DNA Image Cytometry of the Liver of Chronic Hepatitis C Virus Infected Patients and Hepatocellular Carcinoma in Sharkia Governorate in Egypt

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Abstract: In the present investigation forty three (11 HCC, 12 cirrhosis and 20 chronic hepatitis) cases were chosen from the paraffin blocks and unstained slides of fine needle aspirates biopsies which were preserved in the archive of the Early Cancer Detection Unit (ECDU) belonging to the Faculty of medicine, Zagazig University, Egypt. All the cases were previously diagnosed and proved by PCR to have HCV. 50 µm thick paraffin sections and fine needle aspirates smears were prepared for evaluating DNA ploidy and S- phase fraction (SPF) by DNA image analysis. The results indicated that, the hepatocyts of chronic hepatitis C cases have a regular multiplicity of normal diploid DNA (Euploid polyploidization). All of the studied HCCs cases and only one cirrhotic case revealed aneuploidy. It was concluded that, patients infected with chronic HCV are predominantly affected by HCC and the DNA image analysis techniques can be considered as early predictors of cellular abnormality and different malignant criteria, which can lead to early and well diagnosis as well as rapid manipulation of the patients.
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1. Introduction

Objective analysis of microscopic images of cells and tissues for purposes of classification and measurements of cell components has been a goal of human pathology and cytology since the 19th century (Koss, 1987). During the last decade, image cytometry has become an established technique in the field of analytical cellular pathology. The new development of image analysis technology and the availability of commercial instruments capable of making reliable and so measurements, provide an advantage for quantitative immunohistochemistry (Wells *et al.,* 1992). According to Wells *et al.* (1996), all specimens of exfoliative-and aspiration cytology are suitable for static DNA measurements after staining according to Feulgen. Formalin fixed and paraffin embedded tissues are suitable after combined mechanic and enzymatic cell separation.

According to Cohen (1996), image cytometry enables use of internal controls and use of small amount of tissue. On the other hand, technical problems include nuclear overlap, which may give false optical density and a time consuming, quantitation required many nuclei to be cut apart or rejected. For minimal nuclear overlap and sharp focusing 50µm tissue sections are required.

Cell DNA content measurements, including determination of tumor ploidy and S-phase fraction; have been performed on a wide variety of human tumors for more than 20 years. There are two techniques to perform DNA quantitation: flow cytometry and static cytometry. The analysis in both techniques, is based upon the principal that DNA-specific dyes should stain the cellular DNA in a stoichiometric manner (i.e. the amount of stain is directly proportional to the amount of DNA within the cell (Batsakis *et al.,* 1993).

The prognostic value of DNA ploidy in HCC however has been questioned because of contradictory results (Ezaki *et al.,* 1988 and Ng IOL *et al.*, 1994). This discrepancy may be attributed to the limitations of sample size, patient pool, methodological differences, potential differences in the natural history of disease in various geographic locations, and last but not least potential features of HCC, such as multicentric origin and intratumor heterogeneity, which may lead to disparate sampling (Lapis *et al*., 1995).

The diagnosis of liver cell dysplasia and HCC is often difficult to establish in both cytologic material and needle-core biopsy specimens. The potential for misdiagnosis is greatest in attempting to discriminate well-differentiated HCC from dysplastic hepatocytes in cirrhosis (Crawford, 1990). Previous monophotometric IA studies have shown statistically significant differences between benign and malignant hepatocytes. The mean values of nuclear area, perimeter, and maximum diameter measured in fine needle aspirates (FNA) were significantly different between cases of HCC and benign hepatocytes in

cirrhotic nodules (Kondo *et al.,* 1999). Statistically significant differences in histologic nuclear density expressed as nucleocytoplasmic ratio have been demonstrated in histologic sections of HCC and benign hepatocytes in normal, atrophic and cirrhotic liver (Kondo *et al.,* 1999). However reliable classification criteria based on IA measurements have not been developed because there is considerable data overlap between benign and malignant hepatocytes. The accurate diagnosis of HCC may be difficult, because specimens frequently consist only of cytological materials or needle core biopsies. Erler *et al*. (1993 & 1994) suggested that, image analysis methods offer a potentially useful adjunct in discriminating between benign and malignant hepatocytes, because the require on a small amount of tissue and do not rely on architectural pattern. The present investigation is aimed to study DNA image cytometry of chronic hepatitis c virus infected patients and hepatocellular carcinoma in Sharkia Governorate, Eegypt

2. Patients and Methods

The present study has been carried out in the Early Cancer Detection Unit (ECDU) belonging to the Faculty of Medicine, Zagazig University, Egypt. Forty three (11 HCC, 12 cirrhosis and 20 chronic hepatitis) cases were chosen from the paraffin blocks and unstained slides of fine needle aspirates biopsies which were preserved in the archive of ECDU. All the cases were previously diagnosed during the period from January 1998 to December 2002 and proved by PCR to have HCV.

Preparation of smears:

Unstained slides of fine needle aspirates biopsies which were preserved in the archive of ECDU were stained with Feulgen method for DNA image cytometry.

Preparation of nuclear suspension for DNA image analysis:

Five 50 μm sections in 15 ml 16x 100 mm centrifuge tubes were deparaffinized by adding 10 ml of xylene for 20 minutes and this step was repeated twice. The sections were next rehydrated using a sequence of 10 ml of 100 %, 95 %, 70 % and 50 % ethanol incubation for 20 minutes each at room temperature (RT) and each step was repeated twice. The tissue was then washed in 2 changes of distilled water (d.w.) for 20 minutes and left in 10 ml d.w. for 24 hours.

Nuclear suspension preparation was done according to the method described by Hedley *et al*.(1983), with slight modifications. The tissue was resuspended in 1.5 ml of 0.5 % pepsin solution (Sigma Chemicals Co., St. Louis, MO, USA) in normal saline (0.9 % NaCl in d.w. w/v), adjusted to pH 1.5 with concentrated HCl. This mixture was incubated in a water bath for 30 minutes at 37 C°, then vortexing at 5 minutes intervals for 30 minutes. The proteolytic reaction was terminated by adding 2 ml of 10 % fetal bovine serum (FBS) in phosphate buffered saline (PBS) (10% FBS/PBS). The samples were immediately filtered through 40 μm nylon mesh. After centrifugation for 10 minutes at 1000 rpm (round per minute), the nuclear pellet was resuspended in 2 ml 5% FBS/PBS, then centrifugation for 10 min. at 1000 rpm. The supernatant was decantated and the pellet was flattened on a cleaned glass slides. These slides were air dried at room temperature overnight, then post-fixed in 10% neutral buffered formalin for 30 minutes and washed in d.w. for 10 min. and left to dry overnight at RT.

Cytometric Measurements:

1- Reagents:

Feulgen method using CAS DNA staining commercial Kit (Qualitative DNA staining Kit, Cell analysis, Inc., Elmhurst, IL, USA) was used. The Feulgen staining solution was prepared by mixing one stain reagent vial and 100 ml of 0.1 N HCl. The stain solution was stirred on a magnetic stirrer for 60 min. and it was tightly blocked (closed) to prevent SO2 loss. Solution was filtered immediately before using. The rinse solution was prepared by addition of the entire contents of one rinse reagent vial to 300 ml of 0.05 N HCl in a 500 ml flask and mixed well until completely dissolved. The Feulgen reaction produced specific blue staining of nuclear DNA.

2- DNA Staining and Analysis according to Schulte and Wittekind (1990):

Air-dried prepared slides and the unstained fine needle aspiration (FNA) smears were treated for 60 min. in 5N HCl to hydrolyze nuclear DNA. The slides were directly transferred to freshly prepared Feulgen stain in tightly capped coplin jar for 60 minutes, and then rinsed in 3 changes of rinse solution for 30 seconds, 5 minutes and 10 minutes, subsequently. After that, the slides were washed in distilled water and placed in 1% acid alcohol for 5 minutes. They were then dehydrated in 2 changes of absolute alcohol for 2 minutes each. The slides were then cleared in 2 changes of xylene (2 minutes, each) and mounted with DPX and coverslips.

The stained slides were analyzed with Hund CML image analyzer and software *(Hund H500, Wetzlar, FRG)*. The instrument calculates the DNA index (DI) and coefficient of variation (CV) of measured peaks. For each slide, 20 lymphocytes were

used as an internal diploid DNA content standard for the slide. Four hundred (400) non-overlapping hepatocyte nuclei were then measured. Peak statistics are based on user demarcation of the histogram. The CV of each peak was calculated in the standard fashion in the standard deviation of demarcated divided by the mean.

3- DNA Histogram Interpretation:

1-Histograms were regarded as uninterpretable for ploidy if the CV for the DNA diploid Go/G1 peak was < 8

2-The histogram was considered diploid when the single peak occupied the diploid position (DI range: 0.9 – 1.1), and fewer than 15% of cells were present at the tetraploid position.

3-If an additional distinct peak was present, the tumor will be classified into one of the 5 non-diploid categories depending on DI. Thus tumors will be considered DNA hypodiploid for DI<1.0, hyperdiploid for DI in the range 1.1- 2.1 and hypertetraploid for a DI greater than 2.1. If more than one non-diploid peak was observed, the tumor will be classified as multiploid. For tetraploid tumors, the additional peak should be in the tetraploid region and should contain ≥ 15% of cells in the presence of recognizable G2/M peak. The term aneuploid is used to describe hypodiploid, hyperdiploid and hypertetraploid subgroups of non-diploid tumors as one group.

3. Results

In the present study, observations of Image cytometry are illustrated in table (1). According to the criteria of Bocking *et al*. (1992), a single G1 peak was regarded as diploid DNA and two or more G1 peaks as aneuploid. The CV values were relatively broad (3.0 – 7.0), but were still within an acceptable range. All the cases of chronic HCV were diploid. The mean of SPF value for the studied chronic HCV cases was (7.12 ± 3.67), and for the cirrhotic cases was (12.23 ± 5.18), while for the HCC cases was (26.46± 7.14); the difference is a highly significant (F= 90.55 and P = 0.0001) (Table 1). Also, statistical analysis showed a highly significant correlation (P<0.0001) between the DNA ploidy and the average SPF values of the HCCs (table 1 and Fig. 1).

Table (1): Cell Cycle fractions and DNA ploidy according to different pathological changes of the studied cases.

Histopathol.changes / Cell Cycle	Chronic Hepatitis N	Cirrhosis	HCC	Test of sig.	P
S-phase					
x ± SD	7.12 ± 3.67	12. 23 ±5.18	26.46 ± 7.14	F= 90.55	0.0001
Range	2. 20 - 0.75	5.85 – 17.90	21.00 – 38.30		S.
DI1					
x ± SD	0.99 ± 0.08	1.03 ± 0.03	1.035 ± 0.4	F=	0.093
Range	0.91 – 1.08	0.95 –1.07	0.99 – 1.08	252	N. S.
DI 2					
x ± SD	1.98 ± 0.07	2.01± 0.11	1.59 ± 0. 29	F=	0.0001
Range	1.91 – 2.08	1.67 - 2.09	1.23 – 2.3 2	12.88	S.
Prolif. F.					
x ± SD	24.37 ± 4.36	36.14 ±6.28	64. 23 ± 7.08	F=	0.0001
Range	17.90 –33.80	27.5 – 46.60	53.50 – 74.9	61.93	S.
DNA	No %	No %	No %		
Diploidy	20 100.0	11 91.7	0.0 0.0	X 2	0.004
Aneuploidy	0 0.0	1 8.3	11 00.0	8.45	S.

By correlating the DNA index (DI1) and the DNA ploidy, average of (DI1) was 0.99±0.08 for chronic HCV cases, while it was 1.03±0.03 and 1.035±0.4, for cirrhotic and HCC cases respectively, revealing no significant association between histopathological lesions and DI1 value in this study (F=252, P=0.093) as illustrated in table 1 and Fig. 2.

By correlating the DNA index (DI2) and the DNA ploidy, there was a high significant correlation between the average of (DI2), which was 1.98±0.07 for chronic HCV cases, and 2.01±0.11 and 1.59±0.29 for cirrhotic and HCC cases respectively and for different histopathological leions, where (F=12.88, P <0.0001) as illustrated in table (1) and (Fig. 3).

Concerning the mean of proliferation fraction values, it has been found that, it showed a high significant correlation with the type of histopathological lesions. The mean values of proliferation fraction of the studied cases as recorded in table (1) were significantly high (64. 23 ± 7.08) for

HCC comparing to (24.37 ± 4.36 and 36.14 ± 6.28) for chronic HCV and cirrhotic cases, (F=161.93 and P< 0.0001).) as illustrated in table (1) and (Fig. 4).

The DNA ploidy progressively changed with the increased hitopathological changes particularly with the presence of malignancy. By correlating DNA ploidy with the histopathological changes using Chi square , a high significant correlation was recorded, where, (*p* value = 0.004) as illustrated in table (1) and Fig. (5).

According to DNA image analysis data, aneuploid cases were 12 representing (28%) of the studied cases. One of them was cirrhotic (Fig. 10) and 11 cases were HCCs as shown in (Figs. 11, 12, 13, & 14). The remaining cases (31, 72%) were diploid as shown in Figs. (6, 7, 8, & 9). Among the 7 cases of well differentiated HCCs, 6 cases were aneuploid and one case was tetraploid, resulting in (100%) of foci being either aneuploid or tetraploid (Fig. 13). Also, the two (100%) moderately differentiated HCC cases were abnormal, each of them was obviously aneuploid. The two cases of poorly differentiated HCC revealed also abnormal histograms, one of them was aneuploid and the other one was multiploid (Fig. 14).

The DNA index (DI) of the aneuploid peaks ranged from 1.1 to 1.9 or below 0.9 and above 2.1. Among the HCC foci, tetraploidy as defined by a large G2/M peak and a small S-phase population was presented in one sample. Whether a high G2/M includes near-tetraploid aneuploid populations or is merely a manifestation of polyploidy is particularly difficult to resolve in the liver, which has a sizable polyploid population both normally and in HCC. It has been also noticed that, human hepatocellular tumor growth was associated with a decreased tendency toward polyploidy. Observations of the present study indicated that, the poorly differentiated HCCs has a higher frequency of DNA abnormal populations.

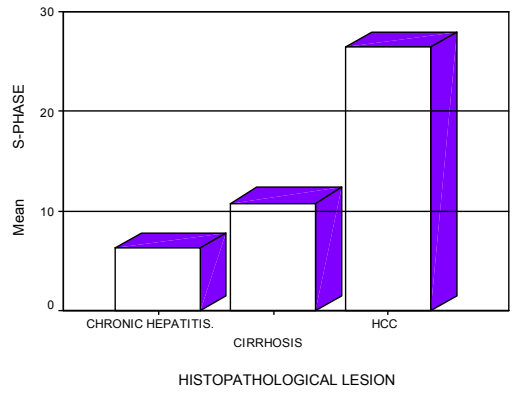

Fig. 1: Comparison of the mean value of S-Phase fraction of different pathological changes.

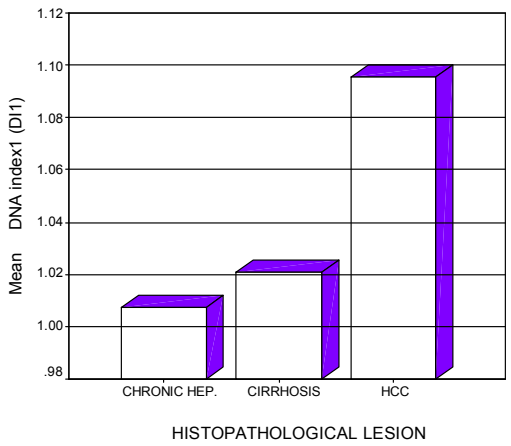

Fig. 2: Comparison of the mean value of DNA index 1 (DI1)of the different pathological changes.

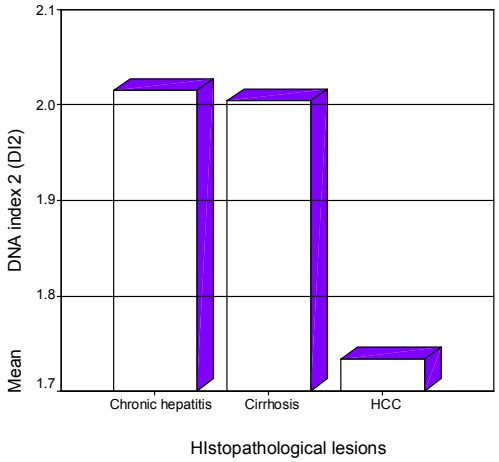

Fig. 3: Comparison of the mean value of DNA index (DI2) of the different pathological changes

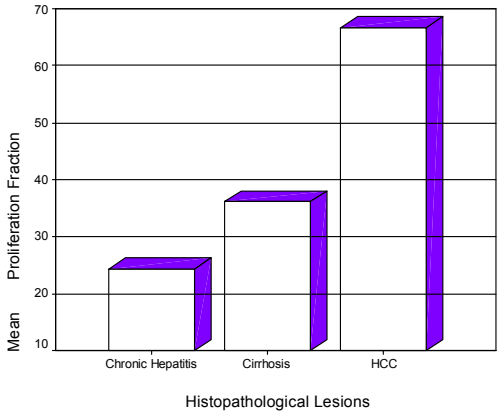

Fig. 4: Comparison of the mean value of proliferation fraction the different pathological changes

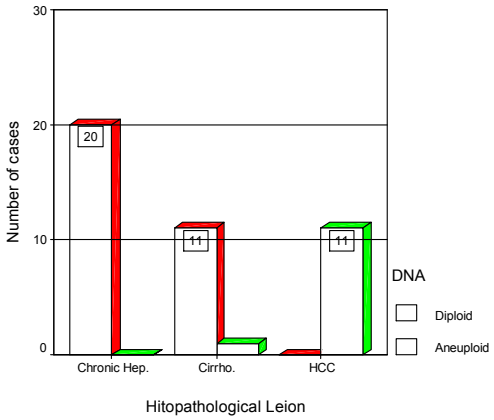

Fig. 5: Comparison of the DNA ploidy and the different pathological changes.

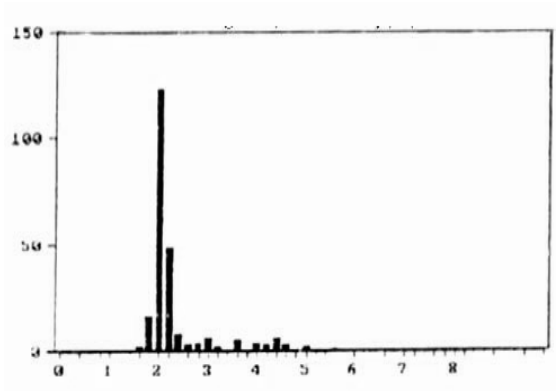

Fig. 6: DNA histogram of a female patient aged 29 years and infected with HCV showing normal diploid peak (non-aneuploid) using (Helmut Hund GmbH D6330 Wetzlar 21, Germany), (SPF=2.98) and (DI1 = 1.00).

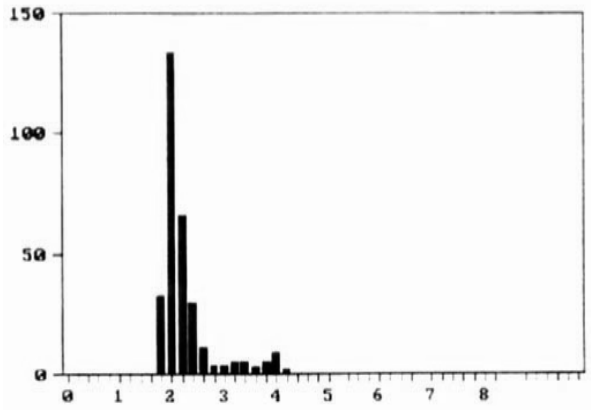

Fig. 7: DNA histogram of a male patient aged 32 years and infected with HCV showing normal diploid peak (non-aneuploid) using (Helmut Hund GmbH D6330 Wetzlar 21, Germany), (SPF=4.23) and (DI1 = 1.00).

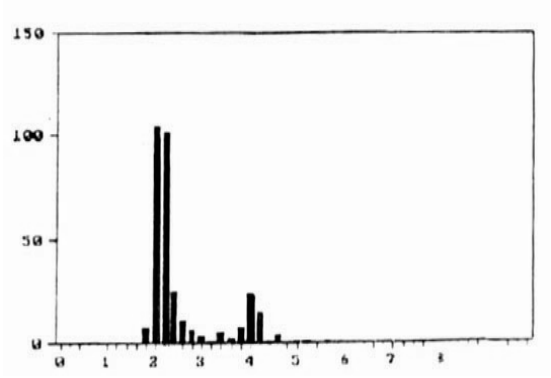

Fig. 8: DNA histogram of a male patient aged 41 years and infected with HCV showing normal diploid peak (non-aneuploid) using (Helmut Hund GmbH D6330 Wetzlar 21, Germany), (SPF=7.11, DI1 = 0.99, DI2 = 1.97).

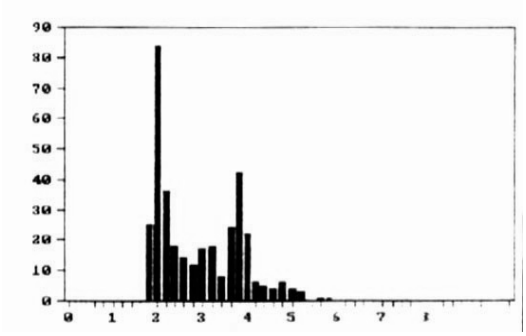

Fig. 9: DNA histogram of a female patient aged 57 years and infected with HCV and marked cirrhosis showing normal diploid peak (non-aneuploid) using (Helmut Hund GmbH D6330 Wetzlar 21, Germany), (SPF=10.28) and (DI1 = 1.03, DI2 = 2.07).

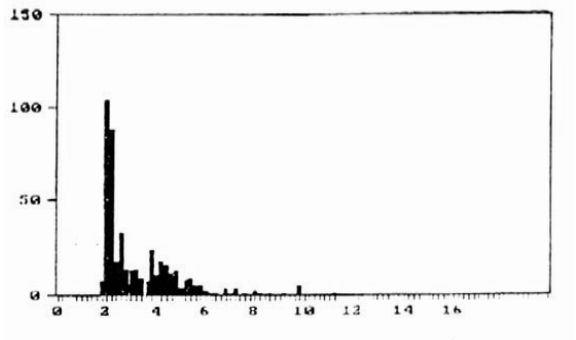

Fig. 10: DNA histogram of a male patient aged 49 years and infected with HCV and marked cirrhosis showing aneuploid peak using (Helmut Hund GmbH D6330 Wetzlar 21, Germany), (SPF=13.47) and (DI1 = 1.06, DI2 = 1.34).

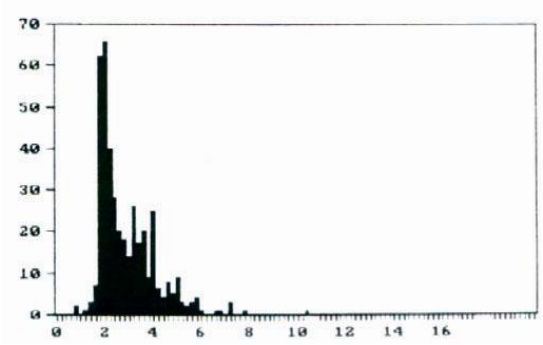

Fig. 11: DNA histogram of a female patient aged 67 years and infected with HCV and well differentiated HCC showing aneuploid peak using (Helmut Hund GmbH D6330 Wetzlar 21, Germany), (SPF=24.68) and (DI1 = 1.04, DI2 = 1.61).

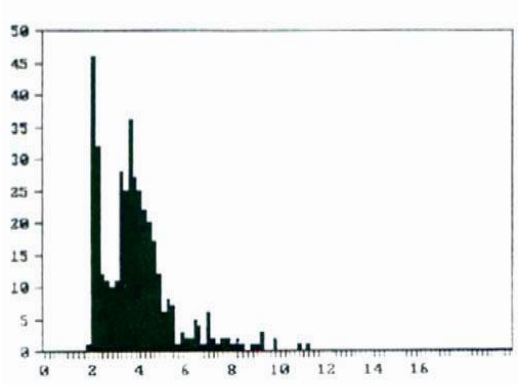

Fig. 12: DNA histogram of a male patient aged 67 years infected with HCV and well differentiated HCC showing aneuploid peak using (Helmut Hund GmbH D6330 Wetzlar 21, Germany), (SPF=26.17) and (DI1 = 1.02, DI2 = 1.98).

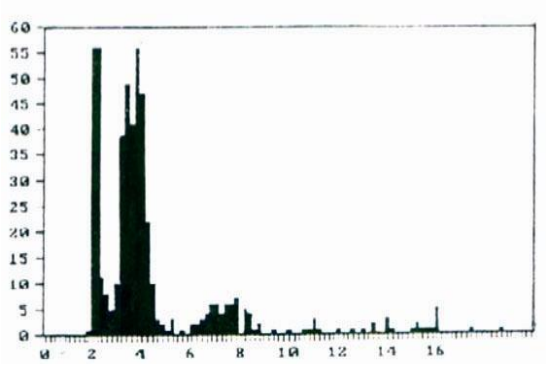

Fig. 13: DNA histogram of a male patient aged 61 years infected with HCV and moderately differentiated HCC showing tetraploid peak using (Helmut Hund GmbH D6330 Wetzlar 21, Germany), (SPF=33.65) and (DI1 = 1.09, DI2 = 1.66).

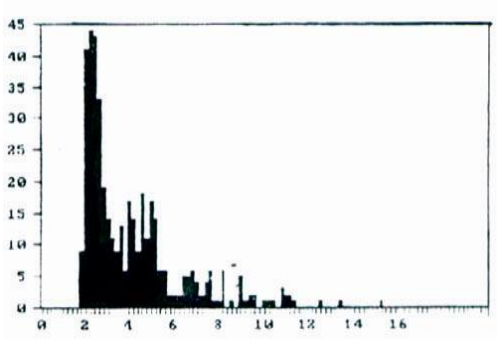

Fig. 14: DNA histogram of a male patient aged 61 years infected with HCV and poorly differentiated HCC (trabeculr pattern) showing multiploid peak using (Helmut Hund GmbH D6330 Wetzlar 21, Germany), (SPF=31.73) and (DI1=1.03, DI2=1.23).

4. Discussion

The development of techniques for measuring the quantity of DNA in a population of tumor cells has resulted in the refinement of diagnostic criteria and development of new treatment protocols based on prognosis and the likelihood of response to therapy (Look *et al*., 1995, Hyman *et al.,* 2002, Clingoz *et al.,* 2003, Keller *et al.,* 2003 and Lorenzato *et al.,* 2004**).** The presence of normal hepatocytes and stroma as well as inflammatory cells served as internal controls for each specimen. As the staining intensity of fixed nuclei varied from one sample to another, the lymphocyte external diploid control was excluded from DNA analysis. The first peak to the left was regarded as representing the diploid cells and the DNA index (DI) values of other peaks were calculated with this peak as a reference. Consequently, tumors were considered to be diploid when a single peak was observed; tumors with more than one peak were non-diploid and the first peak was always regarded as the diploid peak (Bocking *et al*., 1992). Evaluating of cell ploidy of the smears of (FNA) which have been also stained with blue Feulgen are somewhat better than those prepared from paraffin sections because the nuclear chromatin is better spread on the slide. The findings of Rubin *et al*. (1994) in that direction are, in accordance with our observations. The same observations were also noticed by Coon et al. (1987), who showed that, formalin-fixed tissue has additional disadvantages of producing histograms with a higher CV and more subnuclear debris than fresh specimens. A high CV may be due either to a true-near diploid aneuploid population or to deterioration of DNA as a result of poor fixation or embedding (Thomas *et al*., 1992).

DNA index (DI) is a crude measure of true DNA content. The range of diploid value (0.9 to 1.1) would include cells that had gained or lost up to 10%

of their DNA. Thus, it is possible that, diploid clones harbor cytogenetic abnormalities not detectable by ploidy analysis and aneuploid clones with the same DNA index could contain very different karyotypic abnormalities (McEntee *et al*., 1993). Thus cytogenetic differences could exist between primary and recurrent tumor cell populations with the same DNA index (DI). Also, of the diploid clones may actually be near diploid.

In the present study, DNA image cytometry of the chosen cases of chronic HCV showed non aneuploid (diploid) histograms for each of them (100%). Also, the mean of coefficient of variation (CV) of G0/G1 peaks of the samples extracted from paraffin-embedded tissue was (5.5%) and (3.8%) of the FNA cases and the DNA index (DI) was (1.0 ± 0.7) and these values may be entirely consistent with the values obtained with normal diploid populations, as was also observed by Bocking et al. (1992).

The occurrence of aneuploidy in cirrhotic livers without regard to the presence of dysplasia has been evaluated in the present study. Image cytometry of the DNA inclusions of the cirrhotic liver tissues revealed that, two cases representing (8.33%) of them were aneuploid. This finding is in agreement with Lin *et al*. (1990), who noticed that, two of (17) cases were aneuploidy, suggesting that, the occurrence of dysplastic cells is sometimes seen in cirrhotic livers. In addition, when aneuploidy was assessed in cirrhotic livers harboring HCC, the nonneoplastic tissue contained DNA aneuploid cells, while the frequency of DNA aneuploidy was much less in cirrhotic livers of patients without HCC (Ballardini *et al*., 1999**).**

In the present study, one case (9.1%) of the HCCs was diploid, 9 cases (81.8%) were aneuploid while, one case (9.1%) was multiploid. The two diploid and multiploid histograms represented well differentiated and poorly differentiated HCC, respectively. These findings are approximately in accordance with An *et al*. (1997) who observed that, 14 HCC were diploid, 24 0f 40 aneuploid, and 2 cases of them were multiploid. On the other hand, few studies, however, have compared the ploidy status large cell dysplasia with normal, cirrhotic and HCC cases (Rubin *et al*., 1994**).** An *et al*. (1997)**,** showed that, 92% of the dysplastic cases were aneuploid compared with 60% of the HCC cases and 43% of the benign cases exhibited aneuploidy. Zeppa *et al*. (1999) studied 84 HCCs diagnosed by FNA; eight of them were well differentiated HCC. They found that, 68 cases were aneuploid and 16 were euploid (9 diploid and 7 polyploid). Four of the eight cytologically suspect cases were aneuploid. Statistical analysis showed an association between size and cytologic grading, and between aneuploidy and multiple tumors. They concluded that, DNA ploidy evaluation by static cytometry of hepatic tumors may be useful in the diagnosis of cytologic samples and could represent independent prognostic parameter in predicting the survival outcome of patients with HCC.

The role of liver cell dysplasia in the evolution of HCC is a matter of scientific debate. A progression over time from cirrhosis to dysplasia and then to HCC has been hypothesized (Anthony, 1976)**.** However, Cohen and De Rose (1994) argued that, the mean age of cirrhotic patients with dysplasia is less than the mean age of cirrhotic patients without dysplasia, implying that dysplasia is not chronologically related to cirrhosis. Other studies suggest that, dysplasia lacks features usually associated with HCC, in particular, the reticulum framework in dysplasia is preserved, the serum alpha-fetoprotein is normal, and the nuclear to cytoplasmic ratio is unchanged (Watanabe *et al.,* 1983)**.** A normal nuclear to cytoplasmic ratio suggests that "dysplastic cells" are simply polyploid. The presence of polyploid cells in normal liver is well-established (Saeter *et al*., 1988).

Many studies examined the DNA ploidy SPF as potential prognostic factors in human liver diseases and HCC (McEntee *et al*., 1993; Rim *et al*., 1993 and Ng IOL *et al*., 1994). The conclusions reached by investigators range from attributing no prognostic significance to these parameters to considering them significant and independent prognostic factors (McEntee *et al*., 1993). In the current study, DNA ploidy and the fraction of cells undergoing DNA synthesis, expressed as S-phase fraction (SPF) were evaluated in each of chronic hepatitis, cirrhotic and HCC cases. The DNA ploidy was similar in chronic HCV and most of the cirrhotic cases, which was non-aneuploid (diploid), except two cirrhotic cases were aneuploid. The SPF value for the aneuploid HCCs was significantly higher than for the diploid cirrhotic cases, which also in turn have a significantly higher SPF than chronic HCV examined cases.

The proportion of aneuploid HCC cases in this study was considerably higher than that which was previously reported by other investigators (Chen *et al*., 1991; Kopper *et al*., 1991 and Nagasue *et al*., 1992). In this context, it is worth mentioning that, a DNA image cytometric and flow cytometric studies, which were carried out on paraffin embedded human HCC, using the same technique showed that 70% of the tumors were aneuplod (Rim *et al*., 1993).

Different tumors and host factors have been related to prognostic significance of HCC. An association between DNA ploidy and tumor size was made by Japanese scientists (Fujimoto *et al*., (1992) and higher incidence of aneuploid tumors has been

found in poorly differentiated HCC. In the present findings, significant correlation was found between DNA ploidy and cytological grade. In reviewing the literature on DNA ploidy versus age in human HCC, the reports are contradictory (Nagasue *et al*., 1992). Morphometric parameters are useful in discriminating benign from malignant hepatocytes. Studies have also compared the nuclear morphometric characteristics of dysplastic hepatocytes with those of benign and malignant hepatocytes (Marchevsky *et al.,* 1994& 1997)**.** On the other hand, few experimental studies were carried out in this field and took in consideration the role of some nuclear parameters such as *p53* in the liver DNA ploidy. Yin *et al.* (1998) showed that, the hepatocytes of the heterozygous or homozygous *p53*-mice as well as mice expressing one allele of *p53* ser246 do not undergo normal polyploidization with aging and show an increase in the number of cycling (G1-, S-, M- phase) cells. Also, they concluded that, loss of *p53* removes blocks in the cell cycle, leading to increase proliferation, whereas expression of the *p53* ser246 mutation stimulates G0 to G1 and/or M to G1 transition of hepatocytes.

In the present work, it has been noticed that, image analysis is potentially useful as an adjunctive tool for the evolution of liver biopsy material that is potentially difficult to interpret and can be used to distinguish objectively normal hepatic tissue from hepatic dysplasia, HCC and non-neoplastic liver. These findings agree with An *et al*. (1997)**,** who concluded that, foci of morphological atypical hepatocytes found in liver with cirrhosis may contain cells with a distinct DNA aneuploid peak detectable by image cytometry. Foci with high grade dysplasia had a higher frequency of aneuploidy compared with foci of low-grade dysplasia, although this tendency was not statistically significant. These findings support the recognition of liver cell dysplasia as a morphologic entity containing an aneuploid subpopulation. As such, this lesion may be a precursor for the development of HCC. On the other hand, high AFP levels have been found in cases with poorly differentiated HCC, but the relationship between DNA ploidy and AFP production is questionable. According to Chen *et al.* (1991), hyperploid tumors have been associated with high AFP levels and diploid tumors with low AFP wheras Nagasue *et al.,* (1992) found no correlation between DNA ploidy and AFP production.

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