Journal of Carcinogenesis & Mutagenesis - Open Access

Research Article
OPEN ACCESS Freely available online
doi:10.4172/2157-2518.1000106

P53- The Molecular Guardian Crashes in Gastric Adenocarcinomas - A Study in an Ethnic Kashmiri Population

Safiya Abdullah[1], Syed Sameer A[1,2], Dil-Afroze[1], Nidda Syeed[1], Das BC[3] and Mushtaq A. Siddiqi[1]*

[1]*Departments of Immunology and Molecular Medicine, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, India*
[2]*Departments of Clinical Biochemistry, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, India*
[3]*Institute of Cytology & Preventive Oncology, Noida, UP, India*

Abstract

Genetic instability underlies the etiology of multistep gastric carcinogenesis. The *p53* mutations observed in tumors represent the expression of such instability by allowing the accumulation of genetic alterations caused by multiple mechanisms. The present study was conducted to investigate the nature and frequency of TP53 mutations in patients with gastric adenocarcinomas of Kashmir valley. Tumor samples from 30 patients with primary gastric adenocarcinomas undergoing radical gastrectomy were evaluated. The mutational status of the p53 (exons 5 to 8) was screened by PCR-SSCP analysis followed by direct sequencing. Of all 30 gastric adenocarcinomas including ten intestinal types and twenty diffuse types, 20% patients (6/30) harbored mutations in the *p53* gene. Overall, twenty-one mutations were found in *TP53* in 30 patients included in this study. Mutations were found at codon 142 (3 cases) of exon 5, codon 144 (1 case) in exon 5, codon 147 (1 case) in exon 5, codon 157 (1 case) in exon 5, codon 169 (2 cases) in exon 5, codon 170 (3 cases) in exon 5, codon 172 (1 case) in exon 5, codon 173 (3 cases) in exon 5, codon 179 (3 cases) in exon 5, codon 180 (1 case) in exon 5, codon 213 (1 case) in exon 6, the insertional mutation was between codon 216 & 217 (1 case) in exon 6 and codon 287 in exon 8 (1 case). The mutation pattern comprised of 12 insertions, 6 substitutions (all transversions) and 3 deletions. All the twelve insertions represented frame-shift mutations. The six single-base substitutions leading to aminoacid substitution included four missense mutations and a single silent mutation. The mutation effect data was found to be significant ($p< 0.05$). This study exhibited significant amount of mutation in exon 5 (OR=90.25 and $p<0.05$ within the CI of 12.47-652.89) of *TP53* in the gastric adenocarcinoma patients from Kashmir valley. Comparison of mutation profile with other ethnic populations and regions reflected both differences and similarities indicating co-exposure to a unique set of risk factors. The differences could be due to exposure to explicit environmental carcinogens, different lifestyle, dietary or cultural practices of Kashmiris being an ethnic population that need further investigations. The direct sequencing results, therefore, shall help in understanding the molecular events associated with progression and metastasis in gastric carcinoma.

Conclusions: *p53* gene mutation incites the pathogenesis of human gastric adenocarcinomas.

Keywords: Gastric cancer; *p53*; Kashmir; India

Abbreviations: PCR: Polymerase Chain Reaction; SSCP: Single Strand Conformational Polymorphism

Introduction

Gastric cancer is the 2rd most common tumor in the world which represents bulk of global cancer burden. It has a very poor prognosis and is the second most common cause of death from cancer worldwide. This is attributable to its bleak 5 year survival rate, of about 25% and has not changed appreciably in 60 years. In 2001 alone, stomach cancer affected 850,000 people, of which 522,000 men and 328,000 women died of stomach cancer [1]. Globally, incidence of gastric cancer shows a wide geographic variation; being particularly high in Japan, Chile, Costa Rica, Colombia, China, Portugal, Iceland, Finland and Scotland [2], considerably lower in US, UK, Canada, Greece, New Zealand, Sweden and Honduras [3] Two-thirds of these cases occur in the developing countries [4]. In Asia, areas with a low incidence include India, Pakistan and Thailand [5] Incidence rates in men are twice those in women, in both low-risk and high-risk areas. However, in most countries there has been a considerable decline in both the incidence and mortality of gastric cancer during the past six decades [6,7]. Thus, since 1930, the annual mortality rate in US has dropped from about 38%-7% per 100,000 populations for men and from 28%-4% per 100,000 for women due to the identification of several predisposing factors in recent years [8,9]. Overall, it still remains among the leading killer cancers representing 3% of all cancer deaths [10-12] Migrants from high to low incidence countries show a significant decrease in gastric cancer occurrence in their off-springs, suggesting that the cause is related to environmental factor starting early in life [13-15].

Demographically, Kashmir is one of the three provinces of the Jammu and Kashmir State in north India, situated at an altitude of 1800-2400 m above sea level. It comprises of a non-migrant population who have special social, personal and dietary habits that endowed this population with a common ethnic origin. The exact prevalence in Jammu & Kashmir is not known since whatever little work has been done on gastro-esophageal cancers in this part of the world was hospital-based and no population-based epidemiologic studies have been undertaken. In Kashmir, the clinical experiences have revealed a very high prevalence of gastric cancer, although

***Corresponding author:** Dr. Mushtaq A. Siddiqi, Professor & Chief, Department of Immunology and Molecular Medicine, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir, India, Tel: +91-194-2401013; Ext: 2262; Fax: +91-194-2403470; E-mail: vc.tmuk@gmail.com

Received September 28, 2010; **Accepted** October 23, 2010; **Published** October 23, 2010

Citation: Abdullah S, Sameer S A, Dil-Afroze, Syeed N, Das BC, et al. (2010) *P53*- The Molecular Guardian Crashes in Gastric Adenocarcinomas - A Study in an Ethnic Kashmiri Population. J Carcinogene Mutagene 1:106. doi:10.4172/2157-2518.1000106

there is scarcity of data available at present in this regard. The age standardized incidence rates for gastric cancer were 36.7/100,000 per year among men & 9.9/ 100,000 per annum in the women [16]. These figures were three to six times higher than those recorded by cancer registries in Bangalore, Madras and Bombay. Thus, among north Indian states Kashmir has a high incidence of gastric cancers.

Multiple genetic alterations underlie the multi-step progression to cancer. Genomic instability induced by these genetic alterations that determine replication errors could contribute to the inactivation of tumor suppressor genes and increase the mutation rate. The identification of a growing number of genes, primarily tumor suppressor genes contribute to the development and progression of human solid tumors [17-20]. Structural alterations of the p53 product have been frequently detected in a wide variety of human tumors [21,22]. The recessive or dominant negative mutations instigate the loss of p53 function, which can affect the DNA-binding domain of the protein (exons-5 to 9), as well as the interaction with other cellular or viral onco-proteins [23,24] The wild-type p53 protein exerts pleiotropic effects through the transcriptional activation of different target genes that control important checkpoints in the modulation of cell cycle progression [25-30]. The accumulation of wild-type p53 protein results in two pathways; Cell cycle arrest and programmed cell death, which are mutually involved in tumor suppressor functions [31]. *TP53* induces a transient suppression of the cellular growth at the Gl/S checkpoint [32] and causes an irreversible induction of the pathways leading to p53-dependent programmed cell death [33,34] and DNA repair. Therefore, p53 mutations lead to disruption of these pathways conferring a selective growth advantage for tumor cells resulting in increased proliferation activity and tumor development [35,36] Besides, mutated p53-bearing cells have altered controls through the cell cycle progression and prevent apoptosis. Thus, may play a role in the mechanisms of resistance to chemotherapeutic genotoxic agents [37-40].

According to the classification by Lauren [41], stomach cancer is classified into two main histological types: diffuse and intestinal. Several studies in the last decade [42-44] point to precise combinations of genetic and epigenetic alterations that differ in both subtypes, although a few of them appear to be common as well. The most evident genetic changes found in both types of gastric cancers include loss of heterozygosity, hypermethylation of several genes in addition to mutational abnormalities of p53 tumor suppressor gene [44-47]. These alterations are also frequently observed in pre-cancerous lesions such as intestinal metaplasia and dysplasia, which are precursors of the intestinal type of gastric cancer [45,48]. Most mutations of p53 gene or genetic and/or epigenetic changes of upstream and/or downstream located genes in the p53 network result in a loss of function of the wild-type gene product. However, most but not all mutant p53 proteins have a prolonged half-life and accumulate in cells [49,50]. Both p53 accumulation and its absence in the nucleus of malignant cells could thus be used as a valuable prognostic marker and predictor of clinical outcome of gastric tumors [45,50-52].

The present study is aimed at evaluation of involvement of *TP53* gene mutation in incidence and/development of gastric cancer in patients from Kashmir valley. This study also addresses the documentation of the data, the first on gastric cancers from this part of the world. Further, to analyze the differences and similarities in the mutation profiles from various regions, a comparison was made between the mutation patterns of *TP53* obtained in present study and the data compiled at International Agency for Research on Cancer (IARC) *TP53* data base {http://www.iarc.fr/p53/homepage.htm}[57].

Material and Methods

Patients and samples

Patients attending Gastroenterology and Surgery Departments at Sheri-I-Kashmir Institute of Medical Sciences, Srinagar, Jammu & Kashmir, India, between July 2008 and July 2009 for gastric cancer management were recruited for the study, with prior informed consent. Patient underwent endoscopy and histopathological examination to establish the clinical profile. Tumor samples from patients with primary gastric adenocarcinomas undergoing radical gastrectomy were evaluated. A total of 30 gastric cancer cases under study included surgically resected gastric tissue (which incorporated tumor tissue, normal tissue and lymph nodes wherever involved) was collected from these sporadic gastric cancer cases. All samples were snap frozen at -70°C until analysis. A questionnaire was used to collect information on clinico-epidemological characteristics of the patients including age, family history of the disease, body mass, provisional diagnosis, lymph nodes involved, clinical tumor stage and histopathological grade of tumor.

DNA isolation, PCR-SSCP analysis and sequencing

High-molecular-weight DNA was isolated from single cell suspension of blood and tissue samples of gastric cancer patients by Proteinase-K digestion and phenol-chloroform extraction [53].

PCR amplification using 4 set of primer pairs (Table 1) were used to amplify exons 5 through 8 (DNA binding domain) of *TP53*. PCR was carried out in MJ Research Minicycler at respective annealing temperature (Table 1) using standard protocol. SSCP analysis of PCR product was carried out on 6% non-denaturing Polyacrylamide gel (PAG) utilizing either non-radioactive silver staining or radioactive procedures [54-56]. In non-radioactive SSCP analysis [55], PCR products mixed in denaturing buffer (95% formamide, 10mM NaOH, 0.05% xylene-cyanol FF and 0.05% Bromophenol blue) in 1:1 ratio were heat denatured at 95°C for 5 minutes, immediately cooled on ice for 20min, 6 µl of which were loaded on 6% PAG and electrophoresed in 0.5X Tris-borate EDTA buffer at ±17°C at 4W constant power for

Gene	Amplicon	Nucleotide positions in genomic DNA	Primer sequence*	Annealing Temperature (°C)	Product size(bp)
p53	Exon5	13005-13024 13295-13314	1)TGTTCACTTG TGCCCTGACT 2)AGCAATCAGTGAGGAATCAG	55	310
p53	Exon6	13271-13280 13475-13494	1)TGGTTGCCCAGGGTCCCCAG 2)TGGAGGGCCACTGACAACCA	62	224
p53	Exon7	13941-13960 14158-14177	1)CTTGCCACAG GTCTCCCCAA 2)AGGGGTCAGCGGCAAGCAGA	62	237
p53	Exon8	14442-14461 14579-14598	1)TCCTGAGTAGTGGTAATCTA 2)GCTTGCTTACCTCGCTTAGT	58	156

*1) Sense primer 2) Antisense primer

Table 1: Primers used for screening different exons of *TP53*.

PATIENT ID	AGE	RURAL/ URBAN	CLNICAL TUMOR STAGE	EXON	CODON NUMBER	NUCLEOTIDE NUMBER	MUTATION cDNA SEQUENCE	BASE CHANGE	AMINO ACID CHANGE	MUTATIONEFFECT
15N	45/M	R	GRADE III	5	169	13185	506delT	ATG>A_G	NS (Met>Arg)	FS
15T				5	147	13118	439G>T	GTT>TTT	Val>Phe	MS
				5	179	13214	535 C>G	CAT>GAT	His>Asp	MS
15L				5	170	13189	510InsT	ACG>ACTG	NS (Thr>Thr)	FS
				5	173	13197	518delT	GTG>G_G	NS (Val>Gly)	FS
				6	213	13397	636C>G	CGA>GGA	Arg>Gly	MS (at CpG)

Table 2: Clinico-epidemiological details and nature of *TP53* mutations in germline gastric adenocarcinoma patients from Kashmir valley.

PATIENT ID	AGE/ SEX	RURAL/URBAN	CLNCAL TUMOR STAGE	EXON	CODON NUMBER	NUCLEOTIDE NUMBER	MUTATION	BASE CHANGE	AMINO ACID CHANGE	EFFECT
8T	47/ F	R	GRADE II	5	142	13105	426InsA	CCT>CC**A**T	NS (Pro>Pro)	FS
				5	172	13195	516InsC	GTT>GT**C**T	NS (Val>Val)	FS
				5	179	13214	535InsG	CAT>**G**CAT	NS (His>Ala)	FS
12L	52/M	R	GRADE III	5	170	13189	510InsT	ACG>AC**T**G	NS (Thr>Thr) (at CpG)	FS
				5	173	13197	518delT	GTG>G_G	NS (Val>Gly)	FS
				6	216&217	13775	649InsT	_GTG>TGTG	NS (Val>Cys)	FS
16L	62/M	U	GRADE III	5	142	13105	426InsA	CCT>CC**A**T	NS (Pro>Pro)	FS
				5	144	13110	431A>T	CAG>CTG	Gln>Leu	MS
				5	179	13215	536InsG	CAT>C**G**AT	NS (His>Arg)	FS
				5	180	13217	538InsA	GAG>**A**GAG	NS (Glu>Arg)	FS
23L	57/M	R	GRADE III	5	142	13105	426InsA	CCT>CC**A**T	NS (Pro>Pro)	FS
				5	157	13150	471C>A	GTC>GTA	(Val>Val)	Silent
				5	173	13196	517InsC	GTG>**C**GTG	NS (Val>Arg)	FS
24L	54/M	R	GRADE III	5	169	13185	506T>G	ATG>AGG	Met>Arg	MS
				5	170	13189	510InsT	ACG>AC**T**G	NS Thr>Thr	FS

Table 3: Clinico-epidemiological details and nature of *TP53* mutation spectrum in sporadic gastric adenocarcinoma patients from Kashmir valley.

18-22h. Gels were then silver stained. In radioactive SSCP analysis, radiolabelled PCR products (using α32-pCTP) mixed in denaturing loading buffer (95% formamide, 20mM EDTA, 0.05% xylene-cyanol FF and 0.05% Bromophenol blue) in 1:10 ratio were heat denatured at 95°C for 5 minutes, 3µl of which were loaded on 6% PAG and electrophoresed at 200V in 0.5X Tris-borate EDTA buffer at ±17°C for 18-22h. Gel was then transferred onto 3mm Whatmann paper, covered with saran wrap and dried in vacuum drier at 90°C for 1h.The saran wrap was then replaced by X-ray film and kept at -70°C for 48h. The mobility shift in DNA bands were visualized by developing the x-ray film in a developer. Purified PCR products of the samples showing mobility shift on SSCP analysis and randomly chosen samples were used for direct DNA sequencing using Automated DNA sequencer ABI prism 310. To minimize the sequencing artifacts induced by PCR, products from at least 2 different PCRs were sequenced using forward and reverse primers with Big Dye terminator cycle sequencing ready reaction mix (Applied Biosystems) based on fluorescence-labeled dideoxy nucleotides as chain terminators. Purified single-stranded extension products were then resolved on ABI Prism 310™, DNA sequencer.

Statistical analysis

All statistical analyses were performed using S-PLUS software. Chi-square test for homogeneity of proportions was used to determine significance of mutation pattern and mutation effect data. Odds ratio was utilized to determine associations of presence of mutations with various Clinico-epidemological characteristic such as age, provisional diagnosis, lymph nodes involved, clinical tumor stage and histopathological grade of tumor. Statistical significance was considered when $p \leq 0.05$. The prevalence and pattern of *TP53* mutations obtained in patients from Kashmir was compared with compiled data reported for gastric cancer in IARC *TP53* mutation database, release 9, 2004 (http://www.iarc.fr/ p53/homepage.htm).

Statistical analyses were performed using Pearson $\chi 2$ test and Fisher's exact test. The data were computerised and statistical tests were performed with the program, Statistical Package for Social Sciences (SPSS version 10.05). The tests were considered significant when their overall p values were below 0.05.

Results

Mutational screening of exons-5 to 8 of *TP53* gene in 30 sporadic tumors included in this study exhibit *TP53* mutations in 6/30 (20%) patients with gastric adenocarcinoma. Five out of six patients exhibited mutations somatic in nature. However, one patient did exhibit germline mutation. Overall, the six patients revealed a total of 21 mutations; these were exhibited at codon 142 (3 cases) of exon 5, codon 144 (1 case) in exon 5, codon 147 (1 case) in exon 5, codon 157 (1 case) in exon 5, codon 169 (2 cases) in exon 5, codon 170 (3 cases) in exon 5, codon 172 (1 case) in exon 5, codon 173 (3 cases) in exon 5, codon 179 (3 cases) in exon 5, codon 180 (1 case) in exon 5, codon 213 (1 case) in exon-6, the insertional mutation was between codon 216 & 217 (1 case) in exon-6. The mutational pattern included 12 insertions, 6 substitutions (all six were transversions) and 3 deletions (Table 3). Mutation pattern data of *TP53* revealed a high % age of insertions (12/21) (57.14%), 6/21(28.57%) base substitutions included 1/6 (16.7%) G:C>T:A, 1/6 (16.7%) A:T>T:A, 1/6 (16.7%) C:G>G:C, 1/6 (16.7%) C:G>G:C at CpG, 1/6 (16.7%) T:A>G:C and 1/6 (16.7%) C:G>A:T; all transvertion mutations (Table 4). All the 12 insertions represented frame-shift mutations, six single-base substitutions including four missense mutations (leading to aminoacid substitutions) and single silent mutation. The mutation effect data was found to be significant

(p= 0.011). This study exhibited significant amount of mutation in exon-5 (90.48%) and only 9.5% in exon-6 of *TP53* in the gastric adenocarcinoma patients from Kashmir valley.

The mutation effect data revealed significantly high % age of frameshift mutations (71.4%) (15/21) compared to missense (23.8%) plus silent mutations (4.8%) (p=0.0002) [Table 4]. All missense mutations occurred in heterozygous state. Significant amount of mutations were found in exon-5 (p=90.25 and p>0.05 within the CI of 12.47-652.89) of *TP53* while exons-7 and 8 did not show any mutation at all. Further, in exon-5 an insertion (426InsA) at nucleotide# 13105 in codon 142 resulting in frame-shift mutation was identified in three different patients and another insertion (510InsT) at nucleotide# 13189 also resulting in frame-shift mutation in codon170 occurred in three different patients. A deletion (518delT) at nucleotide# 13197 in codon 173 resulting in frame-shift mutation was identified in two different patients. In exon 5, four different mutations (1 missense and 3 frameshift) at codon 142, 144, 179 and 180 were found in same patient (Table 3). None of the *TP53* mutation bearing patients harboured mutations at the hot-spots reported in gastric cancers as per UMD TP53 database (http://p53.free.fr/Database/p53_database.html). However, all the mutations identified at various codons in our study tally to either the moderate (between 11-100) or maximum (>100) number of mutations as indicated by the distribution of p53 mutations in various cancers.

The presence of *TP53* mutations when compared with various clinico-epidemiological attributes of gastric adenocarcinoma patients showed some association, though statistically not significant, of *TP53* mutation with age, positive lymph node status of patient as well as clinical tumor stage (II & III) and moderately and rural/urban status of patient (Table 5).

MUTATION PATTERN DATA (21) (%)		MUTATION EFFECT DATA (21) (%)	
Insertions	12 (57.14%)		
Substitution	6 (28.57%)	Missense	5 (23.8%)
		Frame-shift	15 (71.43%)
G:C>T:A	1 (16.7%)	Silent	1(4.8%)
A:T>T:A	1 (16.7%)		
C:G>G:C	1 (16.7%)		
C:G>G:C at CpG	1 (16.7%)		
T:A>G:C	1 (16.7%)		
C:G>A:T	1 (16.7%)		
Deletion	3 (14.3%)		
χ^2 = 9.00		χ^2 = 22.28	
df= 2		df= 2	
p-value= .001		p-value=.000	

Table 4: Mutation pattern and mutation effect data of *TP53* in gastric cancer patients from Kashmir valley.

Feature	Mutant TP53 (%)	95% CI	OddsRatio
Age			
≤45yrs	0/10	0.027-2.526	0.259
>45yrs	6/20 (66.6%)		
Rural	5/24 (20.83%)	0.124-13.967	
1.316			
Urban	1/6 (16.67%)		
Lymph node/s involved			
Yes	5/6 (83.3%)	0.091-11.028	1.00
No	1/6 (16.7%)		
Clinical Tumor stage			
II	1/10 (10%)	0.033-3.327	3.327
III	5/20 (25%)		

Table 5: Association of *TP53* gene mutation with Clinico-epidemiological features of gastric adenocarcinoma patients of Kashmir (n=30).

Discussion

The occurrence of *TP53* mutations in gastric adenocarcinoma patients in high and low incidence and racially diverse populations has been well established. However, the information regarding the involvement of this gene in the incidence of and predisposition to gastric cancer in ethnic Kashmiri families is lacking. Of particular mention is the fact that Kashmir is a province of the north Indian state of Jammu and Kashmir situated at an altitude of 1800-2400 m above sea level with extremely cold climatic conditions and comprises of a non-migrant population who have special social, personal and dietary habits that endowed this population with a unique ethnic origin. Thus, this study focused on the evaluation of involvement of *TP53* gene mutation in incidence and/development of gastric cancer in patients from Kashmir valley is significant and warrants documentation, the first on gastric cancers from this part of the world.

Various investigators have examined the mutational profile of gastric cancers by examining exons-2 through 11, although most studies restrict their examination to exons-5 through 8. The reported incidence of p53 mutations in invasive carcinomas ranges from a low of 0% to a high of 76.9% [58,59]. Nonetheless, our study revealed the overall frequency of mutations in *TP53* to be 20% in gastric adenocarcinoma patients of Kashmir which is comparable with the existing reports in the literature [60-71]. In the R12 release of IARC [57] mutation prevalence data base for *TP53,* the overall mutation rate in gastric cancers was documented to be 45%. It is likely that these differences in the frequency of *TP53* mutations in gastric cancers are due to such factors, as ethno-geographic diversity of populations studied, small sample size, differences in exposure to endogenous or exogenous carcinogens, differences in life style & food habits, social and cultural differences, which are yet oblique but their role in the molecular events associated with progression and pathogenesis of human cancers is worth addressing in the future studies.

The mutational spectrum of p53 in gastric cancers is wide. The mutations in exons 5 through 8 of *TP53* in our study on the gastric adenocarcinoma patients of Kashmir were found to be unequally distributed. On comparison, a high percentage of mutation was found in exon-5 [90.48% (19/21) Vs 36.28% (78/215) reported in IARC] and exon-6 [9.52% (2/21) Vs 15.35% (33/215) reported in IARC]. Surprisingly, no mutation was found in exons-7 and 8 [0% Vs 22.32% (48/215) & 23.72% (51/215) reported in IARC]. Five out of six patients exhibited mutations somatic in nature. However, one patient exhibited a single germline mutation (Table 2). This patient exhibited a germline mutation at codon169 in exon-5 nucleotide#13185. There are several sites where mutations are more common than others. More than one mutation was present in a single tumor- insertions 426InsA at nucleotide# 13105 in codon142 and 510InsT at nucleotide# 13189 in codon170 resulting in frame-shift mutations, each occurred in three different patients, deletion (518delT) at nucleotide# 13197 in codon173 also resulting in frame-shift mutation was identified in two different patients. In exon-5, four different mutations (1 missense and 3 frameshift) at codons 142, 144, 179 and 180 were found in the same patient. Similar results displaying multiple mutations in a single tumor were also documented by Flejou et al [72] as within a given tumor there can be heterogeneity of the p53 mutational status [73].

A peculiarity of *TP53* mutation pattern data of Kashmiri patients was significantly high prevalence of insertions (57.1%) compared to 2.07% documented in the IARC mutation pattern data on all gastric cancers, R12 release [57]. The frequency of the deletion mutation (14.3%) found in Kashmiri patients was also higher than the reported

frequency from rest of compiled world data of 6.63%. Mutations resulting in loss or gain of nucleotide base pairs may represent the second highest endogenous mutagenic event for p53 gene in human cancers [74]. Insertions and deletions in the p53 gene can be explained by slipped-mispairing mechanism as proposed for germinal mutations of a small number of eukaryotic genes [75]. Almost all deletions and insertions could be due to DNA sequence features of monotonic base runs, adjacent or nonadjacent repeats of short tandem sequences, palindromes and runs of purines or pyrimidines (homocopolymer runs) as increased length of monotonic runs correlates positively with increased frequency of events. Thus, deletions and insertions in the *p53* tumor suppressor gene may reflect both spontaneous and carcinogen-induced mutagenesis. The mutation effect data revealed significantly high %age of frameshift mutations (71.4%) (15/21) compared to 5.46 % reported in the IARC mutation pattern data [57] but lower frequency of missense mutations (5/21) (23.8%) and silent mutations (1/21) (4.8%) as against 71.6% and 8.53% documented in IARC mutation effect data [57]. Complex frameshift mutations can be explained by the formation of quasi-palindromes, with mismatch excision and replication using one strand of the palindrome as a template.

The overall frequency of substitutions mutation was 28.6% (6/21) was observed in our study, all these substitutions were base transvertions. A large fraction of the p53 mutations in gastric adenocarcinomas of the ethnic Kashmiri population are base transversions, a type of mutation that is infrequent in other tumors aside from lung [76], breast [77] and hepatocellular [78] carcinomas. To our knowledge this is the first report implicating the role of base transversions in gastric cancers. Thus, the different mutation spectrum with high transversions may imply that the exogenous mutagens outweigh the endogenous processes in this cancer.

C:G>G:C mutations found in our study were equivalent at CpG or non-CpG sites (16.7%), an observation unique to our population. In contrast, the IARC mutation spectrum data on all gastric cancers document a higher frequency of 35.1% at CpG sites Vs 23.2% at non-CpG sites [IARC R12 release, 2007] [57]. Presence of alkyl nitrosamine in food stuffs, leading to O^6-alkyl guanine adducts and base mispairing during replication, resulting in G>A (or C>T on the other strand of the DNA) transition [79] has been implicated in the etiopathogenesis of oesophageal and gastric carcinomas [80,81,82]. However, to establish a correlation between the enhanced tranvertions in our patients and the presence of carcinogens already reported or unreported to be present in the local food stuffs needs further investigations.

In summary, mutation pattern of *TP53* revealed certain peculiarities in having maximum mutations in exon-5, high frequency of deletions and insertions besides no mutation at hotspot codons. The study, therefore, suggests *TP53* as a potential molecular marker and prognostic tool. Nevertheless, these observations need further investigations in a bigger cross section of the gastric cancer patients. In future, it will be interesting to explore if exposure to particular environmental carcinogens, different life style, dietary and cultural practices adopted by Kashmiris could generate the mutation pattern observed in present study. More complete analysis of all p5J-coding exons would give a more thorough picture of mutational patterns of the DNA-binding domain and focus on the factors that eliminate the p53 DNA binding function and thus would alter p53 function and cell cycle kinetics every time they occur.

References

1. Parkin DM, Bray FI, Devesa SS (2001) Cancer burden in the year 2000. The global picture. Eur J Cancer 37: S4-S66.
2. Wu CW, Chi CW, Lin W (2002) Gastric cancer: prognostic and diagnostic advances. Expert Rev Mol Med 1-12.
3. Dunham LJ, Bailer C (1968) World map of cancer mortality rates and frequency ratio. J Natl Cancer Inst 41: 155-203.
4. Devesa SS, Silverman DT (1978) Cancer incidence and mortality trends in the United States 1935-74. J Natl Cancer Inst 60: 545-571.
5. Cotran RS, Kumar V, Robbins SL (1994) Basic Pathology. 5th edition 779-780.
6. Kumar V, Cotran RS, Robbins SL (1997) Basic Pathology. 482-483.
7. Boring CC (1993) Cancer statistics. CA cancer J Clin 43: 7.
8. Pavithran K, Doval DC, Pandey KK (2002) Gastric cancer in India Gastric Cancer. Gastric Cancer 5: 240-243.
9. Noguchi Y, Yoshikawa T, Tsuburaya A, Motohashi H, Karpeh M, et al. (2000) Is gastric carcinoma different between Japan and United State? Cancer 89: 2237-2246.
10. Ekstrom AM, Hansson LE, Signorello LB, Lindgren A, Bergstrom R, et al. (2000) Decreasing incidence of both major histologic subtypes of gastric adenocarcinoma-a population-based study in Sweden. Br J Cancer 83: 391-396.
11. Mayor RJ (1998) Gastrointestinal tract cancer. In: Harrison's Principles of Internal Medicine. McgrawHill 14th edn: 568-578.
12. Jemel A, Murray T, Samuels A, Ghafoor A, Ward E, et al. (2004) Cancer statistics 2004. CA Cancer J Clin 54: 8-29.
13. Crew KD, Neugut AI (2006) Epidemiology of gastric cancer. World J Gastroenterol 12: 354-362.
14. Ferlay J, Bray F, Pisani P, Parkin D (2001) Globocan 2000: Cancer incidence, mortality and prevalence worldwide. Lyon: IARC Press.
15. Fuchs CS, Mayor RJ (1995) Gastric carcinoma. N Engl J Med 333: 32-41.
16. Khuroo MS, Zargar SA, Mahajan R, Banday MA (1992) High incidence of oesophageal and gastric cancer in Kashmir in a population with special personal and dietary habits. Gut 33: 11-15.
17. Levine AJ (1995) Tumor suppressor genes: The molecular Basis of Cancer. Edited by J Mendelsohn, PM Howley, MA Israel, LA Liotta. Philadelphia, WB Saunders Co. 86-104.
18. Loeb L (1991) Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 51: 3075-3079.
19. Parsons R, Li GM, Longley MJ, Fang WH, Papadopoulos N, et al. (1993) Hypermutability and mismatch repair deficiency in RER+ tumor cells. Cell 75: 1227-1236.
20. Modrich P (1994) Mismatch repair, genetic stability, and cancer. Science 266: 1959-1960.
21. Levine AJ (1994) The 1993 Walter Hubert lecture: the role of p53 tumor-suppressor gene in tumorigenesis. Br J Cancer 69: 409-416.
22. Greenblatt MS, Bennett WP, Hollstein M, Harris CC (1994) Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 54: 4855-4878.
23. Levine AJ, Momand J, Finlay CA (1991) The p53 tumour suppressor gene. Nature 351: 453-456.
24. Vogelstein B, Kinzler KW (1992) p53 function and dysfunction. Cell 70: 523-526.
25. Kern SE, Kinzler KW, Bruskin A, Jarosz D, Friedman P (1991) Identification of p53 as a sequence-specific DNA-binding protein. Science 252: 1708-1711.
26. Hartwell LH, Kastan MB (1994) Cell cycle control and cancer. Science 266: 1821-1828.
27. Kastan MB, Zhan Q, El-Deiry WS, Carrier F, Jacks T, et al. (1992) A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-teleangiectasia. Cell 71: 587-597.
28. Oliner JD, Kinzler KW, Meltzer PS, George DL (1993) Vogelstein 362: 849-852.
29. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, et al. (1993) WAF1, a potential mediator of p53 tumor suppression. Cell 75: 817-825.
30. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ (1993) The p21 Cdk

interacting protein Cipl is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75: 805-816.

31. Janus F, Albrechtsen N, Dornreiter I, Wiesmüller L, Grosse F et al. (1999) The dual role model for p53 in maintaining genomic integrity. Cell Mol Life Sci 55: 12-27.
32. Lin D, Shields MT, Ullrich SJ, Appella E, Mercer WE (1992) Growth arrest induced by wild-type p53 protein blocks cells prior to or near the restriction point in late Gl phase. Proc Natl Acad Sci USA 89: 9210-9214.
33. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T (1993) p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature 362: 847-849.
34. Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC et al. (1993) Thymocytes apoptosis induced by p53 dependent and independent pathways. Nature 362: 849-852.
35. Zhou X, Wei Wang X, Lexis XU, Koichi H, Makoto N, et al. (1999) COOH-Terminal Domain of p53 Modulates p53-mediated Transcription Transactivation, Cell Growth, and Apoptosis. Cancer Research 59: 843-848.
36. Hulleman E, Boonstra J (2001) Regulation of G1 Phase Progression by Growth Factors and The Extracellular Matrix. Cell Mol Life Sci 58: 80-93.
37. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW (1991) Participation of p53 protein in the cellular response to DNA damage. Cancer Res 51: 6304-6311.
38. Lowe SW, Ruley HE, Jacks T, Housman DE (1993) p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 74: 957-967.
39. Kinzler KW, Vogelstein B (1993) Cancer therapy meets p53. N Engl J Med 331: 49-50.
40. Lowe SW, Bodis S, McClatchey A, Remington L, Ruley HE, et al. (1994) p53 status and the efficacy of cancer therapy in vivo. Science 266: 807-810.
41. Lauren P (1965) The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 64: 31-49.
42. Tahara E, Kuniyasu H, Yasui W, Yokozaki H (1994) Gene alterations in intestinal metaplasia and gastric cancer. Eur J Gastroenterol Hepatol 1: S97-S102.
43. Kountouras J, Zavos C, Chatzopoulos D (2005) New concepts of molecular biology on gastric carcinogenesis. Hepatogastroenterology 52: 1305-1312.
44. Tahara E (2004) Genetic pathways of two types of gastric cancer. IARC Sci Publ 157: 327-349.
45. Smith MG, Hold GL, Tahara E, El-Omar EM (2006) Cellular and molecular aspects of gastric cancer. World J Gastroenterol 12: 2979-2990.
46. Tamura G (2006) Alterations of tumor suppressor and tumorrelated genes in the development and progression of gastric cancer. World J Gastroenterol 12: 192-198.
47. Yokozaki H, Yasui W, Tahara E (2001) Genetic and epigenetic changes in stomach cancer. Int Rev Cytol 204: 49-95.
48. Kufe DW, Pollock RE, Wichselbaum RR, Bast RC, Gansler TS, et al. (2003) editors. Cancer medicine. 6th ed. Hamilton, Ontario: BC Decker.
49. Shiao YH, Palli D, Caporaso NE, Alvord WG, Amorosi A, et al. (2000) Genetic and immunohistochemical analyses of p53 independently predict regional metastasis of gastric cancers. Cancer Epidemiol Biomarkers Prev 9: 631-633.
50. Fenoglio-Preiser CM, Wang J, Stemmermann GN, Noffsinger A (2003) TP53 and gastric carcinoma: a review. Hum Mutat 21: 258-270.
51. Gottlieb TM, Oren M (1996) p53 in growth control and neoplasia. Biochim Biophys Acta 1287: 77-102.
52. Levine AJ, Hu W, Feng Z (2006) The P53 pathway: what questions remain to be explored? Cell Death Differ 13: 1027-1036.
53. Blin N, Stafford DW (1976) A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res 3: 2303-2308.
54. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphism. Proc Natl Acad Sci 86: 2766-2770.
55. Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196: 80-83.
56. Bosari S, Marchetti A, Battitta F, Graziani D, Borsani G, et al. (1995) Detection of p53 mutations by single-strand conformation polymorphism (SSCP) gel electrophoresis- A comparative study of radioactive and non radioactive silver-stained SSCP analysis. Diagn Mol Pathol 4: 249-255.
57. Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian SV, et al. (2007) Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat 28: 622-629.
58. Yamada Y, Yoshida T, Hayashi K, Sekiya T, Yokata J, et al. (1991) p53 gene mutations in gastric cancer metastases and in gastric cancer cell lines derived from metastases. Cancer Res 51: 5800-5805.
59. Correa P, Shiao Y (1994) Phenotypic and genotypic events in gastric carcinogenesis. Cancer Res 54: 1914s-1943s.
60. Liu XP, Tsushimi K, Tsushimi M, Oga A, Kawauchi S, et al. (2001) Expression of p21 (WAF1/CIP1) and p53 proteins in gastric carcinoma: its relationship with cell proliferation activity and prognosis. Cancer Lett 170: 183-189.
61. Maesawa C, Tamura G, Suzuki S, Ogasawara S, Sakata K, et al. (1995) The sequential accumulation of genetic alterations characteristic of the colorectal adenoma–carcinoma sequence does not occur between gastric adenoma and adenocarcinoma. J Pathol 176: 249-258.
62. Poremba C, Yandell DW, Huang Q, Little JB, MellinW, et al. (1995) Frequency and spectrum of p53 mutations in gastric cancer-a molecular genetic and immunohistochemical study. Virchows Arch 426: 447-455.
63. Ricevuto E, Ficorella C, Fusco C, Cannita K, Tessitore A et al. (1996) Molecular diagnosis of p53 mutations in gastric carcinoma by touch preparation. Am J Pathol 148: 405-413.
64. Rugge M, Shaio YH, Correa P, Baffa R, DiMario F (1992) Immunohistochemical evidence of p53 overexpression in gastric epithelial dysplasia. Cancer Epidemiol Biomarkers Prev 1: 551-554.
65. Rugge M, Shiao YH, Busatto G, Cassaro M, Strobbe C, et al. (2000) The p53 gene in patients under the age of 40 with gastric cancer: mutation rates are low, but are associated with a cardiac location. Mol Pathol 53: 207-210.
66. Seruca R, David L, Castedo S, Veiga I, Borresen AL, et al. (1994) p53 alterations in gastric carcinoma: a study of 56 primary tumors and 204 nodal metastases. Cancer Genet Cytogenet 75: 45-50.
67. Shepherd T, Tolbert D, Benedetti J, MacDonald J, Stemmermann G, et al. (2000) Alterations in exon 4 of the p53 gene in gastric carcinoma. Gastroenterology 118: 1039-1044.
68. Strickler JG, Zheng J, Shu Q, Burgart LJ, Alberts SR, et al. (1994) p53 and microsatellite instability in sporadic gastric cancer: when guardians fail. Cancer Res 54: 4750-4755.
69. Sud R, Wells D, Talbot IC, Delhanty JDA (2001) Genetic alterations in gastric cancers from British patients. Cancer Genet Cytogenet 126: 111-119.
70. Taniere P, Martel-Planche G, Maurici D, Lombard-Bohas C, Scoazec JY, et al. (2001) Molecular and clinical differences between adenocarcinomas of the esophagus and of the gastric cardia. Am J Pathol 158: 33-40.
71. Tolbert D, Fenoglio-Preiser CM, Noffsinger, DeVoe G, MacDonald J, et al. (1999) The relation of p53 gene mutations to gastric cancer subsite and phenotype. Cancer Causes Control 10: 227-231.
72. Flejou JF, Gratio V, Muzeau F, Hamelin R (1999) p53 abnormalities in adenocarcinoma of the gastric cardia and antrum. Mol Pathol 52: 263-268.
73. Iwamatsu H, Nishikura K, Watanabe H, Ajioka Y, Hashidate H, et al. (2001) Heterogeneity of p53 mutational status in the superficial spreading type of early gastric carcinoma. Gastric Cancer 4: 20-26.
74. Jego N, Thomas G, Hamelin R (1993) Short direct repeats flanking deletions, and duplicating insertions in p53 gene in human cancers. Oncogene 8: 209-213.
75. Greenblatt MS, Grollman AP, Harris CC (1996) Deletions and insertions in the p53 tumor suppressor gene in human cancers: confirmation of the DNA polymerase slippage/misalignment model. Cancer Res 56: 2130-2136.
76. Hainaut P, Pfeifer GP (2001) Patterns of p53 G→T transversions in lung **cancers** reflect the primary mutagenic signature of DNA-damage by tobacco smoke. Carc**in**ogenesis 22: 367-374.
77. Chen FM, Hou MF, Wang JY, Chen TC, Chen DC, et al. (2004) High frequency of G/C transversion on *p53* gene alterations in breast cancers from Taiwan. Cancer Lett 207: 59-67.

78. Lasky T, Magder L (1997) Hepatocellular carcinoma p53 G > T transversions at codon 249: the fingerprint of aflatoxin exposure. Environ Health Perspect 105: 392-397.

79. Rabes HM (1986) DNA adducts and cell cycle. J Cancer Res Clin Oncol 58: 4023-4037.

80. Lozano JC, Nakazawa H, Cros MP, Cabral R, Yamasaki H (1994) G>A mutations in p53 and Ha-ras genes in esophageal papillomas induced by N-nitrosomethylbenzylamine in 2 strains of rats. Mol Carcinog 9: 33-39.

81. Mir MM, Dar NA, Gochhait S, Zargar SA, Ahangar AG, et al. (2005) *p53* mutation profile of squamous cell carcinomas of the oesophagus in Kashmir (India): A high-incidence area. Int J Cancer 116: 62-68.

82. Siddiqi R, Kumar Z, Fazili B, Spiegelhalder R, Preussmann (1992) Increased exposure to dietary amines and nitrates in a population at high-risk area of esophageal and gastric cancer: in Kashmir India. Carcinogenesis 13: 1331-1335.