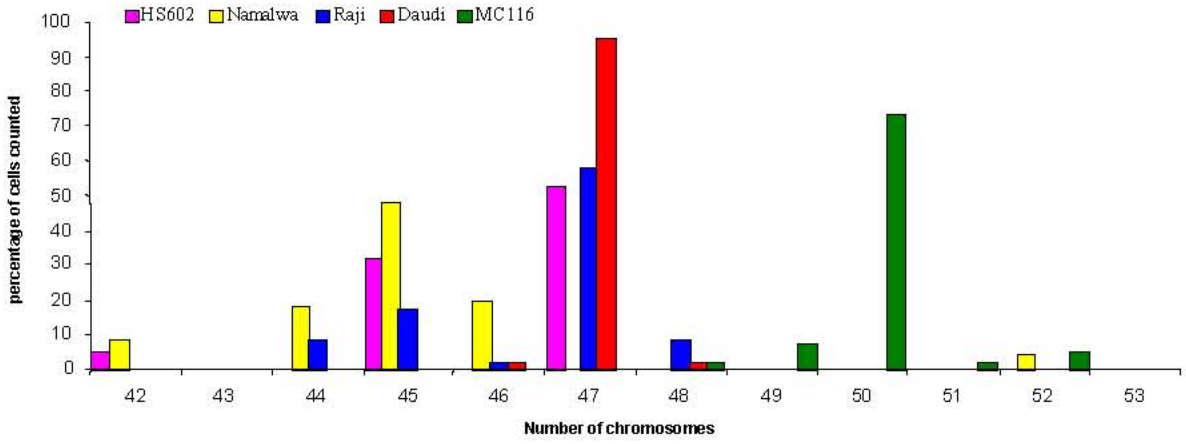

Fig. 2: Proportion of cells exhibiting different chromosome number in human lymphoma cell lines

Table 4: Genetic instability: Karyotypes of cells from lymphoma cell lines, comparing revised and previous work

Cell line	# of cells Studied	Passage No	Previous karyotype	Revised karyotype
Daudi	45	P1, P5, P10	+7 t(8;14)(q23;q32	47, XY, +7, t(8;14)(q24;q32)[39]/47, idem, der(11)t(11;13)(p15;q12)[6]
HS602	40	P1, P5, P10	Unknown	42, XX, -1, 10, -12, -20, -21, +mar[2]/45, XX, -3 [4]/45, XX, -3,
add(3)(q29)[12]/47,				XX, add(3)(q29), +mar[22]
Raji	75	P5, P20	47, XY, +7	47, XY, add(4)(q35), add(8)(p23)t(8;14)(q24;q32), +20[26]
		P1	der(4)t(4;11) (q33;q13)	45, XY, add(4)(q35), -5, +7, add(8)(p23)t(8;14)(q24;q32), -10[15]
		P15	del(7)(q22)	
			der(8)t(8;14;18)	44, XY, add(4)(q35), add(6)(p25), -13, -14, +20, -22[9]
		P10	t(8;14)(q24;q32)	
			t(8;22)(q12;q22)	47, XY, t(4;19)(q35;p12), add(8)(p23)t(8;14)(q24;q32), +22[8]
		P10	del(9)(q22) del(Y)(q12)	47,XY,add(4)(q35),add(8)(p23)t(8;14)(q24;q32),+18[7]
		P10		48, XY, add(4)(q35), add(6)(q27), +7, add(8)(p23)t(8;14)(q24;q32), +20[6]
		P20		48, XY, add(4)(q35), add(6)(q27), +7, add(8)(p23)t(8;14)(q24;q32), +22[4]
MC116	67	P1, P5, P15, P20	45,XO/46,XY	50,XY,dup(1)(q12q31),add(2)(q37),+7,add(7)(p22),del(8)(p23),der(10)t(10;11)(q25;q13),-11,-14,+15,+4mar[45]
		P20	dup(1)(q21-32)	
			t(8;14)(q23;q32)	50, XY, dup(1)(q12q31), -2, +7, t(7;15)(q35;q12), t(8;14)(q24;q32), -9, -10, der(10)t(10;11)(q25;q13), -11, -14, +21, i(22)(q10), +3mar[7]
		P15,P20	del(10)(q23)	49, XY, dup(1)(q12q31), t(8;14)(q24;q32), der(10)t(10;11)(q25;q13), -11, +18, add(18)(q23), +21, +2mar[7]
		P10		52, XY, dup(1)(q12q31), del(6)(q25), +7, t(8;14)(q24;q32),,der(10)t(10;11) (q25;q13), -11, -15, +18, +21, +3mar[4]
		P20		49, XY, dup(1)(q12q31), t(8;14)(q24;q32), der(10)t(10;11)(q25;q13), -11, +15, +18, -22, +3mar[4]
Namalwa	73	P15, P20	47,X,-Y+7	45,t(Y;21)(p11;q12),dup(1)(q21q31),-3dup(3)(q12q27),add(6)(p25),+7,del(8)(q24), add(10)(p15),-13,t(15;21)(p13;q11),- 17,add(18)(q23),+20,-21,-21,+2mar[23]
		P5, P10	ins(1;?)(q11;?)ins(3)	
			(q11-q29)del(3)(p12)	44,t(Y;21)(p11;q12),dup(1)(q21q31),add(2)(q37),-3,dup(3)(q12q27),-5,+7,del(8) (q24),10,add(13)(p13),t(15;21)(p13;q11),add(18) (q23),-21,+mar[17]
		P1	der(6)t(3;6)(p12;p25)	
			t(15;21)	46,X,t(Y;21)(p11;q12),dup(1)(q21q31)hsr(1)(q23),-3,dup(3)(q12q27), add(5)(p15),-6,+7,add(13)(p13),14,t(15;21)(p13;q11),add (17)(q25),+18,add(18) (q23),-21,+2mar[15]
		P10		45, X, t(Y;21)(p11;q12), dup(1)(q21q31), add(2)(q37), -3, dup(3)(q12q27), +7, del(8)(q24), -10, add(13)(p13), -14, t(15;21)(p13;q11), +18, -21, +mar[7]
		P15		42, X, t(Y;21)(p11;q12), dup(1)(q21q31), -3, dup(3)(q12q27), add(5)(p15), add(6)(p25), del(10)(p15), -10, -11, add(13)(p13), -14t(15;21)(p13;q11), +18, -19, -21, +mar[5]
		P5		52, X, t(Y;21)(p11;q12), -1, dup(1)(q21q31), add(2)(q37), -3, dup(3)(q26), -5, +6, +7, +7, +7, del(8)(q22), +12, +12, +13,, add(13)(p13), -14, t(15;21)(p13;q11), +18, +20, -21, +22, +mar[4]
		P20		45, X, t(Y;21)(p11;q12), dup(1)(q21q31), -3, dup(3)(q12q27), add(5)(p15), +7, add(13)(p13), -14, t(15;21)(p13;q11), -21, +mar[2]

additional material on the q37 of chromosome 2, the p22 of chromosome 7 in 67% of the cells and a deletion on the q25 of chromosome 6 in a minority of the cells. About 10% of the cells showed t (7;15)(q37;q12) and isochromosome 22. Namalwa, was even more heterogeneous than the MC116 cell line. A distinctive feature included a condensed dark area, apparently a homogeneously staining region (HSR) attached to the long arm of one of the chromosome 1q, was consistently detected in all examined cells. The most common chromosomal aberrations detected in the analyzed cells were duplication in the long arm of chromosome 1 and 3.

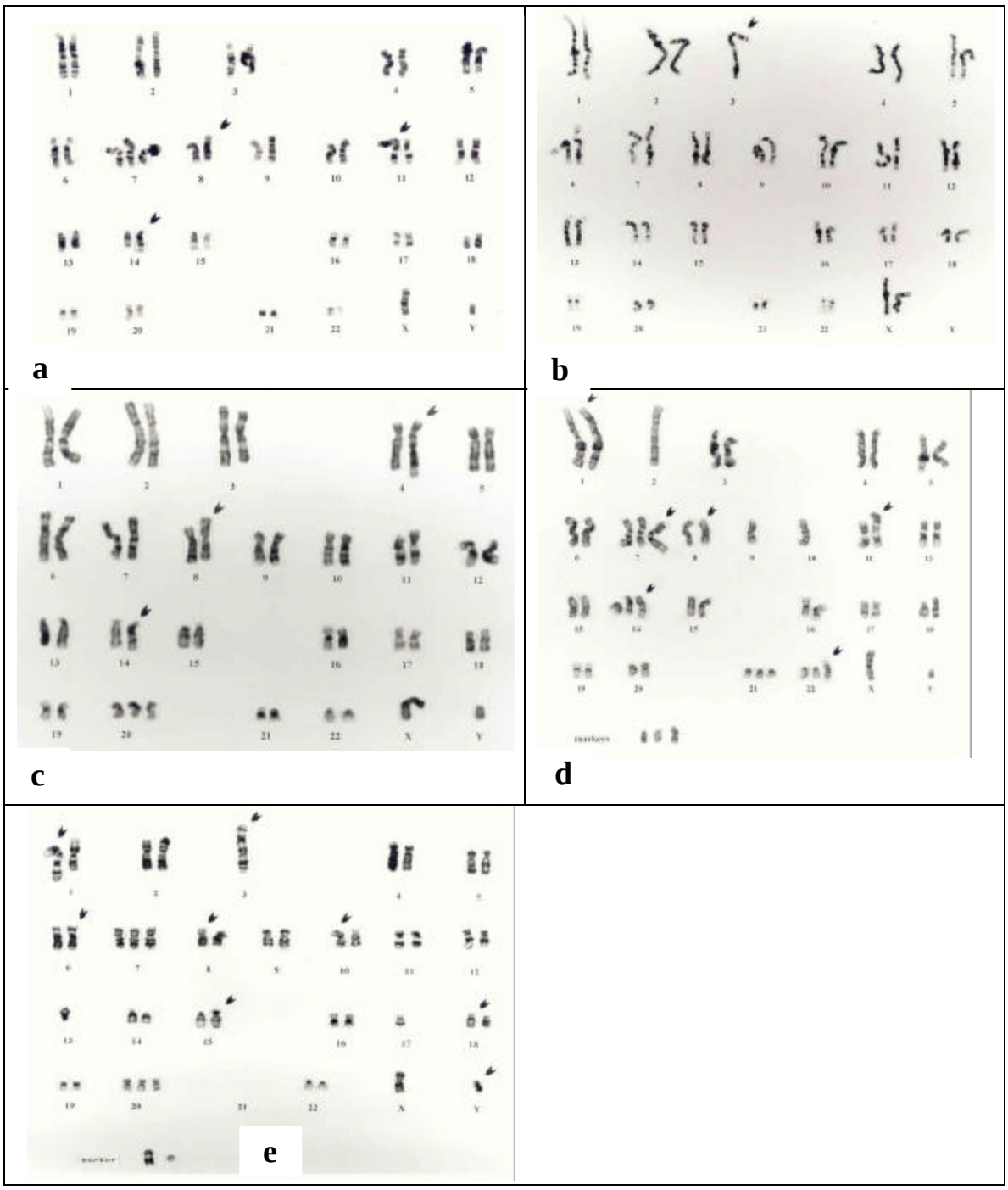

Fig. 3: Representative karyotypes of cell lines depicting structurally abnormal chromosomes indicated by arrows : (a) Daudi, 47,XY,+7,t (8;14)(q24;q32), der (11) t (11;13) (p15;q12); (b) HS602 45,XX,-3add(3)(q29)[12]; (c) Raji47, XY,add(4)(q35),add(8)(p23)t(8;14)(q24;q32),+20[26];(d)Mc11650,XY,dup(1)(q12q31),-2,+7, (7;15) (q35;q12),t(8;14)(q24;q32),-9,-10,dre (10)t(10;11)(q25;q13),-11,+14,+21,i(22)(q10),+3mar[7] and (e) Namalwa 45,X,t(Y;21)(p11;q12),dup(1)(q12q31)hsr(1)(q23),-3,dup(3)(q12q27), add (6)(p25), +7, del(8) (q24),add(10)(p15),-13,t(15;21)(p13;q11),-17,add (18)(q23),+20,-21,-21,+2mar[23]

These reflect interesting facts. Trisomy of 7 constantly figure in all the cell lines barring HS602 and was represented in every cell studied irrespective of cell lines such as Daudi, Namalwa and also appeared in high frequency in MC116, followed by Raji. Like wise, trisomy of 18 was also observed in many cell lines, though in lesser frequency except in Daudi. Neither kind of trisomy could be observed in HS602, instead monosomies of various chromosomes were very much evident. Namalwa also revealed monosomy of about 9 types of chromosomes. All lines showed both type of gross abnormalities for aneuploidy i.e. trisomy or monosomy; while Daudi was exclusively characterized by trisomy and HS602 by monosomy. The cell lines and their passages put to extensive karyotypic analysis in G banded cells are shown in Table 4 and representative karyotypes have been displayed in Figure3.

The out come: cells in every cell lines have undergone complex karyotype changes since studied earlier. Trisomies of 7 chromosomes are conspicuous in Daudi, MC116 and Namalwa in addition to other aberrations. The high level of karyotype complexity and aneusoimies in some cell lines and their passages points to role of CIN responsible for the behavior.

DISCUSSION

In the present study, five human lymphoma cell lines were investigated for genetic instability; comprising three of Burkitt's lymphoma cell lines (Daudi, Namalwa and Raji) and two of undifferentiated lymphomas (MC116 and HS602). Observations on their morphology were generally in unison with prior studies on similar lines [19, 20]. The tendency to grow faster in large clusters rather than single cell was the noted characteristic observed in all cell lines, except HS602. Since grown in suspension, the passage number at the original freezing and subsequently in our laboratory was not known except of Raji. The results obtained were satisfactory for several variations tried during the preparation of the metaphases. Refeeding of cell lines with fresh medium prior to harvesting and the use of different Colcemid incubation timings seem to augment metaphase quality and quantity.

Of the lymphoma cell lines observed, HS602 and MC116 have been karyotyped for the first time, where as, few partial cytogenetic studies were available on Daudi, Namalwa and Raji. The findings show variations in both the numerical and structural chromosomal abnormalities from the earlier observations. This is expected because of many reasons; partially due to differences in passage number and mostly due to the difference in methodology. The cytogenetic of MC116 has however, changed significantly since isolated and subsequently deposited at the ATCC. The Raji show complex karyotypic findings, confirming the earlier observations [21, 22]. Whereas, the Daudi once again proved to be a remarkably stable cell line with less heterogeneity [23, -24]. The studies emphasize the presence of the t (8;14) in three cell lines; Daudi, Raji and MC116 beside the trisomy of chromosome 7 abnormalities [20, 24]. The variation from earlier studies especially in ISCN karyotype reports for Raji and Namalwa, could be due to the use of ISCN 1985 guidelines for the present analysis [22, 25].

These and numerous other studies undertaken, confirm the fact that most cancer cell lines have an abnormal content characterized by changes in chromosomal structure and number [26-28]. Chromosomal aberrations are generally more numerous in malignant tumors than the benign ones and karyotypic complexity and cellular heterogeneity observed is often associated with poor prognosis. Thus, one of the challenges facing cancer researchers, of late, is to understand how cancer cells and cell lines generally acquire genomes with such a high degree of complexity [29]. To understand how these changes may functionally be significant, require a careful analysis of cell lines consistently.

Cytogenetic characterization has routinely tried in many a cancer studies in various ways. Some authors prefer to describe genomic instability into two major types; microsatellite instability, MIN and chromosomal instability, CIN [30]. The MIN involves simple DNA base changes that occur due to defects in the DNA repair processes, while CIN is characterized by grossly abnormal karyotypes, featuring both structural and numerical chromosomal abnormalities. Though suggested to be mutually exclusive [31], present findings points to contrary indicating some overlap in the two pathways. The different clones of MC116, Namalwa and Raji as a result, seem to be evolved sequentially from one another, each acquiring additional abnormalities in the process. Since, cell line can evolve rapidly so that its karyotype differ from the original tumor in short time after explantation [32]; and a repeated cytogenetic examination at different passages of the cell line show the persistence of identical chromosomal aberrations [33, 34], the fact appeared to be partially true when contrasted from the present. Current results indicate that consistent chromosomal abnormalities may have an important bearing on the genesis of lymphomas. Studies on NHL are more challenging because of the technical difficulty in obtaining adequate number of analyzable metaphases and

the complexity of the karyotype frequently observed; nevertheless, cytogenetic studies of the cell lines are necessary to assess the presence of a specific translocation and for the comparison of chromosome abnormalities *in-vivo* and *in-vitro*. This assumes further significance, in view of its involvement in understanding the primary and secondary event of the malignant process [35].

It seems probable that the human lymphoid cell lines can particularly evolve *in-vitro* by the emergence of successive waves of clones that are chromosomally abnormal. When numerical changes affecting whole chromosomes or chromosome arms are considered in a large series of cell lines, the abnormalities seem to accumulate with time in culture [36, 37], the fact corroborated by our observations. The specific and non-random chromosome rearrangements studied extensively in Bukitt's lymphoma cell lines also arrived at the same conclusion [23, 38, 39-41]. The karyotypic analysis of cell lines obtained from Burkitt's lymphoma patients strongly show the retention of specific chromosomal markers *in vitro* [42-44]. In a number of cell lines, however, these marker chromosomes can be subjected to further rearrangements [24].

Although not studied, the role of fragile sites spanning the chromosomal regions can add a new dimension in understanding the cytogenetic of hematological malignancies. The identification of chromosomal changes can coincide with the location of cancer specific chromosomal part and the location of cellular oncogenes [35]. Since fragile sites are shown to be susceptible to break, therefore, these could be the sites for exchange of genetic material between sister chromatids, chromosomal translocation, deletion, gene amplification and even responsible for the integration of oncogenic viruses [45].

Interestingly if the malignant transformation of cells and their propagation in cell lines, confer these cells with the ability to escape normal apoptotic pathways, it could permit the abnormal daughter cells to survive. The scientific literature is rich in studies dealing with factors affecting mitotic segregation errors leading to chromosomal aneuploidy and CIN [46-48]. The role of primary forces that drive a cell to aneuploidy such as, centrosome duplication, chromosome cohesion defects, merotelic attachments of chromosomes and cell cycle/ mitotic checkpoint defects need further to be looked into in these types of studies. The presence of chromosomal aberrations, an outcome of the studied cell lines, is a clear indication of errors in the DNA damage and mitotic/ cell cycle checkpoints. But it needs confirmation.

In spite of extensive researches directed at the study of chromosomes in cancer cell lines, it is still unclear whether genomic instability precedes tumor development *per se* and the primary force for tumorigenesis, or the acquisition of CIN is a more passive secondary consequence of genomic destabilization associated with malignant process. It seems probable that different cancers follow different pathways of initiation. The early oncogenetic steps may vary depending on the tumor type and the time that tumors are studied in the course of disease process.

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