CONSTANTA "OVIDIUS" UNIVERSITY
FACULTY OF MEDICINE
Discipline: Pathologic Anatomy

Epidemiological, Clinical and paraclinical, Morphopathological and Immunohistochemical Aspects of hepatocellular carcinoma developed on a hepatic cirrhosis

SUMMARY

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Hepatocellular carcinoma is the most frequent malign hepatic tumor that occurs within the human species, representing 90% of the total malign hepatic tumors. It represents the sixth largest human neoplasia and the third largest cancer related mortality factor in the entire world.. Every year 500.000 to 1.00.000 new cases manifest throughout the world. The death rate of HCC reaches 94%.

This doctoral dissertation approaches a research theme of a highly scientifical interest connected to the complex study of hepatocellular carcinomas developed against a hepatic cirrhosis background , considering the fact that the incidence of hepatocarcinoma is increasing in Romania and it can manifest itself at any age with a higher rate of occurrence after 60.

An early diagnosis increases the patients' survival chances by receiving treatment in the early stages thus allowing for better control of the disease. Also highly important is the knowledge of morphological, histochemical and immunohistochemical characteristics of hepatocytes , thus allowing the use of newer less toxic therapies which also have better results.

Hepatocarcinomas also have a social and economical impact by affecting people who are still able to work. Therefore any study on the diagnosis of malign hepatic pathology is useful for both doctors and patients.

This dissertation, which is structured according to this goal, is made up of two parts: The first part - ***Current stages of information and research in the field*** - presents a bibliographic synthesis of the knowledge on this subject, structured in three chapters , and the second part - ***Personal contributions to the development of the field*** -
divided into three chapters whose titles mark the essential stages of the research.

Chapter I presents the introductory notions elements of hepatic embryology , laparoscopic anatomy , histiology and physiology.

Chapter II deals with the epidemiology and etiopathogeny of hepatocellular carcinoma, focusing on the different etiological factors involved in its etiology. Also it presents pre-malign hepatic lesions, clinical manifestations and lab work needed in hepatocarcinomas, and ***chapter III*** discusses the classification of hepatic tumors, morpho pathological aspects of hepatocarcinoma with a description of macroscopic and microscopic forms according to usual classification.

Chapter IV expresses concisely the objectives (mainly the effects of the immunohistochemical techniques in establishing a diagnosis algorithm for hepatocellular carcinoma developed on hepatic cirrhosis)

Chapter V defines the material of the study (pieces of hepatic resections from 40 patients diagnosed with hepatocellular carcinoma from 01.01.2005 to 30.12.2009 in Fundeni Clinical Institute) and classical and modern investigation techniques; this is completed by the statistical analysis of prospective-retrospective cases observation , and an interpretation of the statistically representative data that are relevant to the study.

Chapter VI presents the results, correlations and histopathological and immunohistochemical discussions for the studied cases..The immunohistochemical study was based on a panel of immunohistochemical antibodies useful for a confirmed diagnosis through the morphological particularities of hepatocytes (Vegf and p 53) and their degree of increase (Ki 67). This chapter also presents numerous visual representations that complete and support the comments and discussions.

Going through the stages of the research t***he next chapter*** presents the discussions over the obtained results in relation to worldwide research

The last part deals with the conclusions of the study with an emphasis on the most important elements presented in the study.

This theoretical frame is enriched by the results of a rigorous documentation stemming from a bibliography of almost 173 titles, representing both landmarks of the research in the field and extremely recent pieces of information.

The dissertation has 169 photographs, 30 tables and 42 charts.

Both the clinical analysis and the classical and immunohistochemical analysis of pieces of hepatic resections, for a large number of cases provide a significant picture not only on the morphology of the disease, but also on the inter-relations with the micro medium in which it develops, which in turn it influences and is influenced by,

Purpose and general goals of the research

The selection of this theme for the doctoral dissertation is attributed to an increase in the anatomopathological practice of the hepatic pathology dominated by dysplasic and tumoral lesions.

The importance of an accurate diagnosis of these lesions stems from the necessity of using an efficient therapy adapted to every lesion and more importantly to every case.

The goal of this dissertation is to find correlations between the epidemiologic, clinical - paraclinical, morphopathological, and immunohistochemical aspects, that could potentially constitute the basis of algorithms useful for both the identification of the histiological type of tumors and the establishing of the best course of treatment that could reduce mortality and the evolution of hepatic tumors associated with liver cirrhosis.

The degree of epidemiological association was measured through the rating of the frequency of the risk factors within the test group and the control group , as well as through the measuring of the epidemiological association between the risk factors studied and the presence of CHC

Material and Method

The study consists of 40 patients with hepatocellular carcinoma that were admitted to the General Surgery and Hepatic Transplant Department of Fundeni Clinical Institute from January 2005 to December 2009, and a control group that consists of 80 patients who do not have HCC , admitted to 3 different departments: dermatology, ophthalmology and otorhinolaryngology.

The control group was used only for the etiological study. The groups are similar when it comes to the age (+_ 5 years) and the sex of the patients. The patients from the test group were admitted at the same time , do not have any other liver diseases or neoplasms, and the risk factors involved in the respective pathologies are completely different.

The study material is represented by the anamnesis and the clinical examination of patients admitted for the prospective duration of the study and the macroscopic, microscopic and immunohistochemical examination of the pieces of hepatic resection at

the Pathological Anatomy department of the same institution, and the observation charts of the patients for the retrospective part of the study.

For each of the cases the analyzed data was introduced in original study charts and an electronic input form was designed.

The study was centered on creating a morphopathological and immunohistochemical profile of hepatocarcinomas associated or not with hepatic cirrhosis but it also details clinical and paraclinical aspects, that are relevant.

Statistical processing

The data was input in a 2xn table and the processing and analysis of information was done through SPSS version 19.0.

For the descriptive form absolute and relative frequencies were used in the presentation of the group's constituents.

For the quantitative variables were calculated the locating indicators (mean, average) but also the dispersion ones. The frequency distribution of the variables considered was represented graphically.

In order to measure the force of the epidemiological association in the study I used odds ratio (OR represents the report between the quota of the disease for those affected and the quota for those not affected by it) and the etiological fraction of given risk. (FRA%)

In order to determine the level of statistical relevance χ^2 Mantel-Haenszel test was calculated.

Student t test or one way ANOVA was used to examine the association between clinico-pathological features and the biomarkers expression. A p value of ≤ 0.05 was considered statistic significant.

Results and discussions

The test group of the 40 patients with HCCCHC is made up of 32 (80%) males and 8 (20%) females with a ratio of M:F of 4:1, most of them living in an urban environment.

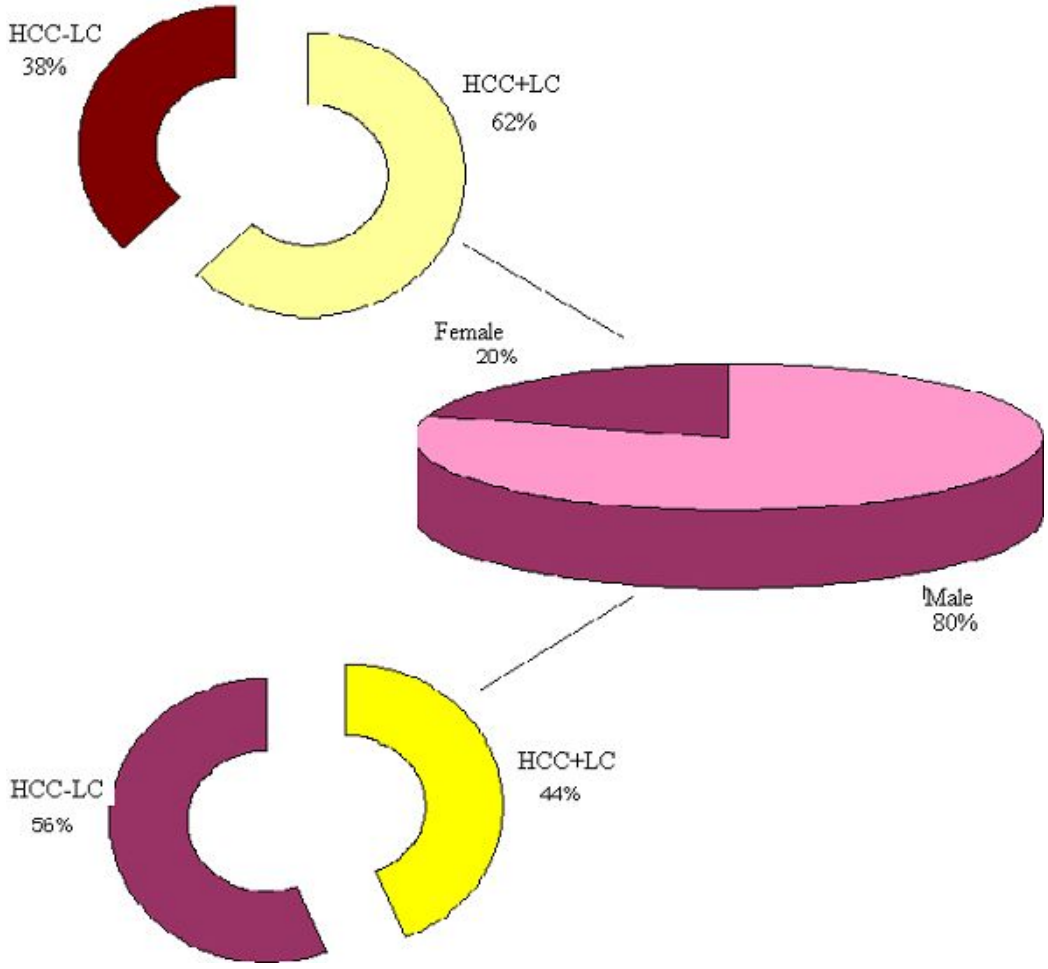

Chart no. 1: The distribution of patients considered for the immunohistochemical study according to sex

The average age of the patients included in the study was 54,6 years, with a standard deviation of ±11.7, the minimum age was 27 years old, and the maximum was 76 years old, though some authors have reported an average age of patients with CHC of 40.

The most frequently affected were people in their 60s, with malign epithelial hepatic tumors in 25 of the patients (52%), the most frequent being the trabecular hepatocarcinoma , both in males and females.

In my study the smokers' ratio is 82,5%, being close to the percent of smokers (70,5%) reported in an article of the International Union Against Cancer (UICC), and the risk of developing CHC is directly proportional to the number of cigarettes consumed daily.

The percent of those who consume alcohol in the study (85%) is 8 times higher than the national average (10%), average reported in an official statistic released by the Health Ministry.

Table no 1: Distribution of patients according to alcohol, tobacco consumption and the presence of hepatitis B and C markers

Risk Factor	Cases		Control		OR	IC 95%	P value	FRA%	χ^2 M-H
	Nr.	%	Nr.	%					
Alcohol consump									
Yes	34	85	50	62,5					
No	6	15	30	37,5	3,4	1,18-10	0,01	58,8%	6,38
Tobacco consump									
Yes	33	82,5	34	42,5					
No	7	17,5	46	57,5	6,38	2,34-18	<0,001	84,3%	17,16
Status HBV									
Ab HBs +	27	70	13	16,3					
Ab HBs -	13	30	67	83,7	10,7	4,05-29	<0,001	90,6%	31,26
Status HCV									
Anti HCV +	8	20	9	11,3					
Anti HCV -	32	80	71	88,7	1,97	0,62 – 6	0,19	49,2%	1,67
Liver cirrhosis									
Yes	19	47	17	22					
No	21	53	63	78	3,3	1,3-8,2	0,003	69,7	8,68

Based on the data calculated and presented in table no. 1, the calculated OR value (of 3,4 for an IC 95% in the interval 1,18-10,25) for alcohol consumption indicates that the risk of developing HCCCHC is significantly greater for people who have admitted a constant consumption of ethanol than for the people who do not present this behavioral risk factor.

As for the risk represented by tobacco consumption , the calculated OR value (of 6,38 for an IC 95% in the interval 2,34-18,06) indicates the fact that the possibility of developing HCC is 6 times higher for the people who stated this type of behaviour.

In patients with Ag HB positive, an OR of 10,7 was calculated with an interval of certainty between 4,05-29. Therefore it can be stated with a high degree of validity that B-type hepatic viral infection represent a risk factor for the developing of HCC (p_v <0,01).

Anti HCV antibodies were found positive in 8 patients with hepatocellular carcinoma, for whom an OR of 1,97 (IC 95% 0,62-6,25) was calculated and an etiologic fraction of attributable risk of 50%. According to the result of this study, we cannot state anything clearly about about type C hepatitis viral infection as the values obtained through the use of statistic test have not reached statistical significance (p_v =0,19).

Therefore the data presented in table no 2 shows that the risk of developing HCC is 8 times higher for those who smoke more than 20 cigarettes daily and 5 times higher for those who consume more than 80 grams of pure ethanol daily , when compared to those who do not smoke or drink alcohol.

The calculations indicate that the risk of developing HCC increases proportionally to the quantity of alcohol ingested. Also statistically significant differences can only be noticed in the case of those who ingest more than 80 g per day of ethanol (p_v =0,002).

Table no 2 : Dosage - effect relation in alcohol and tobacco consumption

Risk Factors	Cases		Control		OR	IC 95%	P value	χ^2 M-H
	Nr.	%	Nr.	%				
Cigarettes								
No	7	17,5	46	57,5				
≤1 packet/day	12	30	14	17,5	5,63	1,65-19,92	0,001	10,23
>1 packet/day	21	52,5	16	20	8,63	2,79-27,72	<0,001	19.07
Alcohol								
No	6	15	30	37,5				
1-79 g/day	16	40	33	41,25	2,42	0,76-8,05	0,09	2,73
≥80 g/day	18	45	17	21,25	5,29	1,57-18,58	0,002	9,45

The general clinical symptoms are subjective and nonspecific and can often be mistaken for hepatic cirrhosis.

The time elapsed, from when the first symptoms appeared until a diagnosis was reached, differ from case to case, from 1 month to 2 years.

Abdominal ache was present in 72,5% of the HCC cases and was often located in the upper right quadrant or epigastrium and was most frequently described as a continuous pain.

Abdominal distension due to an abdominal mass or ascites was present in 50% of the cases

Ascites was found in 55% of the cases, the mean protein level being around 2,5g/100ml.

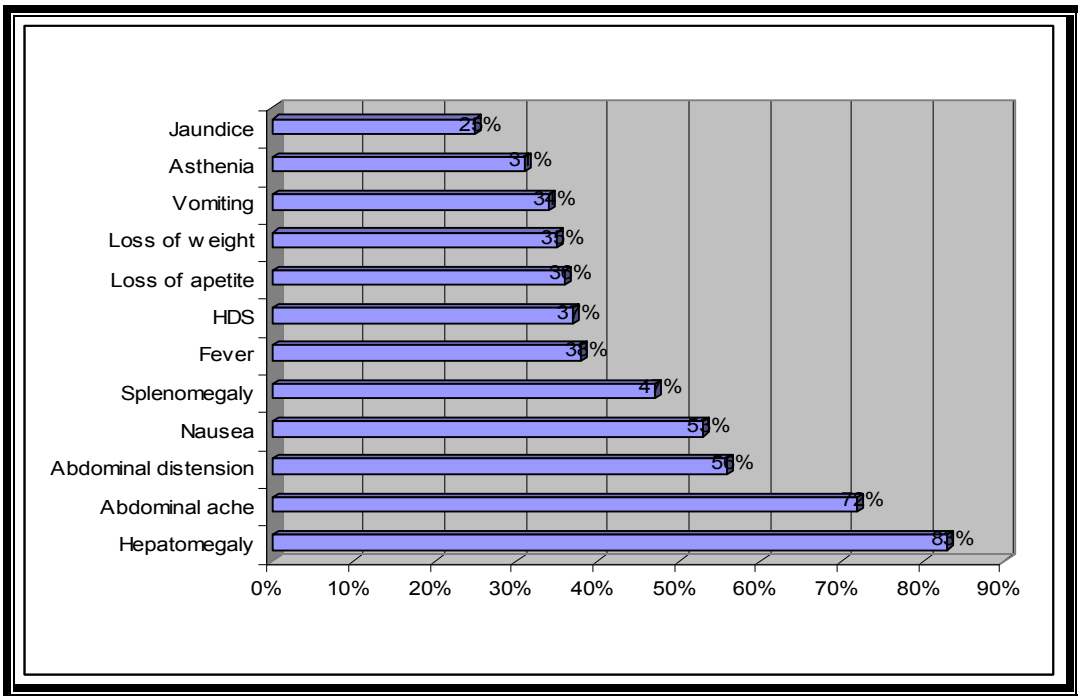

Chart no 2: Symptomatology of hepatocellular carcinoma

Palpable hepatomegaly can be found in 66% of the patients up to 70 years old and in all cases of HCC in patients over 70 years old.

Other symptoms present were: weight loss in 35% of cases, lack of appetite 35%, jaundice 25%, fever 37,5%, constipation.

The mean level of AFP was 552.22 ng/ml with a standard deviation of ±.679.12 and values between 18 şi 4019 ng/ml. 60% of the patients had AFP values higher than 400 ng/ml(Chart no. 3).

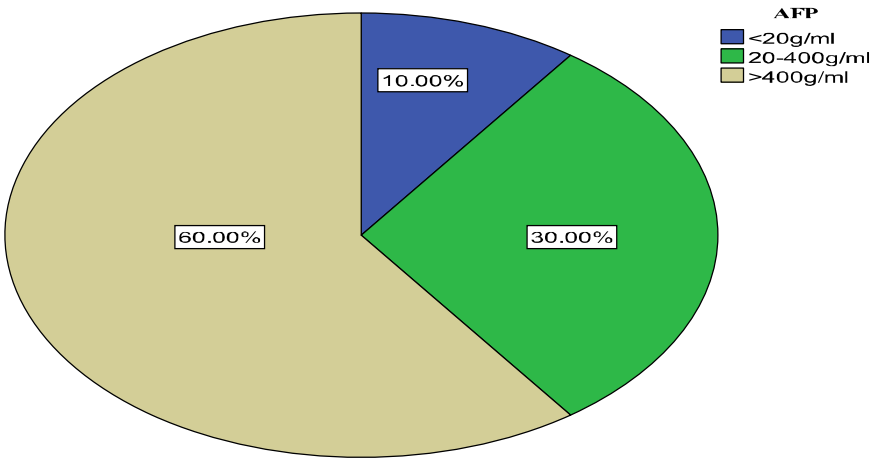

Chart no. 3: Distribution of alpha fetoprotein level

The macroscopic examination was done according to the type of surgical piece and took into account its dimensions, shape, color and consistency.

It can be noticed that there is a prevalent location in the right hepatic lobe for the males and also only one segment for the females. Most frequently one segment or the entire right hepatic lobe were covered by the tumor in the cases of hepatocarcinoma associated with hepatic cirrhosis (Chart no 4).

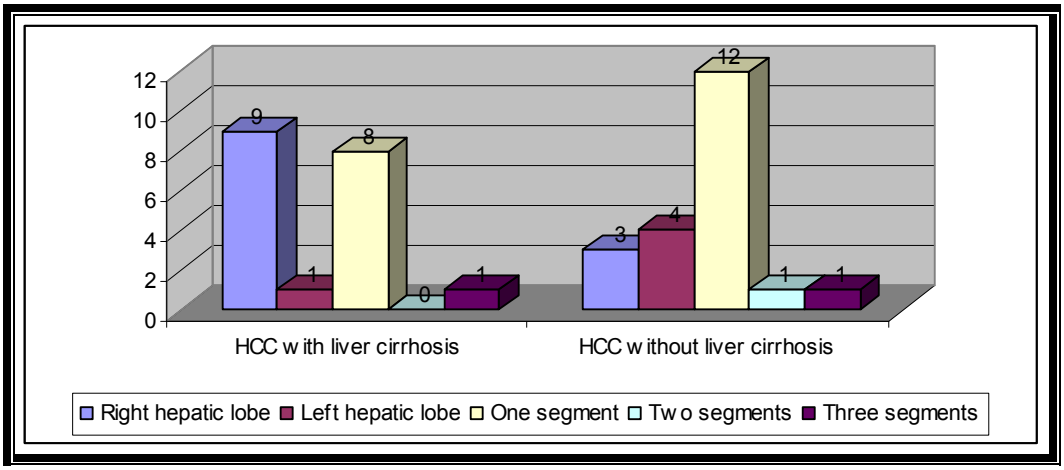

Chart no 4: Distribution according to hepatic cirrhosis presence and tumor location

Both in males and females, most tumor formations had a diameter larger than 5 cm (Chart no. 5). 42% of the HCC cases that were associated with hepatic cirrhosis had a tumor diameter <5 cm, and the rest of 58% had a diameter ≥ 5 cm.

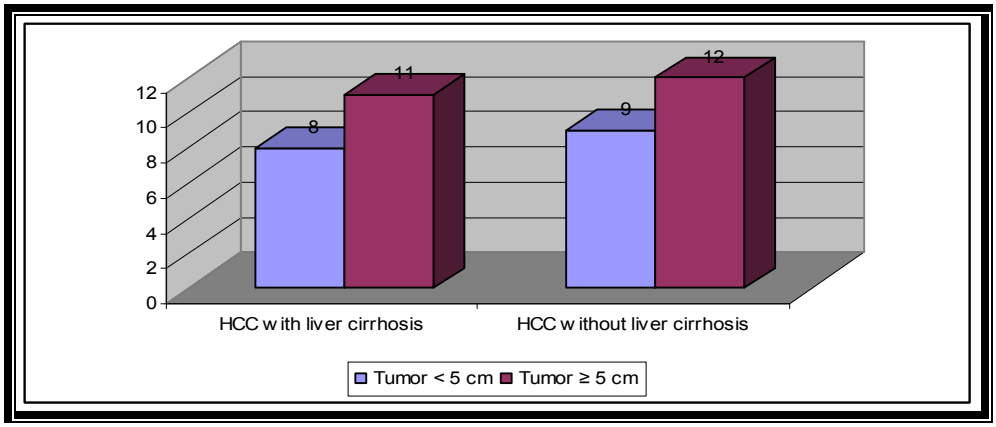

Chart no 5: Distribution of patients according to tumor size

Most tumors were nodular (33 cases) both in female patients (7 cases) and male patients (26 cases). (Chart no 6). The nodular form was also most frequent when age and hepatic cirrhosis were associated (Figure no 1).

This study comprised 30 patients with trabecular hepatocellular carcinoma (Figure no 2), 3 with a pseudoglandular pattern(Figure no 3), 4 patients with a compact pattern(Figure no 4), 2 with fibrolamellar pattern (Figure no 5) and only one case with scirrhous pattern.

Figure no. 1: Hepatocellular carcinoma – macroscopic view .

(W.Y. Lau - Hepatocellular carcinoma pg. 216)

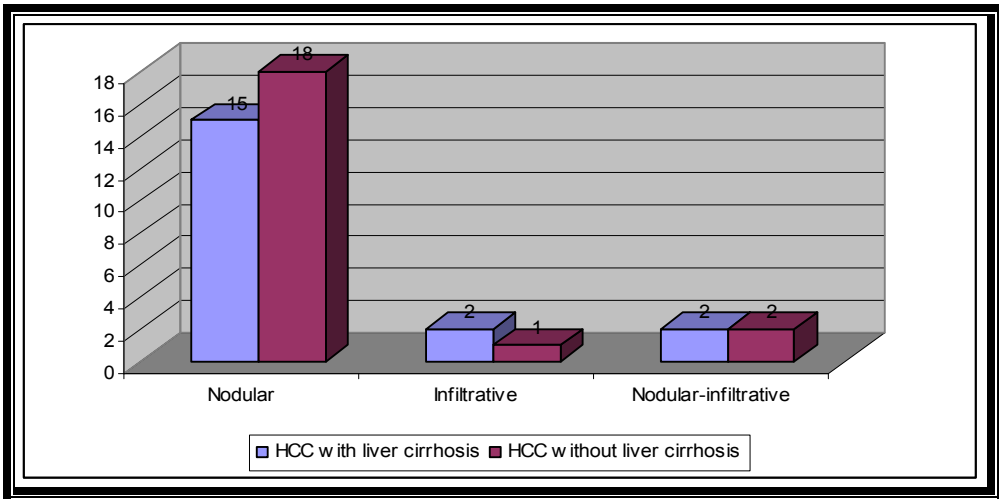

Chart no 6: Distribution of CHC cases according to type of tumor and presence of hepatic cirrhosis.

As for the number of people who presented with cirrhosis that is 19, out of which most presented with the histopathological trabecular type, 14 and 3 hepatocarcinoma had an acinar pattern and 2 were compact type. Schiros and fibrolamellar histopathological types were not present among the cases of hepatic tumors developed on hepatic cirrhosis (Chart no 7)

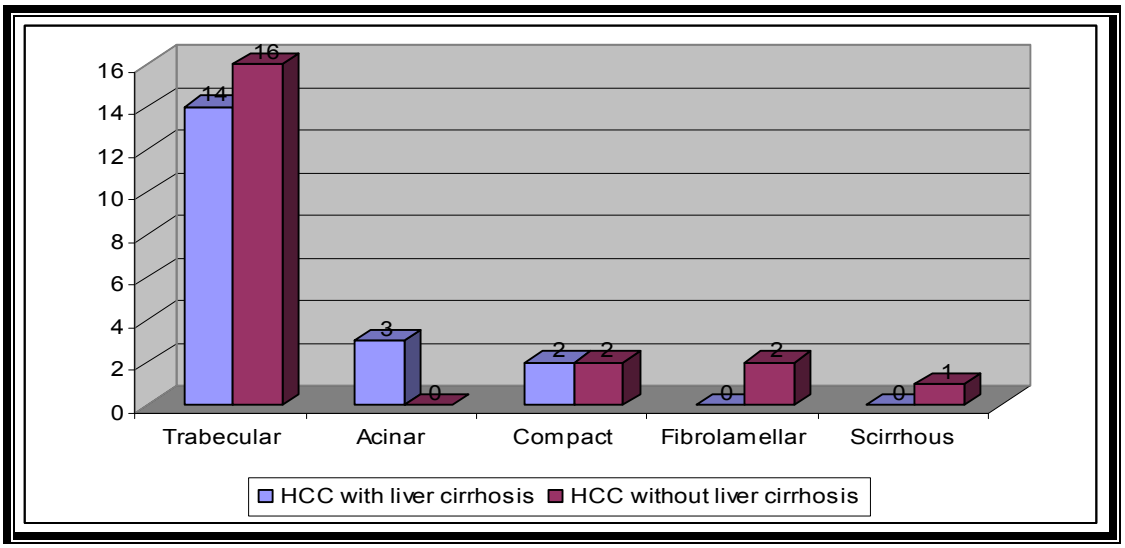

Chart no 7: Distribution of cases according to tumor pattern

From a cytologic viewpoint , a classification of HCC into 3 types was suggested in 1984 : well-differentiated, poorly- differentiated and moderately - differentiated.

Trabecular hepatocarcinoma appears more frequently on cirrhotic liver than other

histopathological types and have associated cytopathological types - hepatocyte-like or pleomorphic.

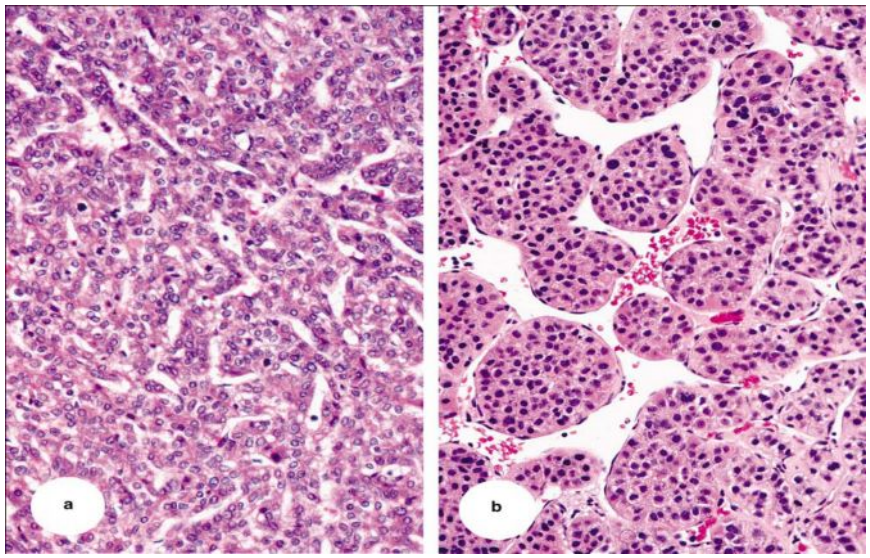

Figure no. 2: Hepatocellular carcinoma: a) Microtrabecular pattern – 3-5 cells, b) Macrotrabecular pattern – 10 or more cells. (http://www.nature.com/modpathol/journal/v20/n1s/full/3800682a.html)

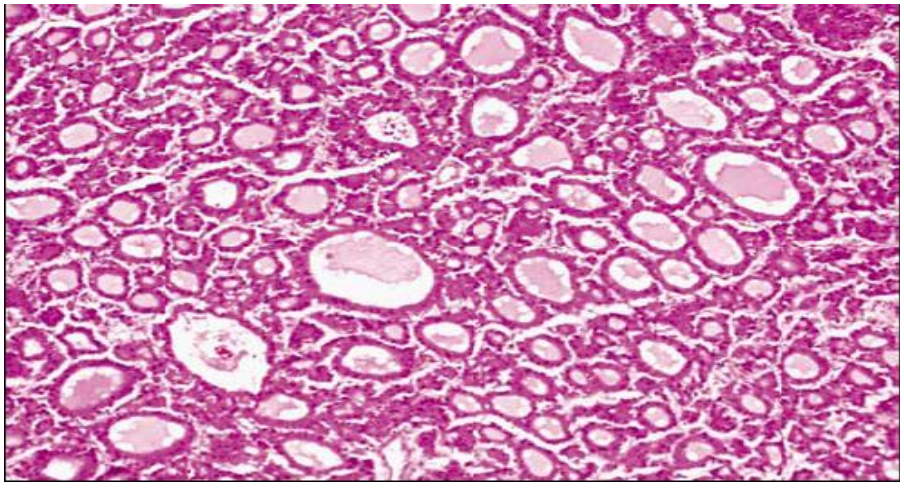

Figure no. 3: Hepatocellular carcinoma – Pseudoglandular pattern
(http://www.nature.com/modpathol/journal/v20/n1s/full/3800682a.html)

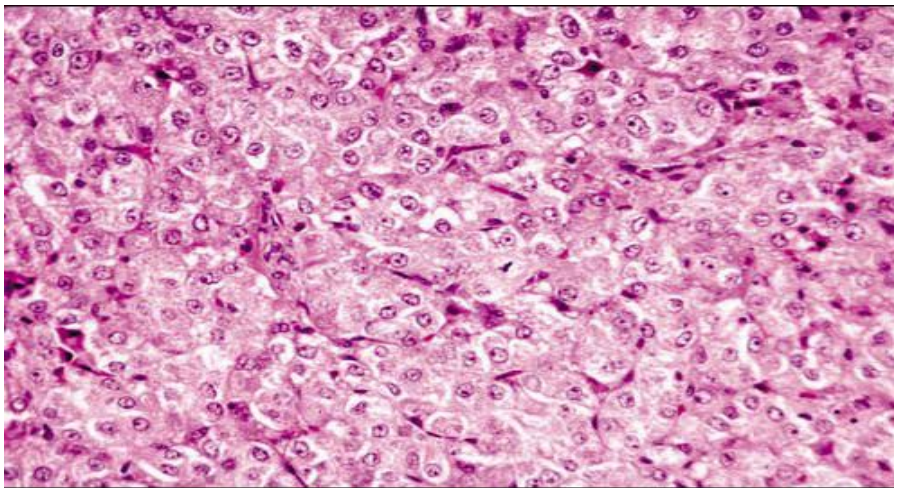

Figure no. 4: Hepatocellular carcinoma – Compact pattern , the trabecul is compressed together with the sinusoidal, which makes the tumor appear compact

(http://www.nature.com/modpathol/journal/v20/n1s/full/3800682a.html)

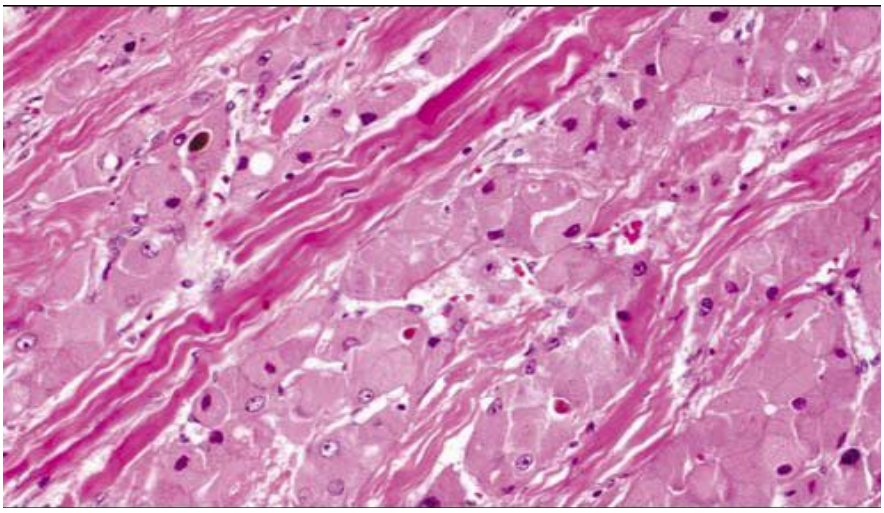

Figure no 5: Fibrolamellar hepatocellular carcinoma

(http://www.nature.com/modpathol/journal/v20/n1s/full/3800682a.html)

A significant number of hepatocellular carcinoma are associated with cirrhosis that can be clinically silent. The patient can evolve from B-type chronic hepatitis to macro-regenerative nodule or border-line cirrhosis, that contain dysplasic lesions that can turn malign.

When HCC is associated with cirrhosis it has a higher tendency to spread throughout the liver, than in a healthy liver. There have been reported cases of HCC developed on a cirrhotic liver connected not only to HBV or HCV, but also to alcoholic

cirrhosis.

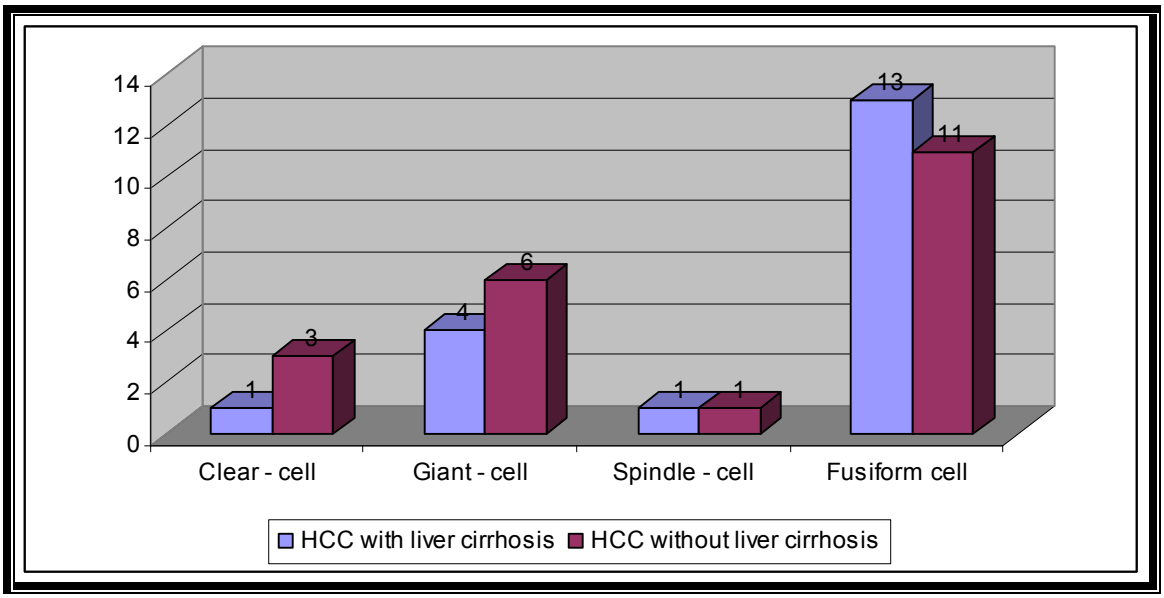

Chart no. 8 : Distribution of cases according to the cytopathological type and association with hepatic cirrhosis.

Considering the cases in which the hepatocellular carcinoma is associated with hepatic cirrhosis these were mostly of the cytologic oncocit- like type (13 cases), pleomorph (4 cases) and 1 each of the clear cell and sarcomatoid types(Chart no 8)(Figures 6-9).

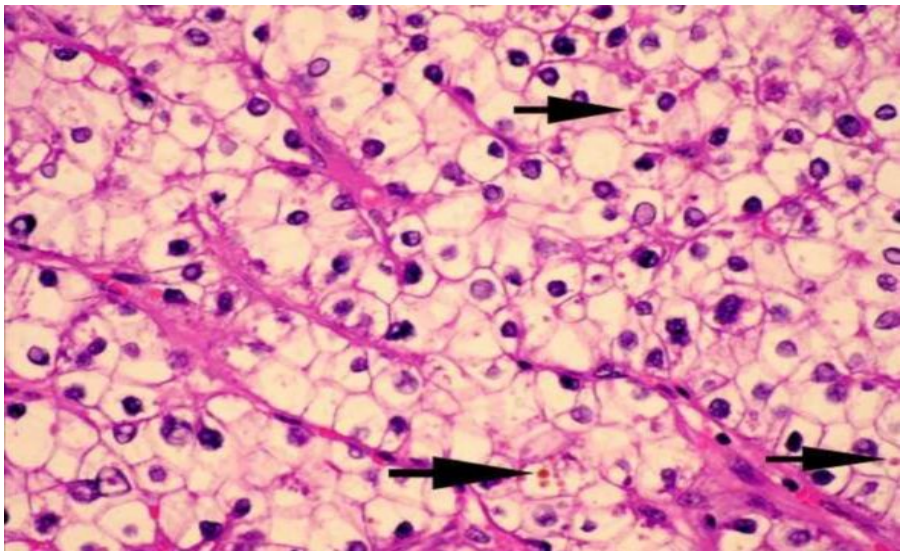

Figure no. 6: Hepatocellular carcinoma - "clear cell" type

(http://www.pathpedia.com/Education/eAtlas/Histopathology/)

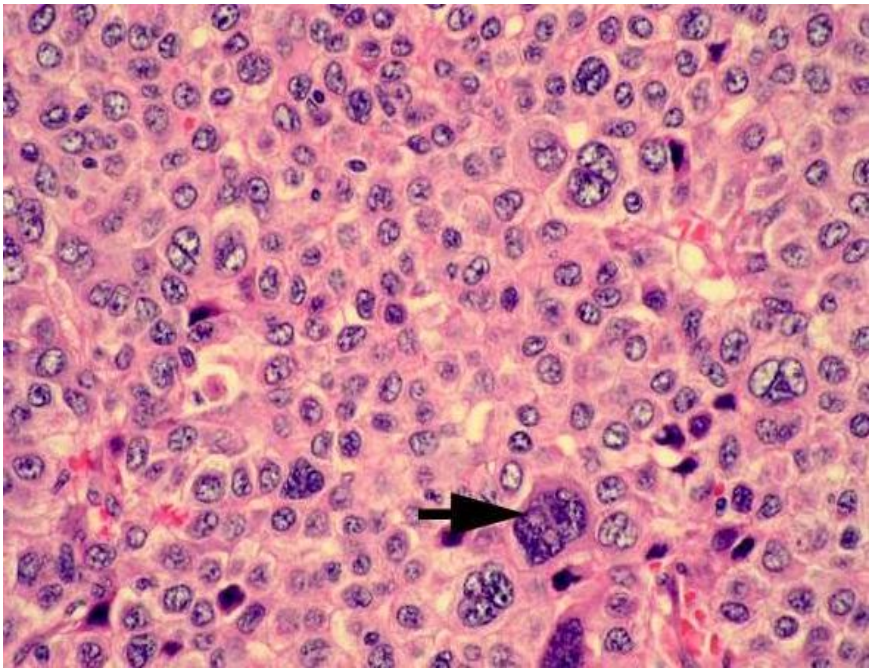

Figure no. 7: Hepatocellular carcinoma, poorly-differentiated, pleomorph/"giant cell" type

(http://www.pathpedia.com/Education/eAtlas/Histopathology/)

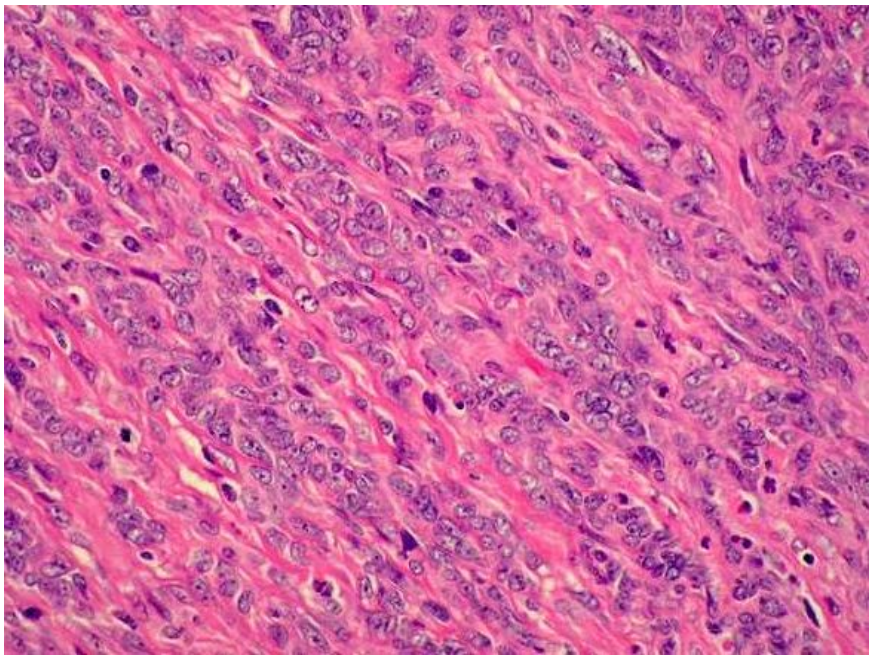

Figure no. 8: Hepatocellular carcinoma - sarcomatoid type/ "spindle cell"

(http://www.pathpedia.com/Education/eAtlas/Histopathology/)

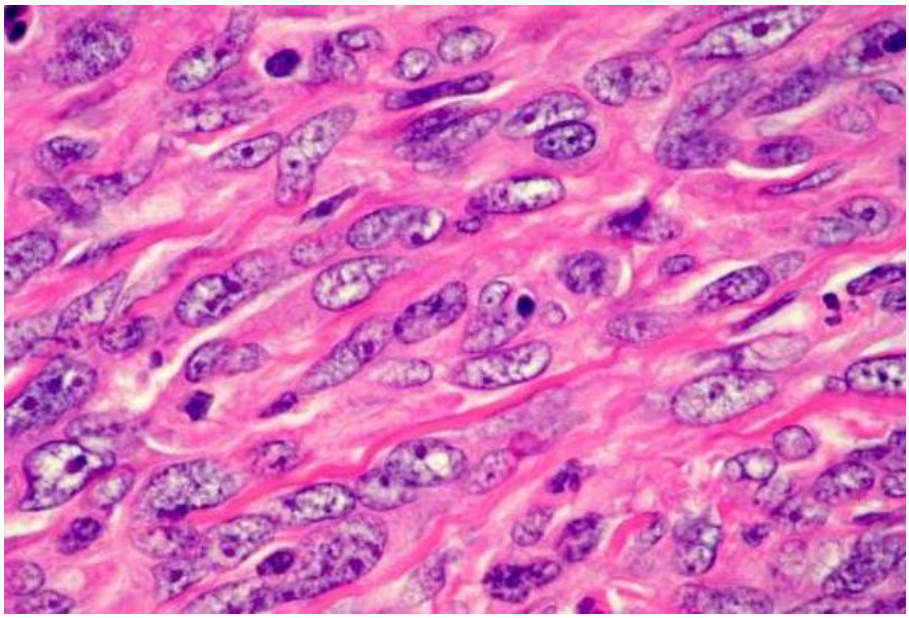

Figure no. 9: Carcinom hepatocelular – fusiform cell type

(http://www.pathpedia.com/Education/eAtlas/Histopathology/)

In the study group there are 3 cases of well-differentiated hepatocellular carcinoma, 17 mildly-differentiated cases and 20 cases of poorly-differentiated HCC.

Of all the cases associated with hepatic cirrhosis , most were poorly-differentiated (Fifure no 10) (11 cases), 7 moderately - differentiated cases and only one case of well-differentiated hepatocellular carcinoma.

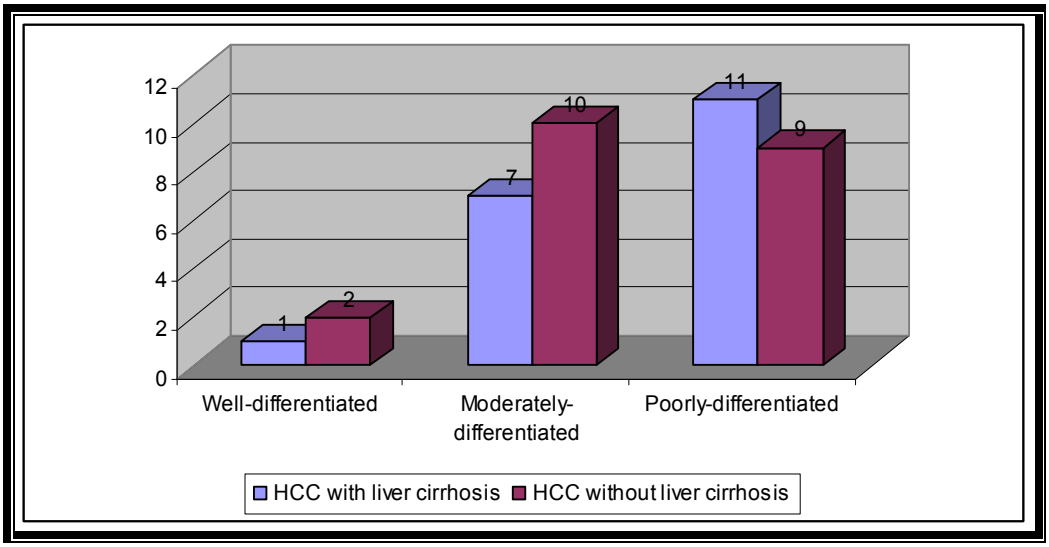

Chart no. 9: Edmondson Steiner stages (histological grading) according to the association of hepatocellular carcinoma with hepatic cirrhosis

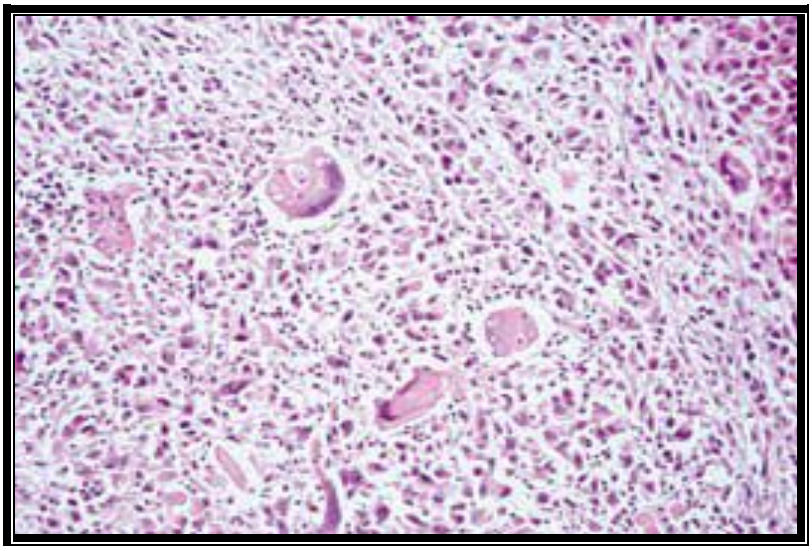

Figure no. 10: Microscopic appearance of a poorly differentiated HCC developed in liver cirrhosis(HE, × 100).

Other histopathological elements present in the study were : vascular invasion (4 cases), pancreatic invasion (1 case), metastases (8 cases) , peliosis (6 cases), as well as atypical mitosis or nonspecific granulomas.

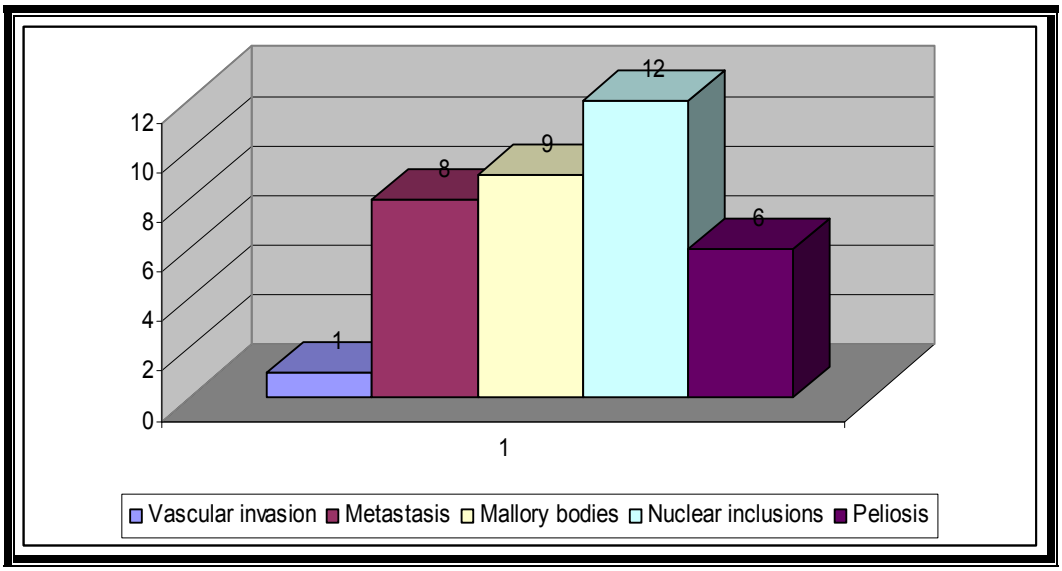

Chart no. 10: Other histopathological elements present in the study

The gall bladder / bile was observed in 11 cases, hyaline eyes in ten cases, nuclear inclusions in 12 cases, Malory bodies in 9 cases, the last two being generaly associated with HCC developed on a cirrhotic liver with tumoral necrosis. (Chart no. 10).

Other special histopathological traits recorded in the study were the presence of glycogen (evidenced by PAS reaction), steatosis , fibrosis (schiros type), the presence of mucus (1 case) and gigantic cells (pleomorph types)

It can be noticed that the immunohistochemical expression of Ki-67 was negative in 22.5% of the 40 cases and Ki-67 positive expression "+", "++", "+++" was found in 11, 7 and 13 cases of hepatocellular carcinoma respectively (Figures no 11,12) .

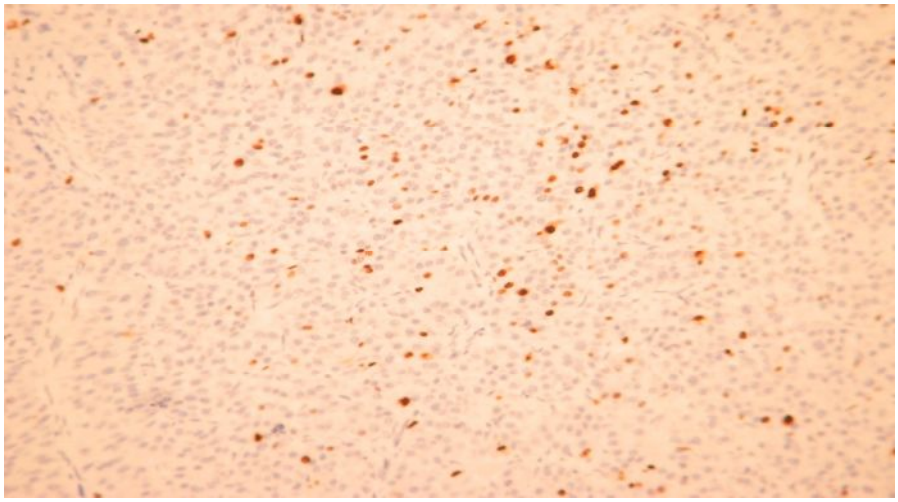

Figure no. 11: Isolated immunoreactivity for Ki-67 in a mildly-differentiated hepatocarcinoma, 200x (Prosonohep 6216)

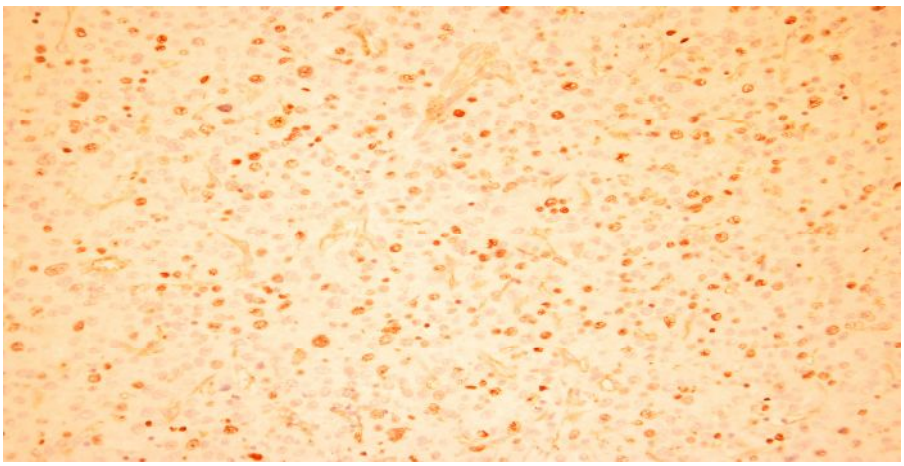

Figure no. 12: Intense Immunoreactivity for Ki-67 proliferation marker (>70% of tumoral cells), 200x (Prosonohep 6216)

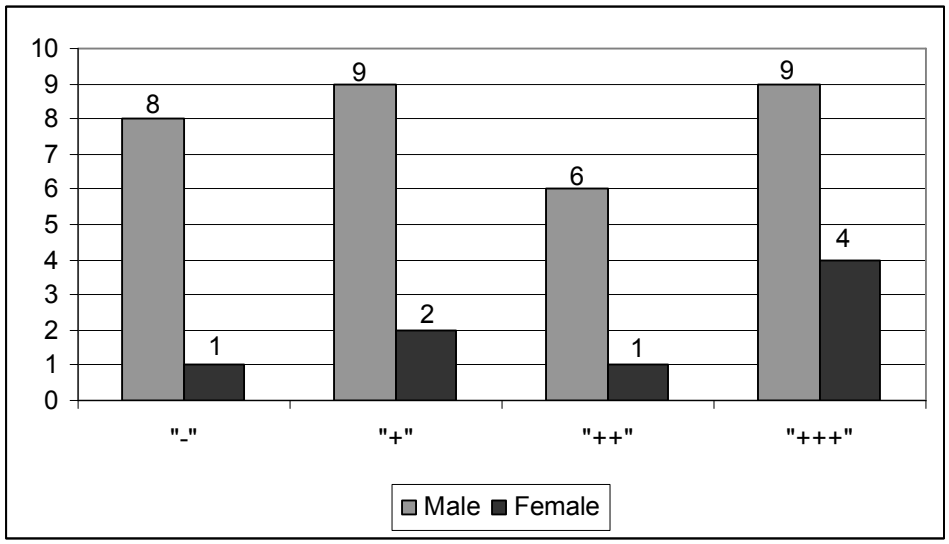

Chart no.11: Immunoexpression of Ki-67 related with sex distribution

For the female (Chart no. 11), immunoexpression predominant were intense positive in 50% of cases, followed by "+" in 25% of cases and 12,5% for "++" and negative. Regarding the distribution in males, we find 9 cases in which the expression Ki-67 was "+", 9 cases with "+ +", 6 cases with "+ ++" and 8 cases without imunoexpression.

To analyze the association level between the Ki-67 values and the age of the people diagnosed with HCC, the Pearson correlation (table 3) factor was used. The results represent a positive correlation, insignificant for the two characteristics (r=0.109, p_v = 0.504, bilateral).

Table no 3: Pearson correlation between Ki-67 values and age of the people diagnosed with HCC

Correlations age - Ki-67	
Pearson Correlation (r)	0.109
p value (p_v)	0.504
N	40

According to tumor size, the 40 cases were divided into two groups, i.e., < 5 cm (n =18) and ≥ 5 cm (n = 22) (Chart no 12).

Among the 18 HCCs measuring less 5 cm, 6 case (33,3%) were "+++", 3 cases (16,6%) were "++" , 4 case (22,2%) were "+" and 5 cases were "-".

Among the 22 HCCs larger than 5 cm in diameter, 7 cases were "+++", 7 cases were "+", 4 cases were "++" and 4 cases were "-"

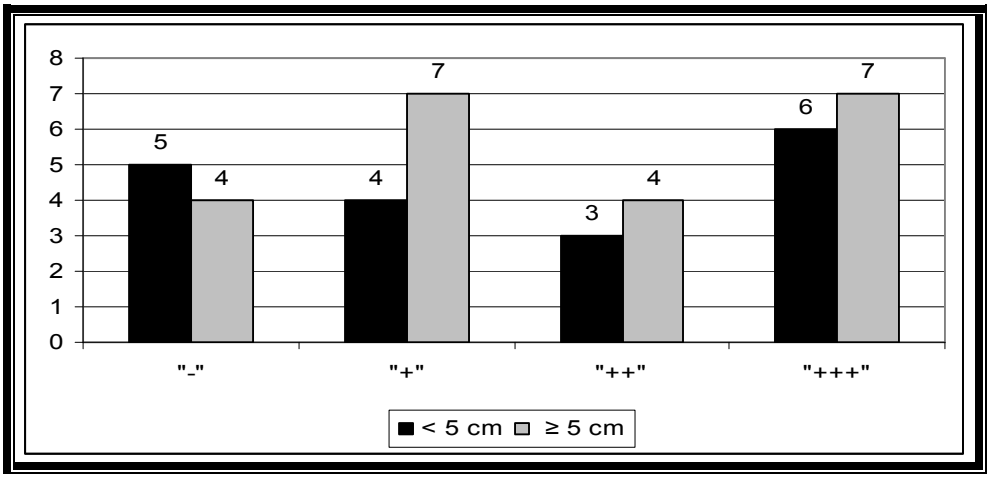

Chart no 12: Imunoexpression of Ki-67 related with tumor size distribution

Table no. 4: Pearson correlation between Ki-67 values and tumor size of the people diagnosed with HCC

Correlations Tumor size – Ki-67	
Pearson Correlation (r)	0.035
p value (p_v)	0.832
N	40

By applying the Pearson correlation test (Table 4) no statistically significant association between the Ki-67 values and dimension of tumors (r = 0.035, p_v=0.832) can be made.

In regard to the relationship between Ki-67 expression and histological grade (Chart no. 13) of HCCs in the 40 nodules that consisted of cancerous tissues of single histological grade in a single tumor nodule (Table 4), 3 well-differentiated HCCs positive one case for each grade of positive imunoexpresion.

Among the 17 moderately differentiated HCCs, 5 cases (29.4%) were intense positive with "+++", 2 cases (11.7%) were positive "++", 6 cases (35.2%) were positive "+", and 4 cases (23.5%) were negative.

Among the 20 poorly differentiated HCCs 7 cases (35%) were intense positive with"+++", 4 cases (20%) were positive "++", 4 cases (20%) were positive "+", and 5 cases (25%) were negative.

Trabecular pattern represents 75% of the cases (Chart no. 14).

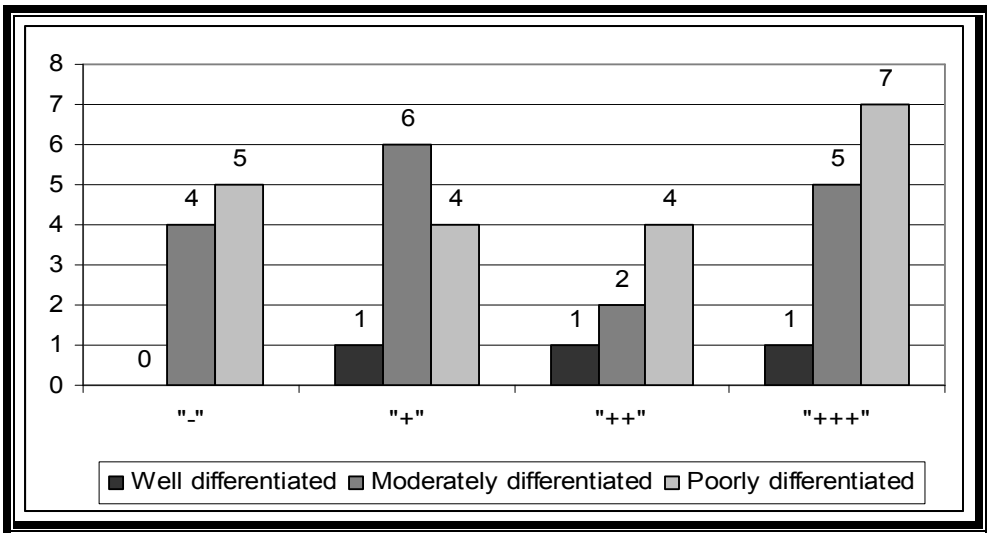

Chart no.13: Edmondson Steiner grading

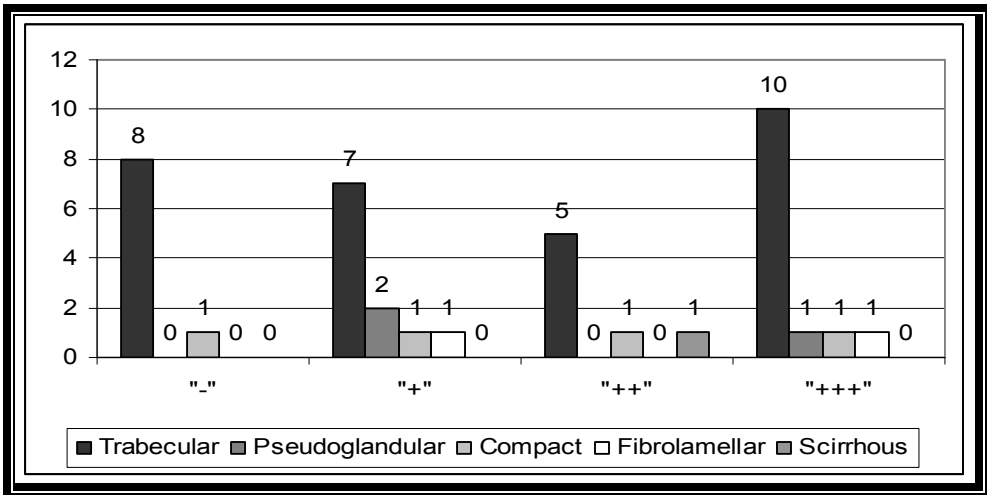

Chart no.14: Expression of Ki 67 in different hepatocellular carcinoma pattern

VEGF-negative expression was found in 10 of 40 HCC patients (25%) and "+", "++", "+++" in 5, 11 and 14 cases of hepatocellular carcinoma respectively.

Capsular infiltration (p = 0.1), vascular invasion (p = 0.2) and metastasis (p = 0.8) were observed more frequently in patients with VEGF-positive expression than in those with VEGF-negative expression.

Table no.5: t-Test in males and females

	Male	Female
Mean	53.90625	20
Variance	983.4425	1435.714
Observations	32	8
Hypothesized Mean Difference	0	
df	10	
t Stat	2.338658	
P(T<=t) one-tail	0.020717	
t Critical one-tail	1.812461	
P(T<=t) two-tail	0.041434	
t Critical two-tail	2.228139	

Statistically, there is a noticeable difference between the mean VEGF of male and female patients. In the male patients, the average value is 53.90% whereas for the female patients the value is 20%. The value obtained for t=2,338, higher that the value for t- table for 10 degrees of latitude which is 2,228. P=0,041. It can be safely stated that the VEGF imunohistochemical expression is significantly statistically influenced by the patients' sex, with higher values in the case of male patients. Results are detailed as follows in table 5.

It can be noticed that the imunohistochemical expression of VEGF was negative in 25% of the 40 cases.

Pareto distribution of VEGF'(chart no 15) imunohistochemical expression points out that for 6 of the 8 women (75%) in the test group, the VEGF imunoexpression was negative. As for the male imunoexpression, predominant were intense positive cases "+++" 40.06%, followed by "++" 32,25%, and "-" 12,5%.

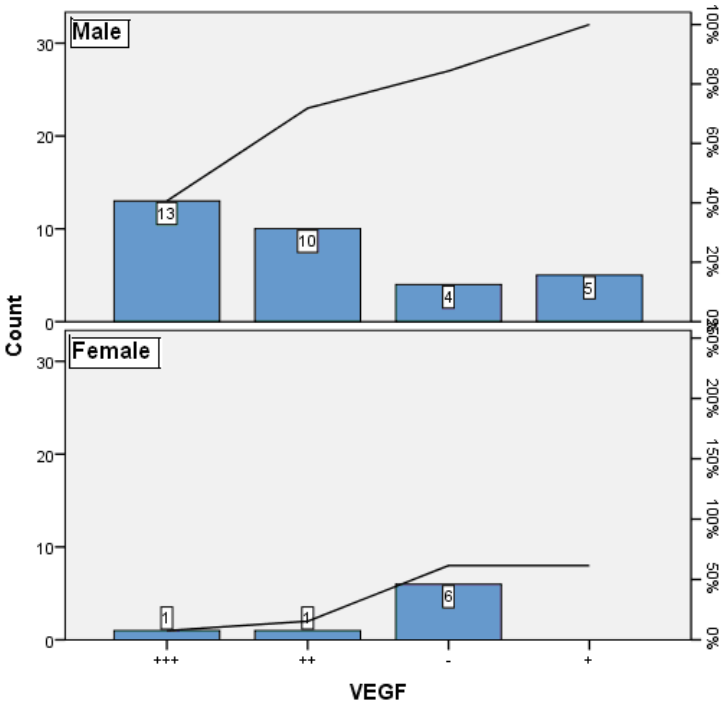

Chart no.15:Pareto analysis of VEGF values according to the sex of the patients

To analyze the association level between the VEGF values and the age of the people diagnosed with HCC, the Pearson correlation (Table 6) factor was used. The average value of VEGF was 47,12%, and the average age was 54,6 . The results represent a negative correlation, insignificant for the two characteristics (r=-0,016, p=0,921, bilateral).

Table no.6:Pearson correlation between VEGF values and age of the people diagnosed with HCC

	Age VEGF
Pearson Correlation	-.016
Sig. (2-tailed)	.921
N	40

Chart no. 16 displays a scatterplot graphic where VEGF values are represented according to age. It is noticeable that the trendline is a horizontal one therefore the VEGF values are not in any manner influenced by age.

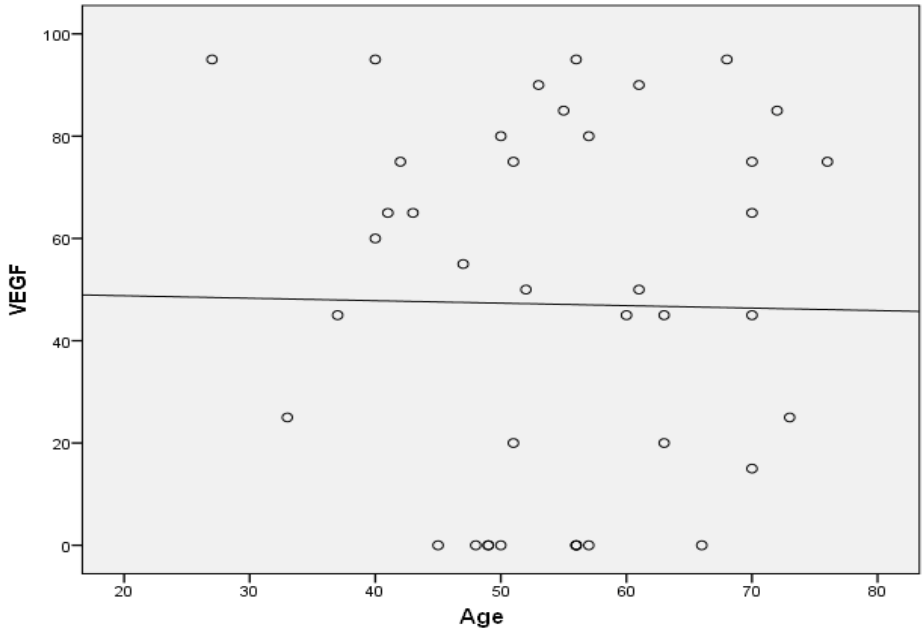

Chart no.16: VEGF values according to age

According to tumor size, the 40 cases were divided into three groups, i.e., ≤1.5 cm (n =4), >1.5 and ≤3.0 cm (n =6), and >3.0 cm (n = 30).

Among the 4 HCCs measuring 1.5 cm or smaller, 1case (25%) were "+++", 2 cases (50%) were "++" , 1 case (25%) were "+".

Among the 6 HCCs whose diameter was 1.5 to 3.0 cm, 2 cases (33.3%) were "+++", 2 cases (33.3%) were "++" , 1 case (16,6%) were "+" and 1 case (16,6%) were "-"

Among the 30 HCCs larger than 3.0 cm in diameter, 11cases (36,6%) were "+++", 7 cases (23,3%) were "++" , 3 cases (10%) were "+"and 9 cases (30%) were "-".

In regard to the relationship between VEGF expression and tumor size, most of the 30 HCC cases larger than 3.0 cm were either positive "+++"or"++" . In addition, 9 of the 10 negative cases were relatively large, and their diameter ranged between 3,5 and 8 cm.

By applying the Pearson correlation test (Table 7) no statistically significant association between the VEGF values and dimension of tumors (p=0.398) can be made.

Table no.7: Pearson correlation test between VEGF values and dimension of tumors

VEGF Tumor size	
Pearson Correlation	.137
Sig. (2-tailed)	.398
N	40

Although the graphic 17 shows a slightly increasing trendline (VEGF expression positively increased with the increase in tumor diameter), the determinant coefficient r2 is of a very small value (0.019) therefore the dimension of the tumor has only a slight effect on the VEGF imunoexpression.

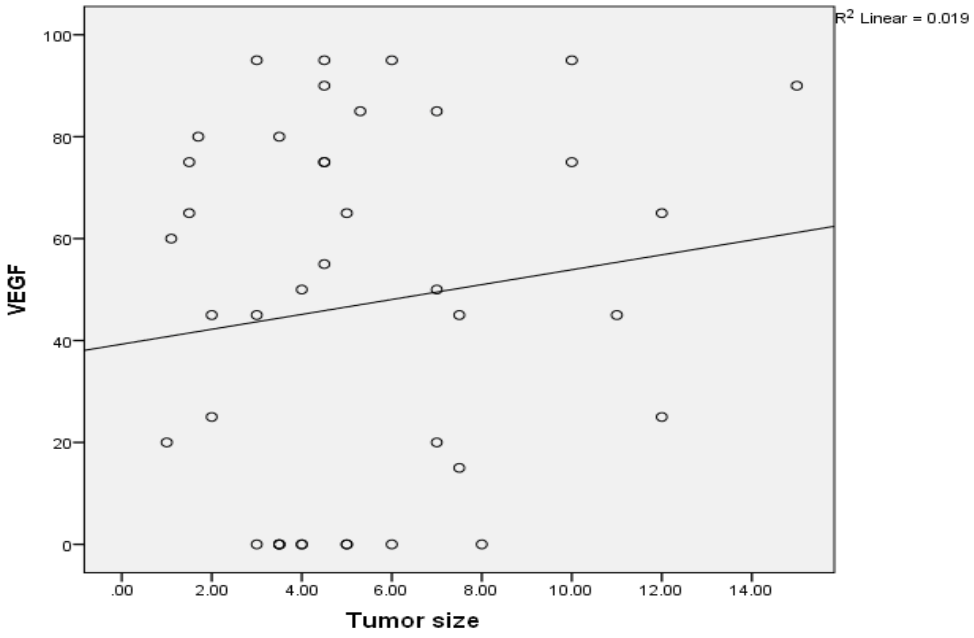

Chart no.17: VEGF values according to tumor size

In regard to the relationship between VEGF expression and histological grade of HCCs in the 40 nodules that consisted of cancerous tissues of single histological grade in a single tumor nodule (Table no.8), 3 well-differentiated HCCs were either intense positive (n=2 [66,6%]) or negative (n=1 [33,3%]).

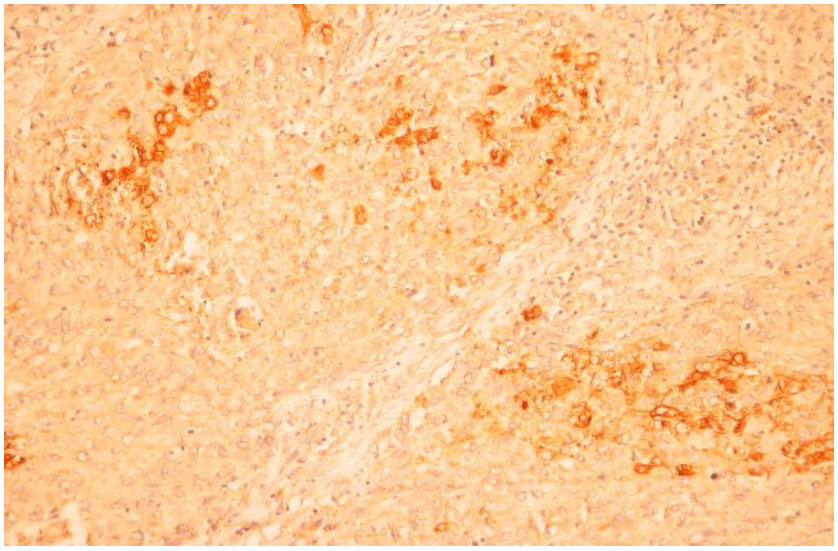

Figure no. 13: Cellular Immunorectivity focal present for VEGF in tumoral tissue , 200x (Prosonohep 6216)

Among the 17 moderately differentiated HCCs, 6 cases (35,5%) were intense positive with"+++", 2 cases (11.7%) were positive "++",4 cases (33.4%) were positive "+", and 5 cases (29.4%) were negative.

Among the 20 poorly differentiated HCCs 5 cases (25%) were intense positive with"+++", 7 cases (35%) were positive "++", 3 cases (15%) were positive "+", and 4 cases (20%) were negative.

Table no. 8: Analysis of mean VEGF according to histological grading

Histologic Grading Edmondson-Steiner	Mean	N	Std. Deviation
Well differentiated	56.67	3	50.083
Moderately differentiated	45.88	17	37.426
Poor differentiated	46.75	20	32.577

Higher VEGF means can be noted in the case of an well differentiated (56,67%) histological grading. Lower means are noticeable for moderately differentiated tumors (45,88%).

In order to determine whether the observed differences were statistically significant the statistic One Way Anova test was used (Table 9). The value received for p is 0.889 therefore the null hypothesis cannot be rejected, concluding that the histological grading does not influence the value of VEGF expression.

Table no.9: One Way Anova test for histological grading

One Way Anova test	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	302.194	2	151.097	.117	.889
Within Groups	47592.181	37	1286.275		
Total	47894.375	39			

The mean for trabecular pattern (which represents 75% of the cases) is 44.5% (Table no.10). The highest VEGF expression is recorded with the Pseudoacinar pattern (81.67%). The lowest mean is recorded in the case of the Compact pattern (25%).

Table no.10: Mean VEGF according to tumoral pattern

Pattern	Mean	N	Std. Deviation
Trabecular	44.50	30	34.625
Pseudoacinar	81.67	3	15.275
Compact	25.00	4	37.859
Fibrollamelar	80.00	2	21.213
Scirrhous	45.00	1	.
Total	47.12	40	35.044

By the use of the Anova test (Table 11) it is proven that the differences observed in the VEGF expression and pattern are not statistically significant.

Table no.11: One Way Anova test for tumor pattern

One Way Anova test	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7910.208	4	1977.552	1.731	.165
Within Groups	39984.167	35	1142.405		
Total	47894.375	39			

In this study the nuclear build-up of p 53 protein was observed in 72,5% of the cases, while in a study done by Moghaddam and collaborators the nuclear build-up of p 53 protein was observed in 24% of the cases (6 out of 25) in variable proportions , and the remaining 76% of the cases had a negative immunoexpression for p 53.

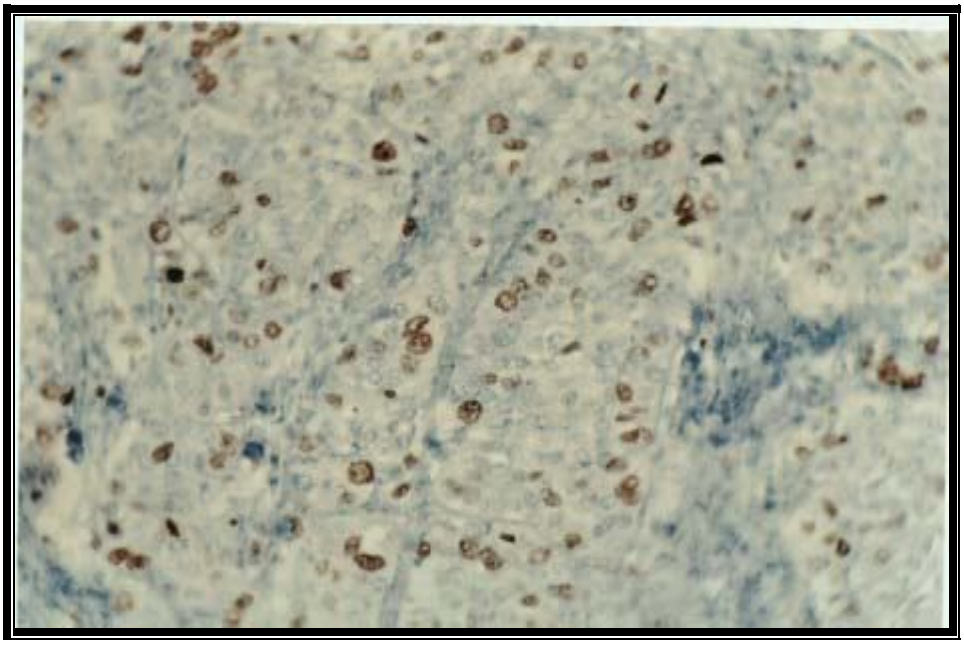

Figure no. 14: Positive immunoreaction to p 53 in CHC (Prosonohep 6216)

This study postulates that the tumors with an increased Ki 67 coefficient and also increased p53 and VEGF values , tend to have a higher risk of reoccurrence. 7 cases of small size tumors and 23 cases of large size tumors presented an increased Ki 67

coefficient, respectively 2 and 13 cases of increased protein p53 values and 1 and 17 for VEGF.

The manifestation of p53 protein in advanced malign lesions indicates that this anomaly may occur in the advanced stages of HCC. There can be noticed a strong connection between the Ki 67 expression and that of p53 protein. All cases positive for p53 had a trabecular or compact structure with a high degree of proliferation.

The immunohistochemical correlations for Ki 67, p53 and VEGF may help when it comes to deciding in favor of a curative resection in HCC.

Some authors reported that the Ki 67 coefficient or the expression of p53 protein may give information about the clinical stages of the disease. Thus the Ki 67 coefficient corresponds to the histological degree of differentiation, being significantly lower in small incapsulated tumors, and higher in large incapsulated tumors.

This dissertation approaches new morphopathological data, that may enlarge basic information about hepatocarcinoma , through both conventional and modern methods associated with clinical observation.

Although it is difficult to foretell the changes in the field of epidemiology , some experts suggested that the global incidence of HCC will continue to rise in the next few years reaching a potential plateau in 2015-2020.

This immunohistochemical study proves the value of molecular markers in a positive differential diagnosis and in the monitoring of hepatic tumors. It also constitutes a valid argument for the introduction of routine immunohistochemical examinations in the diagnosis of patients with hepatic tumors, in the detection and screening of the early stages of different degrees of malignity.

The study was limited by the material, whose structure cannot be made up of homogeneous groups that could permit a more exact comparative analysis. Other limitative factors were the quality of the archived materials used for the retrospective part of the study as well as the panel of markers available.

The results of IHC marking present many variables, among which the more important can be considered : the site of prelevation, and respectively of the marking itself, and also the subjectivity of interpreting the correlation due to the invariable imunocoloration intensity.

These result give new perspectives on the clinical diagnosis, the stage and prognosis of hepatic cancer, and also state the need for an extensive study on the quantification of tumoral hepatic markers in larger clinical trials.

The future perspectives of IHC attempt to identify newer more specific markers for diagnosis and post-therapeutic monitoring and also to identify new molecular targets useful in future therapeutic methods.