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Mutagenic Potential of Bile Specimen From Carcinoma Gall Bladder

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ABSTRACT

BACKGROUND: Gall bladder cancer is the most common type of malignancies of the gastrointestinal tract (80-95%). Our aim was to study the mutagenic potential of bile specimens in gall bladder cancer and also to study the epigenetic alteration in gall bladder cancer and gall stone disease.

MATERIAL AND METHODS: 95 bile samples (35 from ca gall bladder and 60 from cholelithiasis/ cholestasis) were collected and studied for genetic mutations in tumor suppressor genes through tester strains of Salmonella typhi.

RESULTS : Two strains TA1535 and TA1538 were found with rfa mutations and third strain TA97a as found with uvrB mutations. All the genes p15, p16 and CDH1, that we had screened were found to be methylated in the promoter region in all the 35 gall bladder cancer patients (p<0.001). while the rest 60 cholelithiasis cases didn't show methylation.

CONCLUSION : The epigenetic alterations in carcinoma of gall bladder patients may probably be induced by the degrading potential of the bile salts.

KEYWORDS : Cholelithiasis , Cholestasis , Gall bladder cancer

INTRODUCTION :

Gall bladder cancer is the most common type of malignancies of the gastrointestinal tract (80-95%). Most of the cancers are detected during histological examination after cholecystectomy. Females are more prone to disease than males. A direct association was found between the presence of cholelithiasis and the development of carcinoma gall bladder.[1] Tumors of the bile duct obstruct the flow of bile from the liver and are potentially involved in the impairment of major liver functions. The symptoms of tumor formation may be associated with the disturbances in metabolic, storage, synthetic and catabolic function of the liver.[2] The composition of vesicular and micellar proteins of human gall bladder and a comparative map of two dimentional gel electrophoresis of serum ,bile fluids , RBCs & liver cells has shown presence of eight serum proteins in bile.[3] The largest catalogue of human bile protein components consists of 87 unique proteins in the fluid which includes the presence of Mac-2 binding protein in the bile. Besides Mac-2 binding protein is reported as a potential bile marker in biliary tract carcinoma. But limited sample set of study makes it a statistically non significant marker.[4] Pathological epigenetic changes are more and more being considered an alternative to mutations and chromosomal alterations in disrupting gene function. These epigenetic changes include global DNA hypomethylation , hypermethylation , gene specific hypo and hypermethylation , chromatin alteration and loss of imprinting. All of these may lead to abnormal activation of growth promoting gene or abnormal silencing phenomenon and is a major mechanism for silencing of tumor suppressor genes.[5] As early disease diagnosis is a major intricacy in the management of carcinoma gallbladder , surgical resection is possible only in one third of patients.[6] No chemotherapy has shown efficacy in GBC. Adjuvant drug therapy with 5-fluorouracil and Mitomycin C is evaluated but with no specific efficacy.arecently gemicitabine , leucovanin and triapine are reported in phase –II trial.[7] The aim was to study the mutagenic potential of bile specimens in gall bladder cancer and also to study the epigenetic alteration in gall bladder cancer and gall stone disease.

MATERIAL METHODS :

This was a prospective study conducted at Chirayu Medical college & Hospital,Bhopal(MP) during the period of December 2014 till December 2015 . 95 bile samples (2-3 ml) were collected from the patients of carcinoma gallbladder (n=35) & cholelithiasis /cholicystitis (n=60) during cholecystectomy . Samples of surgically removed tissue were also collected from these patients to study the epigenetic alterations in the major tumour suppressor genes. The demographic data was collected from patients record and statistically analysed. The tissue samples were processed for histopathological examination.

Eight histidine dependent mutant tester strains of salmonella typhi TA1535, TA1538, TA97a & TA100 were used in the study for Ames Test. Working culture of all tester strains were prepared and studied for mutagenicity. Histidine and Biotin requirements for growth of tester strains on selective agar plats, crystal violet sensitivity to comfirm the presence of deep rough (rfa) mutations , UV sensitivity to confirm deletetion of uvrB gene and ampicilline and tetracycline resistance to know the presence of plasmid R factor were performed. Genomic DNA was isolated using phenol-chloroform isoamyl alcohol method and its quantification was done by running in 0.8 % agarose gel. Methylation was confirmed by methylation specific polymerase chain reaction. The amplified PCR product was run on 6% native PAGE in 1XTBE buffer and than silver staining was done .All these findings were corelated & statistically analysed.

Results :

Total 95 samples of bile (35 carcinoma and 60 cholelithiasis and cholecystitis) were collected. Mean age being 47.76 years for cholecystitis, 36.6 years for cholelithiasis and 50.1 years for carcinoma. In carcinoma patients (n=35) 85.71%(30) were Adenocarcinoma, 5.71%(2) had Adenosquamous carcinoma, 5.71%(2) small cell carcinoma and 2% (1) had other types of carcinoma.

Table 01: Different doses of bile juice in different strains of Salmonella typhi and their mutagenic response [A]. Strain TA1535

Dose of bile juice/ MQwater	Tester strain	rfa mutation revertant colonies/ plate (mean ± SD)	PI
1000µl	TA1535	-	-
2 µl/998 µl	TA1535	159±14.2	-
5 µl/995 µl	TA1535	3854±19.79	-
10 µl/990 µl	TA1535	3381±19.79	5.66
15 µl/985 µl	TA1535	1838±181	48.71
20 µl/980 µl	TA1535	438±30.40	87.77

[B]. Strain TA1538

Dose of bile juice/ MQwater	Tester strain	rfa mutation revertant colonies/ plate (mean ± SD)	PI
1000µl	TA1538	-	-
2 µl/998 µl	TA1538	2660±21.9	-
5 µl/995 µl	TA1538	2737±6.36	-
10 µl/990 µl	TA1538	2692± 25.55	-
15 µl/985 µl	TA1538	2669±15.5	-
20 µl/980 µl	TA1538	2440±89.09	6.87

[C]. Strain TA97a

Dose of bile juice/ MQwater	Tester strain	uvrB mutation revertant colonies/ plate (mean ± SD)	PI
1000µl	TA97a	-	-
2 µl/998 µl	TA97a	247±13.43	-
5 µl/995 µl	TA97a	5420±152.7	-
10 µl/990 µl	TA97a	1880±152.7	65.31
15 µl/985 µl	TA97a	1736±124.4	67.97
20 µl/980 µl	TA97a	1224±299.8	77.41

It was observed that two strains TA1535 and TA1538 were found with rfa mutations and third strain TA97a as found with uvrB mutations. This result indicates that bile salts act as inducer to the carcinogenesis of gall bladder. In the study other strains TA98 and TA100 did not show any such type of variation in the colonies. This shows that the advanced stage of gall bladder cancer contains more quantity of degrading bile salts. All the genes p15, p16 and CDH1, that we had screened were found to be methylated in the promoter region in all the 35 gall bladder cancer patients ($p<0.001$). while the rest 60 cholelithiasis cases didn't show methylation.

Discussion :

The lower mean age of the patients of gall bladder diseases and carcinoma indicates the genetic predisposition for this condition in population.The epigenetic alteration, like promoter hyper methylation of CDH1, DKN2A aand CDKN2B was found in all included 35 GBC cases. The reason for methylation in all the gall bladder carcinoma tissue may be due to their advanced stage (T2N1; P stage II) ,as also diagnosed by the pathologists. Unlike Chilean, Chinese, Japanese or American cholelithiasis cases; none of Indian subject included in this study (n=60) showed hypermethylation of any mentioned genes.[1] Since gall stones associated tissues are unrelated to metastasis and not all gall stones lead to cancer ; the absence of hypermethylation is a likely finding.By and large total bile proteomics is not widely reported in literature. The composition of vesicular and micellar proteins of human gall bladder and a comparative map of two dimensional gel electrophoresis of serum, bile fluids, red blood cells and liver cells have shown presence of eight serum proteins in bile.[3] The largest catalogue of human bile protein components consists of 87 unique proteins in the fluid, which includes the presence of Mac-2 binding proteins in the bile fluid. Besides Mac-2 binding protein is reported as a potential bile marker in biliary tract carcinomas. But limited sample size of study makes it a statistically non significant marker.[4] Genetic mechanisms are not only the cause of altered or impaired gene function in tumorigenesis. Pathological epigenetic changes (non sequence based alterations that are inheritated through cell division) are more and more being considered an alternative to mutations and chromosomal alterations in disrupting gene function. These epigenetic changes include global DNA hypomethylation, hypermethylation, gene specific hypo and hypermethylation, chromatin alteration and loss of imprinting. All of these may lead to abnormal activation of growth promoting genes or abnormal scilencing of tumor suppressor genes.[5]

Conclusion :

The epigenetic alterations in carcinoma of gall bladder patients may probably be induced by the degrading potential of the bile salts which in turn is mediated by Salmonella typhi. The association between hypermethylation and the presence of Salmonella typhi in the gall bladder carcinoma patients can be used as a diagnostic marker. The indirect correlation in the advanced tumorigenesis by the presence of Salmonella typhi in the gall stone cases can be proposed and the Ames test is also very helpful in the diagnosis by simply testing bile from early stages of gall bladder carcinoma.

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