Tissue Expression of PCNA and Caspase3 in the Liver in Patients with Chronic Hepatitis, Cirrhosis and Hepatocellular Carcinoma

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Abstract: Our aim is to evaluate the role of nuclear proliferative activity mainly PCNA and apoptosis mainly caspase3 in cases of chronic hepatitis, liver cirrhosis and HCC. Methods: This is a prospective study, ninety patients with chronic liver disease and HCC were subjected to clinical examination, laboratory investigations for hepatitis C and liver function tests and to abdominal ultrasonography. Liver biopsy was performed for histopathological examination. They were 3 groups: chronic hepatitis (35), liver cirrhosis (25) and HCC (30) and ten control patients with negative serological markers for hepatitis (C&B). Immunohistochemical staining for PCNA and Caspase 3 was done. Results: The highest PCNA expression was in the HCC group and the lowest was in the control group. The PCNA level increased gradually from the control group to the CH group, LC group to reach its highest level in the HCC group. This denotes that cellular proliferation in LC and HCC groups was higher than in the CH and Control groups. CH, LC and HCC groups showed a statistical significant difference relative to control group at a $p < 0.05$ (CH group) and $p < 0.01$ (LC, HCC groups). PCNA expression difference in CH and LC groups is statistically significant relative to the HCC group and to each other at a $p < 0.01$. There is statistically significant difference in PCNA expression levels between HCC grades I and II at $p < 0.05$, and HCC grade III with a statistical significant difference relevant to grades I and II at a $p < 0.01$. The highest caspase3 expression was in the LC group and the lowest was in the control group. The caspase3 level increased from the control group to the CH and LC groups and decreased in the HCC group. The CH, LC and HCC groups showed a statistical significant difference relative to the control group at a $p < 0.05$ (CH group) and $p < 0.01$ (LC, HCC groups). Caspase 3 expression in CH and LC groups is statistically significantly higher relative to the HCC group and relative to each other at a $p < 0.01$. There is statistically significant difference in Caspase3 expression levels between HCC grades I and II at $p < 0.05$, and low Caspase3 expression level in HCC grade III with a statistical significant difference relevant to grades I and II at $p < 0.01$& $p < 0.05$, respectively. Conclusion: Increased PCNA expression in hepatocytes indicating recent replication is increased in HCV chronic liver disease and in HCC. Development of HCC in patients with cirrhosis can to be predicted by liver cell proliferation. Reduced caspase3 expression in liver cirrhosis may indicate malignancy and a higher grade in HCC. Research directed to suppress nuclear proliferation and to enhance apoptosis may be of therapeutic value to control HCC development in cirrhosis.
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1. Introduction

The recently released Egyptian Demographic Health Survey (EDHS) tested a representative sample of the entire country for HCV antibody. The overall prevalence positive for antibody to HCV was 14.7%. Not everyone remains infected but EDHS reported that 9.8% continue to have HCV-RNA [(1)]. It is observed that the prevalence of HBV- and HCV-associated chronic liver diseases in liver biopsy material over the last decade showed a steady rise of HCV-associated diseases and a decline in HBV-associated ones [(2)]. Chronicity of HCV infection leads to chronic hepatitis and liver cirrhosis much more frequently than chronic infection by other hepatitis viruses [(3)], and shows a higher degree of association with hepatocellular carcinoma in several parts of the world [(4)]. Hepatocellular carcinoma is one of the most prevalent malignancies worldwide, being the third largest cause of cancer deaths. HCC is considered a multistage disease whose occurrence is caused by the interaction between genetic and environmental factors [(5)]. Usually, HCC arises from an adenomatous hyperplasia in an already diseased liver and progresses from a well-differentiated stage to less-differentiated forms [(6)]. While the sequential progression of chronic hepatitis to cirrhosis and ultimately to cancer seems to be well established, the exact mechanism of viral hepato-carcinogenesis is yet to be clearly defined [(7).] It has been suggested that the most important factor for the development of HCC is not the integration of viral DNA, but possibly the persistent liver cell necrosis and the resultant irregular regeneration [(8).] Therefore, the

proliferative rate of regenerating hepatocytes may be an important pathogenetic and prognostic factor in chronic liver disease [9].

Inoue *et al.* [10] found that the proliferation capacity of hepatocytes estimated immunohistochemically by proliferating cell nuclear antigen staining was markedly increased at early stage of cirrhosis development. However it was gradually decreased thereafter and suppressed substantially at the time of cirrhosis manifestation, the proliferative capability of hepatocytes is exhausted during continuous hepatic damage, this is considered to be a main cause of the development of liver cirrhosis.

A number of markers have been used in the assessment of the proliferative status of cells, like PCNA. The proliferating cell nuclear antigen (PCNA) is a 36 kDa protein that interacts with multiple proteins that play a key role in DNA synthesis and repair, cell cycle regulation, chromatin remodelling and apoptosis [11-12]. PCNA is one of the best markers for evaluating cell proliferation in studies on retrospective material, since the antigen can be localized in routine formalin-fixed paraffin-embedded tissue [13] . The apoptotic process appears to be a host defense mechanism against viral infections and tumourgenesis. However, many viral genomes encode proteins, which repress apoptosis so as to escape from immune attack by the host. Therefore, virus-host interactions may determine viral persistence, extent and severity of liver inflammation and possibly viral hepato-carcinogenesis. Apoptosis of liver cells may play a significant role in the pathogenesis of hepatitis C. It may represent a mechanism for viral shedding rather than for viral elimination, thereby raising the concept that inhibition of apoptosis could ameliorate hepatitis C. Apoptosis may occur in the absence of significant transaminase elevation, thereby explaining the lack of correlation between biochemical activity and liver cell histological injury. Monitoring caspase activation might provide a reliable tool to estimate the efficacy of HCV therapy, and might open challenging therapeutic strategies in HCV infection [14].

The aim of the work is to evaluate the role of nuclear proliferative activity (PCNA) and apoptosis (caspase3) in cases of chronic hepatitis, liver cirrhosis and HCC.

2- Patients and Methods:

Patients

Ninety patients (72 males and 18 females; mean age 40.3 ± 2.4, range 25-65 years) were the subject of this study. Patients were admitted to the Department of Gastroenterology and Hepatology, Theodor Bilharz Research Institute, Giza, Egypt. They included 35 cases of chronic hepatitis C virus infection (CH), 25 cases with liver cirrhosis (LC) and 30 cases of HCC, all with HCV infection. Of the HCC patients (7) patients were grade I, (14) were grade II; and (9) were grade III. The presence of HCV-RNA in patient's sera was detected by real-time polymerase chain reaction. They were subjected to thorough clinical examination, urine and stool analysis, liver function tests, ultrasonography and liver biopsy for histo-pathologic and immuno-histochemical studies. The study protocol was approved by the Ethics Committee of TBRI according to the Institutional Committee for the Protection of Human Subjects and adopted by the 18th World Medical Assembly, Helsinki, Finland [15].

Ten control liver biopsies were taken from individuals subjected to laparoscopic cholecystectomy after their consent. They were 4 males and 6 females with a mean age of 48.3 ± 2.3 years. Their liver function tests were normal and had no serologic evidence of hepatitis B or C viruses.

Liver biopsies were fixed in 10% buffered formalin for 24 hours, and then processed in ascending grades of ethyl alcohol, xylene, wax and paraffin blocks. Sections (4µm) were cut on albuminized glass slides and stained with Hematoxylin & Eosin and Masson trichrome stains. All sections were subjected to light microscopic examination for evaluating the histopathological and basic classification of cases. Five histological features have been observed to be relatively characteristic of (although not pathognomonic for) chronic hepatitis: (1) lymphoid aggregates in portal tracts, (2) degenerative injury-type changes of bile ducts, (3) large droplet steatosis, (4) Mallory body-like material within injured hepatocytes, and (5) lymphocytic aggregates within the lobules [16]. They were evaluated on a five point scale, using 20 random fields at x100 and x400 magnification per slide. Architectural changes, fibrosis and cirrhosis were evaluated on a seven point scale according to Knodell score system [17]. HCC cases were classified into 3 grades (I,II,III)well-, moderately-, and poorly differentiated) tumors [18]. Other liver sections (4µm) were cut on slides, which were treated with TESPA (3-aminopropyl-triethoxysilane, Sigma) for immune-histochemistry (IHC).

Immuno-histochemistry for Detection of tissue PCNA and Caspase 3 antigens:

Immuno-histochemical reaction was performed using an avidin biotin complex (ABC) immune-peroxidase technique according to Hsu and Raine 1981[19] using anti human PCNA and Caspase3 on paraffin sections, which were de-waxed in xylene and hydrated in descending grades of ethanol. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide in 100% methanol for 20 min. Antigen retrieval was performed by microwaving the sections in citrate buffer (PH 6.0) for 15 min at 700 W. Sections were incubated overnight at 4°C with the anti-human primary antibodies against PCNA, (purchased

from Dako, Demark), and Caspase3 (purchased from Santa Cruz Biotechnology Inc.; Santa Cruz, USA) monoclonal antibody, diluted at 1:50,150 respectively in BPS. Next day, after thorough washing in PBS, the sections were incubated with streptavidin-biotin-peroxidase preformed complex and evidentiated using a peroxidase/DAB (diaminobenzidine) enzymatic reaction for PCNA & Caspase3. Staining is completed by 5-10 minutes incubation with 3, 3'-diaminobenzidine (DAB) + substrate - chromogen which results in a brown-colored precipitate at the antigen site for PCNA & Caspase 3 (cytoplasmic stain). Slides were washed in PBS for 5 minutes. Slides were placed in 70%, 95% and then 100% alcohol each for 5 minutes. The cell nuclei were counterstained with Mayer's hematoxylin. The cover slips were mounted using Dpx.

Positive and negative control slides for each marker were included within each session. As a negative control, liver tissue section was processed in the above mentioned sequences but the omission of the primary antibody and PBS was replaced.

Immuno-histochemical scoring of PCNA

Sections were examined under Zeiss light microscopy at x40, the proliferation index (P.I) is the number of positive nuclei stained by PCNA in 1000 hepatocytes then the mean of them calculated, and the mean for each group is taken according to Akyol *et al.*[20]. Zero% was given to unstained sections

Immuno-histochemical scoring of Caspase3:

Expression of caspase3 was cytoplasmic, sections were examined under Zeiss light microscopy at x40, in ten microscopic fields. The mean of the percentage of the positively stained cells was calculated from their mean according to Shen *et al.* [21].

Statistical analysis:

The Statistical Package for Social Sciences (SPSS) for Windows (version 10) computer program was used for statistical analysis. For comparison of more than 3 group's means, one-way ANOVA test, Post Hoc test was used. Comparison between percent positive cases was calculated by Chi-square test. A P value < 0.05 was considered statistically significant.

3- Results:

Subjects were 66 males (73.3 %) and 24 females (26.7 %), their age ranged (23-72 years) with a mean of 46.6±4.5 years, as well as 10 patients with normal liver as control group. They were 4 males and 6 females, their age ranged (30-45 years) with a mean of 39.4±3.7years. Some important demographic data & lab investigations are showmen in table I.

Table 1. Liver biochemical profile of studied groups

Variables	CH (n=35) Mean ± SD	LC(n=25) Mean ± SD	HCC(n=30) Mean ± SD	Control (n=10) Mean ± SD
ALT (0-41) U/L	53.95 ± 29.16 [a',b]	45.8 ± 31.91 [a']	36.87 ± 21.5	19.8 ± 5.59
AST (0-38) U/L	38.3 ± 17.33 [b]	69.8 ± 16.15 [a',c]	72.9 ± 34.75 [a']	20.2 ± 5.87
Tot. protein (6-8) gm/dl	7.76 ± 1.17	8.02 ± 0.64 [a]	7.74 ± 1.14	7.24 ± 0.39
S. albumin (3.5-5) gm/dl	4.46 ± 0.4 [b]	3.62 ± 0.22 [a,b,c]	3.28 ± 0.54 [a]	4.22 ± 0.18
Total bilirubin (0.1-1.2)mg/dl	0.9 ± 0.5 [b]	1.2 ± 0.45 [b]	1.7 ± 1.02 [a]	0.9 ± 0.15
Direct bilirubin (0-0.25)mg/dl	0.46 ± 0.35 [a]	0.55 ± 0.5 [a]	1.09 ± 0.81 [a]	0.21 ± 0.06
Prothrombin Concentration (70-100)%	90.2 ± 10.43 [b]	83.2 ± 10.6 [a,b,c]	76.0 ± 11.29 [a]	98.8 ± 2.53

[a]: p value <0.05 relative to the control group
[a']: p value <0.001 relative to the control group
[b]: p value <0.05 relative to the HCC group
[c]: p value <0.001 relative to the CH group

The highest PCNA expression was in the HCC group and the lowest was in the control group. The PCNA level increased from CH to the LC group to reach its highest level in the HCC group. The CH, LC and HCC groups show a statistical significant difference relative to control group at $p < 0.05$ (CH group) and $p < 0.01$ (LC, HCC groups). PCNA expression in LC group is statistically significantly is higher than CH at $p < 0.01$ and in HCC relative to LC at $p < 0.01$ (Table 2, Figure 1A,B,C, D).

There is statistically significant difference in PCNA expression levels between HCC grades I and II at $p < 0.05$, and there is a high PCNA expression level in HCC grade III with a statistical significant difference relevant to grades I and II at $p < 0.01$ (Table 3).

The highest caspase3 expression was in the LC group and the lowest was in the control group. The caspase3 level increased from the control group to the CH group, LC group and decreased in the HCC group compared to LC. The CH, LC and HCC groups show a statistical significant difference relative to control group at $p < 0.05$ (CH group) and $p < 0.01$ (LC, HCC groups). There is a statistically significant difference in CH& LC groups relative to the HCC group and relative to each other at $p < 0.01$. The HCC group showing a statistically significant difference $p < 0.01$ higher compared to CH group& lower than LC group compared to LC group (Table 4, Figure 2A,B,C,D).

Table 2. PCNA scoring (Proliferating index) in studied groups

Variable	CH (n=35) Mean ± SD	LC (n=25) Mean ± SD	HCC (n=30) Mean ± SD	Control (n=10) Mean ± SD
PCNA scoring	8.5 ± 2.9 *,^	20.7 ± 3.4 **,^,$	46.4 ± 12.7**	0.3 ±0.1

*: p value <0.05 relative to the control group **: p value <0.01 relative to the control group
^: p value <0.01 relative to the HCC group $: p value <0.01 relative to the CH group

Table 3. PCNA expression levels versus different grades of HCC in studied HCC group.

Variable	Grade I (n=7)	Grade II (n=14)	Grade III (n=9)
PCNA Scoring	27.2 ± 8.54	47.9 ± 12.92*	63.0 ± 10.5 **,^

* p value <0.05 relative to grade I **: p value <0.01 relative to grade I
^: p value <0.01 relative to grade II

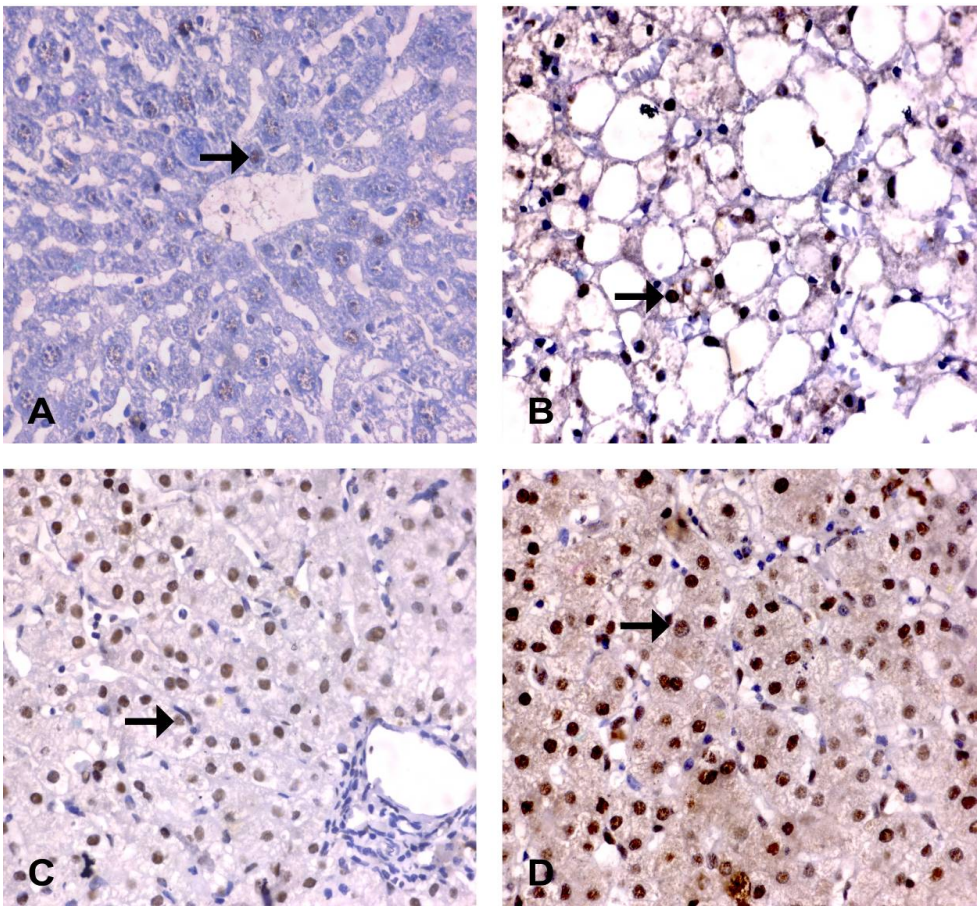

Figure (1): A) A control case showing scattered hepatocytes nuclei positive for PCNA monoclonal antibody (arrow) **B)** A case of chronic hepatitis, showing few positive nuclei for PCNA monoclonal antibody (arrow) **C)** A case of liver cirrhosis showing moderate number pf hepatocytes nuclei positive for PCNA monoclonal antibody (arrow) **D)** A case of HCC showing many showing hepatocytes nuclei positive for PCNA monoclonal antibody (arrow) (Immunohistochemistry, DAB, x 200).

Table 4. Expression of Caspase3 in studied groups

Variable	CH (n=35) Mean ± SD	LC (n=25) Mean ± SD	HCC (n=30) Mean ± SD	Control (n=10) Mean ± SD
Caspase 3	17.5 ± 4.5 *,^	48.5 ± 3.7 **,^,$	32.5 ± 11.9**	1.2 ±0.2

*: p value <0.05 relative to the control group
**: p value <0.01 relative to the control group
^: p value <0.01 relative to the HCC group
$: p value <0.01 relative to the CH group

Table 5. Expression of Caspase3, versus different grades of HCC in studied HCC group.

Variable	Grade I (n=7)	Grade II (n=14)	Grade III (n=9)
Caspase3	47.6 ± 9.22	35.4 ± 7.92*	24.0 ± 3.5 **,^

*: p value <0.05 relative to grade I
**: p value <0.01 relative to grade I
^: p value <0.05 relative to grade II

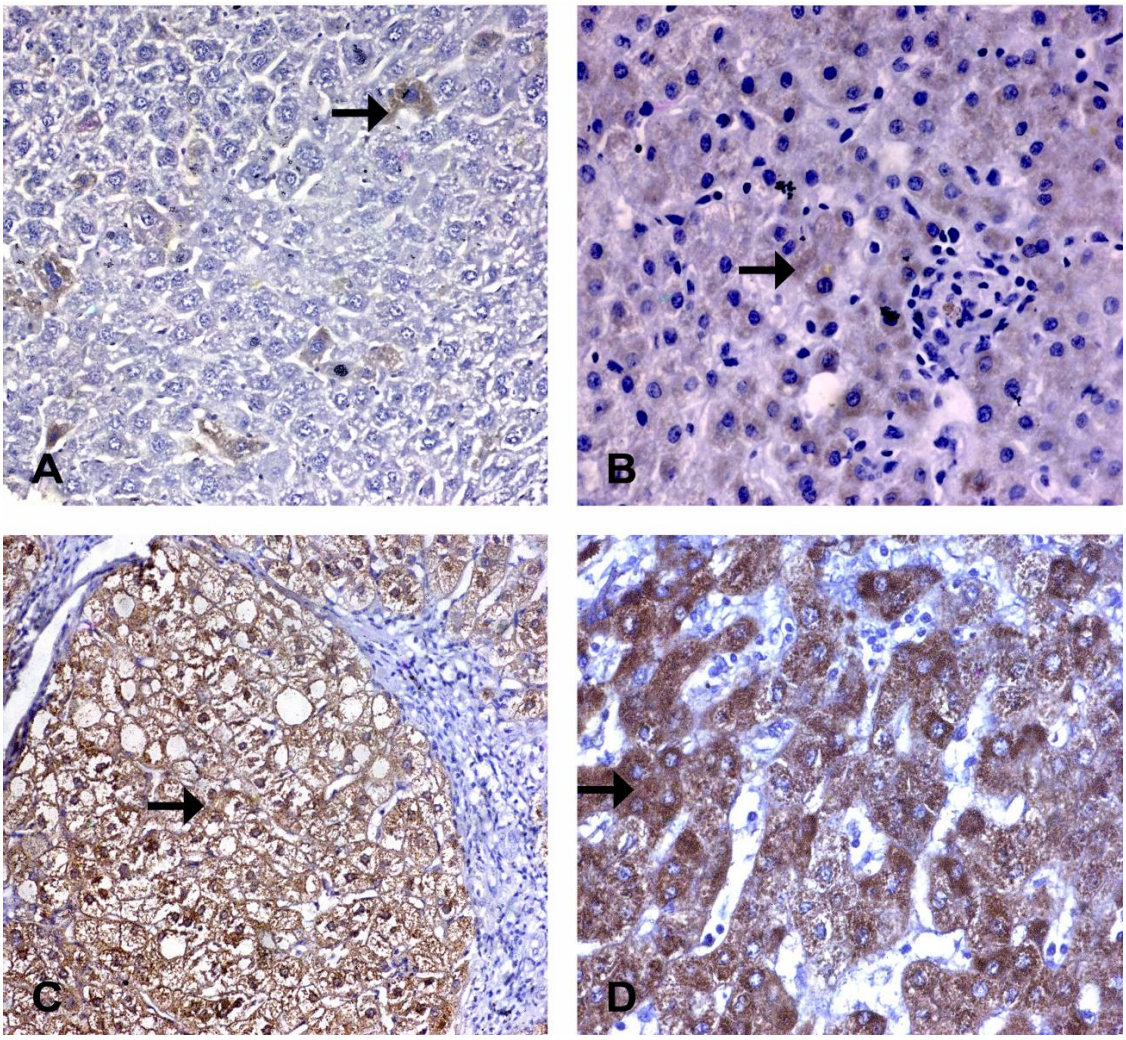

Figure (2): **A)** A control case showing scattered hepatocytes positive for Caspase3 monoclonal antibody. (as cytoplasmic brownish stain) (arrow) **B)** A case of chronic hepatitis, showing few positive hepatocytes for Caspase3 monoclonal antibody (arrow) **C)** A case of liver cirrhosis showing moderate number of hepatocytes nuclei positive for Caspase3 monoclonal antibody(arrow). **D)** A case of HCC showing many hepatocytes positive for Caspase3 monoclonal antibody (arrow). (Immunohistochemistry, DAB, x 200).

Discussion:

In this study we found that PCNA level increased gradually from the control group to the CH group, LC group to reach its highest level in the HCC group. Our data are consistent with other reports [22]. Most normal hepatocytes are in a state of proliferative quiescence

(Go phase) in the adult. This correlates with the low PCNA labeling index (PCNA-LI) observed in normal livers [23].

Lake-Bakaar [24] reported that the fraction of replicating or PCNA-staining cells in chronic HCV liver disease and in HCV-related hepatocellular cancer is increased compared to that in controls. Approximately 20% of hepatocytes in chronic HCV liver disease with or without cirrhosis showed evidence of recent cell division. In contrast, less than 1% of liver cells in controls had recently divided.

PCNA labeled fractions (PLFs) in chronic hepatitis and cirrhosis were no different in the material of Abdul Sathar et al. [25]. Virtually identical PCNA-labeling indices have been reported in chronic viral hepatitis and cirrhosis, though seperately, in some earlier reports [12, 18]. On the other hand, hepatocyte proliferative activity has been reported to decrease at the stage of cirrhosis from a higher rate in active chronic hepatitis. Compared to chronic persistent hepatitis, chronic active hepatitis, particularly of the more severe types, has been reported to show higher replicative rates [26].

Donato et al. [27] reported that the mean PCNA-LI ranged from 0.1% for patients with minimal changes to 3.6% for those with cirrhosis and hepatocellular carcinoma. Both HCV or HBV cirrhotics had similar liver cell proliferation rate but those with HBV had higher prevalence of liver cell dysplasia with respect to those with HCV. They concluded that PCNA-LI was a reliable assay for assessing liver cell proliferation rate in patients with chronic viral hepatitis and correlated with liver disease severity.

As in the study by Nakamura et al. [9] Abdul Sathar [25] did not find significant difference between PCNA-LI in HBV- and HCV-associated diseases, though there appeared to be a somewhat higher rate in the latter. Also, in terms of location, PCNA-labeling did not necessarily occur in the vicinity of piecemeal necrosis, fibrosis or portal inflammation. If this excess of PCNA-LI in HCV-associated chronic liver disease is confirmed by correcting the limitation of small numbers, it may partly explain the intriguing phenomena of some biological features of chronic HCV infection [28] regarding the more frequent association with cirrhosis and quicker progression to hepatocellular carcinoma when compared to those in chronic HBV infection.

The relationship between hepatocyte proliferation and liver functional reserve in cirrhotic patients was analyzed by Delhaye et al. [29] and showed that the PCNA-LI value declines with worsening Child class and is closely associated with serum albumin, a marker of protein synthesis in the liver, whatever the cause of the disease. An interesting aspect of their study resides in the observation that hepatocyte proliferative activity seems to reflect the liver functional reserve in human cirrhosis.

Evaluation of liver cell proliferative activity in the tumoral and non-cancerous surrounding tissue, expressed as PCNA positive cells demonstrated a significantly increased proliferation in tumoral as compared to peri-tumoral tissue [30]. In tumoral tissue, a high PCNA score is associated with a bad prognosis in HCC [31-32].

Hepatocellular proliferation rates observed in our material were generally similar to those reported by others in chronic hepatitis and cirrhosis [12, 18, and 33]. Ojanguren and colleagues [33] found no PCNA labeling in more than half (7/13) of biopsies showing cirrhosis, while Ballardini et al[13] reported absence of strong labeling indicative of pre-mitotic phase in cirrhotic livers that did or did not develop HCC during follow-up. Since replicating cells are always randomly distributed, non-detection of labeling may merely be due to relatively small tissue samples obtained by biopsies, particularly on needle aspiration.

Venturi et al. [34] found that in none of cirrhotic tissues was a PCNA basic spot was detected. These data show that PCNA is differently expressed in HCC and cirrhotic tissues, in terms of structure, isoforms and post-translational modifications, strongly implicating functional alterations in PCNA in the hepato-cancerogenetic process. The PCNA basic isoforms, exclusively detected in hepatic malignant samples, may represent a new signature for neoplastic liver cells compared to cirrhotic tissues. The several PCNA isoforms detected in neoplastic samples compared to non-cancerous tissues might help explain the PCNA over-expression in HCC detected at immune-labelling and immune-staining analysis [35].

In our study, we found that there is statistically significant difference in PCNA expression levels between HCC grades I and II, and there is a high PCNA expression level in HCC grade III with a statistical significant difference relevant to grades I and II. This is in accordance with previously published literature [36-38]. PCNA positively stained cells were 10% in low-grade HCC specimens. In moderately differentiated malignancies, PCNA staining was detected in approximately 30% of the nuclei. High-grade malignancies showed 70% of tumor cells positive to PCNA.

A progressive increase in the PCNA-LI from regenerative to dysplastic nodules to HCC has been observed [39]. Additionally, an increase in the DNA index was correlated with an increase in PCNA labeling, and both were correlated with pathological changes in HCC tumors [40].

Only HCC had a significantly increased PCNA LI compared to benign categories. Thus is probably related to the malignant nature of HCC and may reflect

the uncontrolled proliferation of the neoplastic hepatocytes [41].

Gramantieri et al. [42] suggested that in HCC PCNA participates both in DNA synthesis & repair and that highly proliferating HCC may display a sustained DNA repair .Also PCNA LI was significantly higher in cancer & correlate with tumor size [43].

Recent, study [44] demonstrated an association between small cell dysplasia, but not large cell dysplasia, and an increased HCC risk in patients with HCV related cirrhosis. Interestingly, liver specimens showing small cell dysplasia changes also had a higher proliferative rate by PCNA and a lower apoptotic rate compared with samples with large cell dysplasia. PCNA assay and small cell dysplasia seemed to be more predictive in the context of HCV patients [44]. The finding of PCNA–positive liver cells clustered near areas of spotty, confluent, or piecemeal necrosis in patients with acute and chronic viral hepatitis [42] provides further support to a possible correlation between increased liver cell proliferation rate and high hepatic inflammation scores in patients with chronic liver diseases [45]. An inverse correlation between liver cell proliferation and survival was shown. differences in survival between patients with high versus low PCNA-LI values points to a link between liver cell proliferation, HCC and liver-related mortality in patients with compensated cirrhosis [46].

Venturi et al. [34] concluded that human HCC express specific PCNA isoforms compared to those found in cirrhosis, implicating a role for PCNA functional alteration in hepatocarcinogenesis.

Proliferating activity of tumor defined by PCNA immune-histochemical study has been reported to be related to metastatic potential, recurrence and overall prognosis [47]. In our study, the PCNA LI was significantly higher in more poorly differentiated HCCs, which is consistent with previous reports [48], that cell proliferative activity of HCC cells correlates with their degree of dedifferentiation.

In our study, the caspase3 level increased from the control group to the chronic hepatitis group, liver cirrhosis group and decreased in the HCC group. Farinati et al. [45] reported that cyto-proliferation is more pronounced in chronic HCV-related hepatitis, while apoptosis is not significantly higher than in other types of liver damage, suggesting an imbalance between the two. Apoptosis and cyto-proliferative index are directly related to the extent of liver damage.

Arzberger et al. [49] found an inverse correlation between the strength of an apoptotic stimulus and the infectivity of the virus particles released: the more potent the apoptotic stimulus, the higher the ratio of non-enveloped capsids to virions and the lower their infectivity.

Recent studies provide evidence for variable degrees of liver cell apoptosis in the liver of patients with chronic hepatitis C. Apoptosis does not correlate with transaminase levels, viral load or genotype [50-51]. The lack of correlation of caspase activation to viremia or serum transaminase levels may be related to different types of cell death. *In vivo* studies have shown that the apoptotic process in hepatocytes is accompanied by increased transaminase levels, but the release of transaminases is lower in apoptosis than in necrosis. Therefore, relative differences in the occurrence of apoptosis and necrosis could explain why transaminase levels and caspase activation are not correlated [52].

In our study, Caspase 3 was highest in LC group and was lower in HCC group. In biopsy specimens with low activity (grade 0), 7.7% of the hepatocytes have caspase3 activation, whereas 20.9% of the cells stain positive in grade 3. Kinetic analysis of viral turnover in patients indicates that HCV infection is a highly dynamic process with a short half-life of viral particles and HCV-infected cells [53]. It is estimated that the daily turnover of HCV-infected cells may be as high as 13-25%. Assuming that in patients with chronic HCV infection approximately 50% of liver cells are infected, this would mean that between 6.5 and 12.5% of hepatocytes are killed daily [54]. It is notable that this number is very similar to the amount of cells with active caspases ranging from 7 to 20%, thereby suggesting that the daily liver cell death might occur via apoptosis [54].

We found that there was statistically significant difference in Caspase3 expression levels between HCC grades I and II at $p < 0.05$, and a low Caspase3 expression level in HCC grade III with a statistical significant difference. Sun et al. [55] reported that 53.8% cases of HCC were found to express caspase3 transcripts, while 46.2% of HCC failed to express it. The expression of caspase3 was correlated with HCC differentiation, as 72.2% (13/18) of moderately to well differentiated HCC showed positive caspase3 transcripts, while only 38.1% of poorly differentiated HCC was caspase3 positive. No relationship was found between caspase3 and tumor size or grade or metastasis, although 62.5% (5/8) of HCC with metastasis was caspase3 positive but it did not differ significantly from that without metastasis. Expression of caspase3 alone did not affect the apoptosis index (AI) of HCC. Sun et al. [55] concluded that caspase expression may not be related to cell apoptosis in HCC. In contrast, a previous report had reported reduced expression of caspase-3 by immune-histochemistry in HCCs compared to non-tumor liver tissue [56]. These differences may be due to differences in methodology including use of different primary antibodies. Caspase3 over- expression was not associated with histology or prognosis. However, caspase3 expression has been associated with histological type and grade of tumor, and with prognosis in other tumor types [57].

Conclusion,

Increased PCNA in hepatocytes indicating recent replication is increased in HCV-related chronic liver disease and in HCC. These findings have potential implications for linking chronic HCV infection to cirrhosis and HCC. Development of HCC in patients with cirrhosis can to be predicted by liver cell proliferation status. Reduced caspase3 expression in liver cirrhosis may indicate malignancy and higher grade in HCC. Research directed to suppress nuclear proliferation and enhance apoptosis may be of therapeutic value to control HCC development in cirrhosis.

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