Section: Pathology

Original Research Article

"IMMUNOHISTOCHEMICAL EXPRESSION OF HSP70 IN HEPATOCELLULAR CARCINOMA- A STUDY IN A TERTIARY CARE CENTER"

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is one of the most common and aggressive malignancies due to its higher rate of metastases. HSP70 inhibits key effectors of apoptotic machinery which participate in oncogenesis. HSP70 positivity confers resistance to radiotherapy and high risk for metastasis. This study aimed to evaluate the expression of HSP70 in different grades of HCC.

Materials and Methods: This prospective study was conducted in the Department of Pathology of a tertiary care hospital over a period of 2 years. Hematoxylin and eosin–stained sections from 60 cases of HCC were evaluated and graded microscopically. Immunohistochemical expression of HSP70 was evaluated in different grades of hepatocellular carcinoma.

Results: There were 60 patients in our study, eight of whom were under 40 years, 21 of whom were between 41 and 60 years, and 31 of whom were above 60 years. Only six were female patients and rest were male patients. On studying HSP70 expression associated with liver changes, 51.6% shows expression in association with cirrhosis, 16.1% in association with HbsAg positivity, 6.5% in portal vein invasion and 25.8% in association with steatosis and periportal fibrosis which is statistically significant. Among HSP70 positive cases, 90.3% cases were well differentiated HCC and 9.7% cases were moderately differentiated HCC.

Conclusion: HSP70 expression was higher in well differentiated HCC associated with cirrhosis. HSP70 induces treatment resistance and poor prognosis. This study highlights the significance of immunohistochemical evaluation of HSP70 in HCC patients for risk stratification and targeted therapy.

Keywords: Hepatocellular carcinoma, Heat shock protein, HSP70, prognosis.

INTRODUCTION

Hepatocellular carcinoma is the most common cause of cancer related death with over one million cases globally. Hepatocellular carcinoma is more commonly associated with chronic liver disease. The most common risk factors globally were hepatitis B (HBV), hepatitis C (HCV) and alcohol exposure.[1] The other risk factors include NASH, metabolic syndrome, aflatoxin B exposure, hereditary hemochromatosis, alpha 1 anti-trypsin deficiency and beta catenin activated hepatocellular adenoma.[1,2]

The high mortality ratio in HCC and its poor prognosis due to therapy resistance require extensive analysis of prognostic markers. Heat shock proteins (HSPs) were widely distributed, highly conserved molecules that provide a vital defence mechanism to shield cells from a variety of environmental disturbances.[3]

Different types of heat shock proteins classified based on the molecular weight include HSP100, HSP90, HSP70, HSP60, HSP40 and HSP27.

HSP70 and HSP 27 block the apoptosis and principally participate in oncogenesis.[4]

Presently, 14 HSP70s were identified. High expression of HSP70 induced resistance to radiotherapy through different mechanisms. Thus, HSP70 inhibitors, such as triptolide can be used as radiosensitizers in radiation therapy.[5]

Combination of photothermal therapy (PTT), photodynamic therapy (PDT), or radiofrequency ablation (RFA) with HSP70 inhibitors, which has already shown improved efficiency than PTT, PDT, or RFA used alone in liver cancer, offers successful results in the management of HCC.[5,6] Moreover, HSP70 expression was also found to be higher in hepatitis B virus (HBV)-related early stage HCC patients, and was generally correlated with early recurrence and poor prognosis.[7]

Aim: The study aimed to evaluate the expression of HSP70 in hepatocellular carcinoma and to correlate its expression in different grades of hepatocellular carcinoma.

MATERIALS AND METHODS

This prospective study was conducted in 60 cases of hepatocellular carcinoma in biopsy and resection specimens received at the Department of Pathology in a tertiary care center over a period of 2 years. Ethical approval was obtained from the Institutional Ethics Committee.

Inclusion & Exclusion criteria: Primary hepatocellular carcinoma in patients of all age groups was included in the study. Metastatic tumors and benign lesions of liver were excluded from the study.

Methodology: Formalin fixed and paraffin embedded tissue sections were examined microscopically using Hematoxylin and Eosin stain. WHO 2019 criteria for histological grading of hepatocellular carcinoma was employed.[8] Immunohistochemical expression of HSP70 was evaluated in tumour cells in different grades of hepatocellular carcinoma. IHC scoring criteria for HSP70 used was i) Negative (0) - < 10% tumour cells, ii) 1+ - 10-50% tumour cells. iii) 2+ - > 50% tumour cells. HSP70 showed nuclear and fine granular cytoplasmic positivity in tumour cells. Statistical analysis was done using SPSS VERSION 29 and the results were evaluated. Descriptive statistics, like frequency and percentage analysis, were utilized to describe the data for categorical variables, while the mean and standard deviation were utilized for continuous variables. Qualitative categorical data were assessed for significance using the Chi-Square test, and if the expected cell frequency in 2x2 tables was less than 5, Fisher's Exact was used. p value <0.05 was considered statistically significant.

RESULTS

We analysed 22% biopsy proven cases and 78% cases of resection specimens of Hepatocellular carcinoma with respect to age, gender, segment and lobe of liver involved, associated liver disease, histologic grade and immunohistochemical expression of HSP70.

Analysis of the age distribution showed 1.7% of cases were under 20 years and 45 % of cases were between 61 and 70 years. The minimum age recorded was 17 years, the maximum age was 75 years and the mean age ± standard deviation was 56.6±13.4 years [Chart 1].

According to the gender distribution, 90.0% of the population was male and 10.0% was female [Chart 2].

In our study, both lobes of liver were involved in 3.3%, left lobe involvement in 33.3%, right lobe involvement in 60.0% and caudate lobe was involved in 3.3% of cases.

Segments of 2,3 were the most commonly involved (15.0%) whereas 6,8, 4A, 4B, 4B,5, 4A,2,5, 4 were the least affected (1.7%).

In our study, cirrhosis was seen in 36.7%, portal vein invasion in 5.0%, HbsAg positivity in 15.0% and steatosis in 43.3% of cases in the background liver [Table 1]. In microscopic evaluation, HCC was moderately differentiated in 20.0% and well differentiated in 80.0% of cases.

In our study, HSP70 was positive in 51.7% and negative in 48.3% of HCC [Table 2]. Among HSP70 positive cases, 90.3% cases were well differentiated HCC and only 9.7% cases were moderately differentiated HCC.

According to Pearson's Chi-Square test, the comparison of liver lobe with HCC and HSP70 expression showed $x^2=3.18$, $p=0.365>0.05$, indicating no statistical significance. Liver segment distribution and HSP70 expression were compared using Pearson's Chi-Square test, and the results showed no statistical significance ($x^2=22.636$, $p=0.161>0.05$).

According to Pearson's Chi-Square test, the pathologic changes in liver and HSP70 expression were compared. The results showed a statistically significant correlation with $x^2=8.779$ and $p=0.032<0.05$ [Table 3].

Fisher's exact test results for the grade of HCC and HSP70 comparison were $x^2=4.271$, $p=0.054>0.05$, indicating no statistical significance [Table 4].

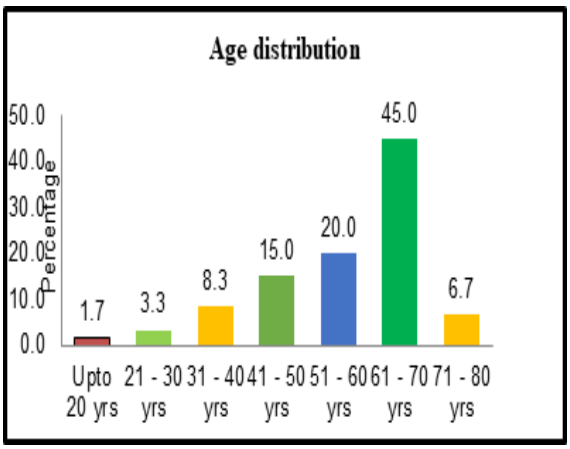

Chart 1- Age Distribution

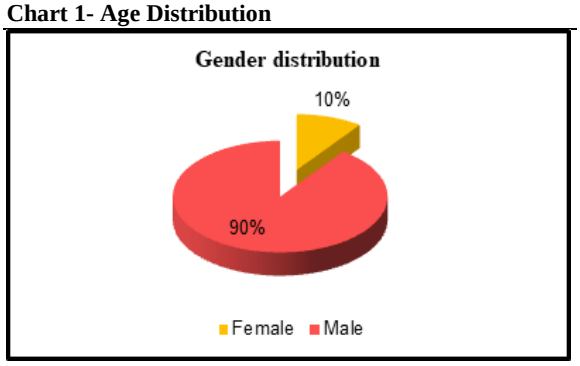

Chart 2- Gender distribution

Figure 1: Gross Specimen Showing Liver with HCC

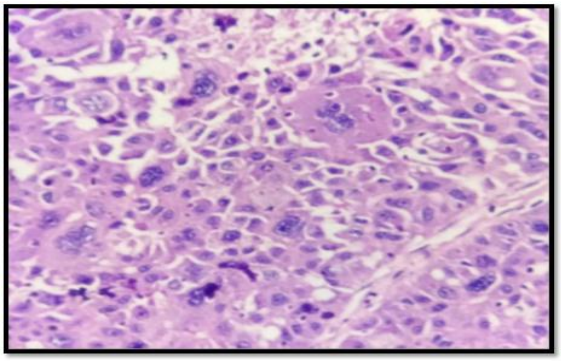

Figure 2: Moderately Differentiated HCC

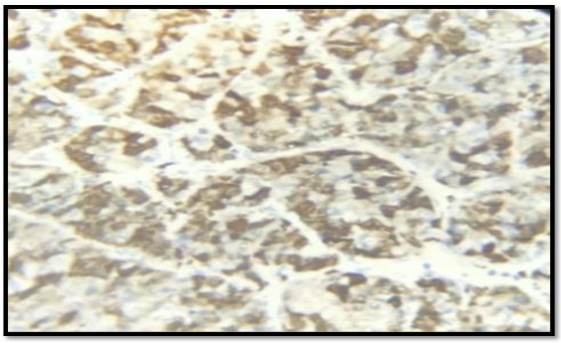

Figure 3: HSP70 Expression in HCC.

Table 1: Liver changes distribution in HCC

Liver changes	Frequency	Percent
Cirrhosis	22	36.7
Portal vein invasion	3	5.0
HbSAg positive	9	15.0
Steatosis	26	43.3
Total	60	100.0

Table 2: HSP70 expression in HCC

HSP70 expression	Frequency	Percent
Positive	31	51.7
Negative	29	48.3
Total	60	100.0

Table 3: Comparison of Liver changes and HSP70 by Pearson's Chi-Square test

			HSP70		Total	□ 2 - value	p-value
			Positive	Negative			
Liver changes	Cirrhosis	Count	16	6	22	8.779	0.032 *
		%	51.6%	20.7%	36.7%		
	Portal vein invasion	Count	2	1	3		
		%	6.5%	3.4%	5.0%		

	HbsAg positive	Count	5	4	9		
		%	16.1%	13.8%	15.0%		
	Steatosis	Count	8	18	26		
		%	25.8%	62.1%	43.3%		
Total		Count	31	29	60		
		%	100.0%	100.0%	100.0%		
* Statistical Significance at p<0.05 level							

Table 4: Comparison of Grade and HSP70 by Fisher's exact test

			HSP70		Total	□ 2 - value	p-value
			Positive	Negative			
Grade	Moderately differentiated	Count	3	9	12	4.271	0.054 #
		%	9.7%	31.0%	20.0%		
	Well differentiated	Count	28	20	48		
		%	90.3%	69.0%	80.0%		
Total		Count	31	29	60		
		%	100.0%	100.0%	100.0%		
# No Statistical Significance at p>0.05 level							

DISCUSSION

Prognostic markers are widely used in Hepatocellular carcinoma due to its high mortality ratio and have high clinical value as determinant factors for effective treatment. [9] AFP is by far the most widely and extensively studied prognostic marker in HCC. However, the controversy has been raised regarding which specific cut-off for recurrence or survival to be selected.[10] Moreover, AFP negative tumours account for 30-40% of pathologically diagnosed HCC patients, which significantly hamper the application of AFP in HCC prognosis.[10] Therefore, new prognostic markers were needed. Many oncoproteins require high levels of HSPs to maintain their function, and increased levels of HSPs have been found to be significantly higher in HCC.[11] Its expression is associated with migration and invasion of tumour cells.[12] Monitoring the expression level of HSP70 is a potential approach to predict disease progression and guide in deciding the next treatment strategy.[13]

On studying HSP70 expression, male patients exhibited higher scores. Resection specimen cases showed higher scores. Patients in the age group of 40-70 years exhibited higher expression of HSP70. Patients with right lobe HCC had higher HSP70 expression. HSP70 expression levels in various tumour grades were examined. 90.3% cases of well differentiated HCC showed expression of HSP70 in tumour cells. Histological grade shows no statistical significance with HSP70. On studying HSP70 expression in cases with liver pathologic changes, 51.6% shows expression in association with cirrhosis, 16.1% shows expression in association with HbsAg positivity, 6.5% shows expression in portal vein invasion and 25.8% shows expression in association with steatosis and periportal fibrosis. Association between HSP70 expression and pathologic changes in liver is statistically significant at p value less than 0.05.

Comparing the study conducted by Inje University Seoul Paik hospital, Korea with 71 patients, 65 patients were male patients, which is comparable to our study. The expression of HSP70 expression is seen in 40 cases. HSP70 shows significant association with HBV associated HCC (52 cases) and cirrhosis in HCC.[14] This was similar to our study report.

Comparing the University of California study, which assessed the effectiveness of using HSP70, glutamine synthase (GS), and glypican-3 together in 107 hepatocellular lesions. Nine out of thirteen patients with extremely well-differentiated HCC, thirteen out of twenty instances of well-differentiated HCC in non-cirrhotic liver, and sixteen out of twenty-three cases of well-differentiated HCC in cirrhotic liver had higher expression of HSP70.[15] Also, this study explained the significance of HSP70 in diagnosing HCC and to differentiate from other benign lesions (dysplastic nodules) which will be negative for HSP70[15,16]. Our study also demonstrated higher expression of HSP70 in well differentiated HCC and showed significant association with clinicopathological changes in liver.

On comparing study by University of Milan School of Medicine, 53 HCC were analysed. HSP70 immunoreactivity was seen in the vast majority of HCCs (39 of 53 cases, 73.58%) out of which, 25 cases include early HCC and grade 1 HCC.[17] HSP70 immunoreactivity is higher in well differentiated tumours. This is comparable with our study.

CONCLUSION

Hepatocellular carcinoma continues to rank among the world's leading causes of cancer death. Its significant risk of metastasis and treatment resistance were the causes of its poor prognosis. Screening for molecular markers of HCC may improve prognosis and lower the mortality. HSP70 aids in the preservation of protein homeostasis in cells. HSP70, being antiapoptotic, encourages the growth of tumour cells in patients who express them. Vascular invasion and higher chance of metastasis were associated with elevated HSP

expression leading to decline in overall survival. Early or well differentiated hepatocellular carcinoma exhibits HSP70 expression. HSP70 inhibitors reduce tumour invasion, migration, and proliferation while also making tumour cells more susceptible to chemotherapy and radiation. HSP70 is therefore a significant prognostic indicator in patients with hepatocellular carcinoma and can be used as a guide for patient risk stratification and targeted therapy.

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