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Research Article

Analysis of Cytogenetic Alterations in Patients affected by Hepatocellular Carcinoma in Tamilnadu Population

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Abstract

The Hepatocellular carcinoma is one of the most lethal cancers from all of the gastrointestinal tract cancer. In HCC the involvement of chromosome 1 and 3 mainly in the region of 1p36.11 particularly in the short arm have a predominant alteration. The objective of this study is to find the region of the altered chromosome and to find the novel biomarker for cytogenetic analysis of HCC cancer in and around the Tamilnadu state. In the present study, totally 10 Peripheral blood samples from HCC cancer patients were obtained. The chromosomal banding is performed by the Trypsin- Giemsa banding technique and the microscope slides were evaluated under the microscope by 100x magnification and statistical data is processed by ANOVA. Further in future we can go for the FISH technique, by specifically we can use the particular probes for the identification of the altered region in chromosome of HCC patients.

Keywords: Hepatocellular Carcinoma (HCC), Chromosomal aberrations (CAs), deletion, Tamil Nadu

INTRODUCTION

Hepatocellular carcinoma (HCC) is the main form of liver cancer. Liver cancer, also known as hepatic cancer, is a cancer that originates in the liver[1] . HCC have different growth patterns, some begin as a single tumor that grows larger and late in the disease it spread to other parts of the liver[2]. The pathological aspects of HCC includes cirrhosis and fibrosis, conditions resulting from chronic inflammation due to chronic HBV and HCV infection or to alcohol intoxication can lead to cancer through several mechanisms and some of which are linked to chronic inflammation such as hepatocyte necrosis, liver regeneration and fibrosis and others that are specific of each virus[3]. The distribution of HCC varies according to geographic location. The disease burden is highest in areas with endemic HBV infection (where HBsAg prevalence is 8% or more), such as in sub-Saharan Africa and Eastern Asia, with incidence rates of over 20 per 100,000 individuals. Mediterranean countries such as Italy, Spain, and Greece have intermediate incidence rates of 10-20 per 100,000 individuals, while North and South America have a relatively low incidence (< 5 per 100,000 individuals)[4]. HCC may connect with the chromosome 1 and 3 mainly in the region of 1p36.11 particularly in the short arm[5].the objec-

tive of the present study is to find the novel chromosomal marker in the Hepatocellular carcinoma patients in Tamilnadu.

Materials and Methods

Subject Recruitment

In the present study, totally 10 Peripheral blood samples from HCC cancer patients were obtained. Peripheral blood of Experimental were collected using a heparinised syringe The study was approved by the Ethics Committee of the participating hospital and the patients were informed and their consent form to be included in the study. The samples were recruited from hospitals in Tamilnadu. The subjects were selected based on their age of above 28 to 68 years.

Peripheral blood Culture and Cytogenetic analysis:

A 2.0ml of venous blood from the experimental subject was drawn into a sterile heparinised syringe and 0.5ml of the blood (about 30 drops) was inoculated under aseptic conditions into a culture vial containing 5.0ml of culture medium, 1.0ml 0f AB serum and 0.2ml of PHA. The cultures were incubated at 37℃ for a period of 72 hours and were shaken periodically twice a day in order to facilitate proper mixing of the medium and cells in the culture. The dividing cells were arrested at the metaphase stage by adding 0.05ml of colchicine solution (0.01%) at 30 minutes before harvesting the culture. The contents of the vials were centrifuged at 1000rpm for 20 minutes at the end of colchicine treatment. The supernatant was discarded and 6ml of pre-warmed hypotonic solution (0.75M KCl) was added to the test tube after disturbing the cell button. The contents of the test tubes were incubated for 7 minutes. After incubation, 1ml of freshly prepared fixative [Methanol and Glacial Acetic acid (3:1v/v)] was added and centrifuged at 1000 rpm for 10 minutes.

New slides were allowed to remain in concentrated nitric acid overnight. These slides were then kept in a horizontal Coplin jar under running tap water for 2-3hrs. The slides were finally rinsed in double distilled water and stored in 70% ethyl alcohol. Before using the slides for spreading they were wiped and dried. Chromosomal preparations obtained were processed and stained with Giemsa to obtain G-bands. The slides were analysed under the microscope [6].

Statistical Analysis:

The statistical significance of the difference in the frequencies of p53 genotypes was calculated by t test. Following, a descriptive analysis, Pearson correlation coefficients were separately calculated between age dependent chromosomal alterations. All the analysis was performed with IBM-SPSS software 20.0 version. Odds ratios (OR) and confidence intervals were calculated to estimate the strength of the association of polymorphism genotype alleles in patients. Mean and standard deviation were calculated to assess the different between the patients and controls and the level of significance was calculated by ANOVA.

Results

A total of 10 subjects which include peripheral blood (n=10; that comprises of males and female) samples respectively (Table 1, 2 & 3). Equal number of controls was recruited. Among the subjects recruited, location site and patient's history were taken in to account. The Mean age of the total subject (peripheral blood samples) study population in which the mean±SD of the total population is 47.7±13.0.

Statistical data of chromosomal aberration in blood sample which resulted with chromosomal type aberration (CSA) as 1±0.774 and chromatid type aberration (CTA) was found out to be as 0.7±0.64. The total chromosomal aberration (TCA) resulted with to be as 0.8±0.874.the p value of the CSA will be 0.825 and CTA was found to be 0.409 and TCA is 1 and correspondingly the confidence interval for CSA is between 0.0073 to 49.45.CTA evaluate as 0.113 to 220.63 and TCA will be 0.00108 to 92.4776.

Discussion

HCC is the main form of liver cancer. Liver cancer, also known as hepatocellular cancer, is a cancer that originates from the liver. HCC is one of the ten most commonly occurring solid cancers worldwide and is the second cause of death from malignancy[7]. The most recent data indicate that its incidence is still increasing in many countries whereas the most effective way of reducing mortality due to HCC is prevention.

Table: 1.Total Subject of study Population

Sl. No	Sample Type	No. Of Cases	Patients Age		Total	Mean± SD
1	Peripheral blood	Male and Female (n=10)	P1	29	47.7	47.7 ± 13.0
			P2	33		
			P3	35		
			P4	40		
			P5	46		
			P6	48		
			P7	53		
			P8	59		
			P9	65		
			P10	69		

Table: 2. Statistical of CSA, CTA and TCA of Hepatocellular Patients

Sl. No	Case	Group	Total	Patients Age	CSA	CTA	TCA	CSA Total Age Mean ± SD	CTA Total Age Mean ± SD	TCA Total Age Mean ± SD
1	Hepato Cellular Carcinoma Patients	n=10	47.7	29	1	2	0	1±0.774	0.7±0.64	0.8±0.774
				33	2	1	0			
				35	1	0	1			
				40	0	0	2			
				46	1	1	2			
				48	1	1	0			
				3	0	1	2			
				59	2	0	1			
				65	0	0	1			
				69	2	1	1			

Table: 3. Odd Ratio of CSA, CTA and TCA of Hepatocellular Patients

Cancer Type	Sample Type	Cytogenetic Parameter	Odd Ratio	P Value	95% CI
Hepatocellular Carcinoma	Peripheral blood	CSA	0.6	0.8205	0.0073 to 49.45 Z=0.227
		CTA	5	0.4049	0.1133 to 220.63 Z=0.833
		TCA	1	1	0.00108 to 92.4276 Z=0.00

Gerald States that malignant Carcinoma of the gastrointestinal parts have approximately 25%-28% overall cancer of gastrointestinal tracts[8] important sites being the Liver, Pancreas, large intestine etc. These kinds of malignancy can be analyzed by chromosomal bandings. These alterations collaboratively help to find out the prefect cytogenetic marker for further cytogenetic downstream process such as FISH Technique[9]. The Cytogenetic Location of 17p13.1, which is the short (p) arm of chromosome 17 at position 13.1. Molecular Location: base pairs 7,668,402 to 7,687,550 on chromosome 17 which is main key chromosome for the TP53 gene mutation in HCC Carcinoma[10] and also the Cytogenetic Location of 1p36.11 which is the short (p) arm of chromosome 1 at position 36.11.

Molecular Location: base pairs 26,696,031 to 26,782,110 on chromosome 1 is also exhibits the most commonly altered genes in the liver cancer genome include p53, CTNNB1, ARID1A, MTDH, AXIN1, CDKN2A.

Conclusion

Discovering the altered chromosomal regions may harbor the tumor suppressor genes or oncogenes that are involved in the multistep process of carcinogenesis or disease pathology. These data provide the superficial understanding of the chromosomal region of the patients affected from the Cancer. Further we will go for the downstream process such as FISH and sequencing technique these technologies may have exact detection of cytogenetic and molecular alteration in Hepatocellular carcinoma.

Conflict of Interest

The authors declare that they have no conflict of interest.

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