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TP53 Arg72Pro polymorphisms and Gastric cancer predisposition in an ethnic Kashmiri population

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Abstract
TP53 (TP53 Arg72Pro) gene polymorphisms at codon 72 have been associated with risk and susceptibility to various cancers. In this case-control study we examined the genotype distribution of TP53 Arg72Pro single nucleotide polymorphisms (SNP) by using a PCR-RFLP approach to determine that if this SNP can modulate the risk factor for gastric cancer development and to check for the possible correlation of this SNP with clinicopathological variables of gastric cancer. We in this study investigated the genotype distribution of this SNP in 120 gastric cancer cases in comparison with 170 healthy subjects. We found significant association of the Pro/Pro mutant related to clinical tumor stage (p=0.000164), lymph node involvement (p=0.00193), Age (p=0.00568) and EGD biopsy (p= 0.0097), but not to other variables. We by these investigations conclude that Arg72Pro SNP is associated with susceptibility to developing gastric cancer and lymph node development in ethnic Kashmiri population.
Keywords: *Gastric cancer,Kashmiri population, SNP (single nucleotide polymorphism), TP53.*

Introduction

Cancer being the leading death cause among the adults worldwide with an estimated death of 9.6 million deaths in year 2018, As India among the international agency for research on cancer indirectly estimated about 635 000 people died because of cancer in 2008, almost representing 6% of all deaths in India and almost 8% total deaths worldwide. Kashmir (North India) being the high incidence belt of gastric cancer and other related cancers were incidence is high for gastric cancer among that men representing 36.70 lac per annum, women 9.9 lac per annum[1].

Genetic polymorphisms are reported to be an important cause of the predisposition to several human cancers. The role of oncogenes and tumor suppressor genes in the pathogenesis of gastric cancer has recently received considerable attention. Allelic deletions of the MCC,APC and P53 tumor Suppressor gene has been reported in 33%,34% and 45% of gastric cancers respectively [2]. Structural alterations of the *TP*53 product have been frequently detected in a wide variety of human tumors[3,4]. *TP53* induces a transient suppression of the cellular growth at the Gl/S checkpoint (Lin 28) and causes an irreversible induction of the pathways leading to *TP*53-dependent programmed cell death[5, 6] 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[7, 8]. 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 genotoxicagents[6, 9-11]. TP53 (Tumor Protein, MW: 53 kDa) is known to regulate the cell cycle and functions as a tumor suppressor. This protein also prevents genome mutation. In humans, a frequent polymorphism is the replacement of medium sized and hydrophobic amino acid proline with large and basic amino acid arginine at codon location 72. The substitution of amino acid has been associated with cancer vulnerability. *TP53* polymorphisms have been studied for cancer risk factor band is a nonsynonymous polymorphism in a proline-rich domain located in exon 4, where a cytosine (*C*; variant allele) for guanine (*G*) substitution results in the substitution of proline (Pro) for arginine (Arg) at codon 72 of the p53 protein (Arg72Pro). The alleles of Arg72Pro function differently in regulating gene transcription, their interaction with p73, targeting proteasome, and how they get degradation by human papillomavirus E6 protein. They act as modulators of apoptosis at differing rates.

Using bioinformatics tools like alternative splicing to elucidate expression of genes at cellular level. Protein-protein interaction network was used to study the interactive network for structural, functional and evolutionary properties of protein.The Eukaryotic linear motifs are used to study the protein-protein interactions at cellular level by using methodology prosplicer, string database, keg mapper and ELM source. There are network of genes and protein interactions functional in Tp53 pathway which is important for cellular haemostasis. Studies on genomics and other high through put sequencing will lead to study various genetic abnormalities for patients where Tp53 is involved. These interaction networks can be used for annotating structural, functional and evolutionary properties of proteins. They are abundant in intrinsically disordered sections of the proteins and offer extensive assortment of properties to proteins. They play key functions in the regulation of cellular machinery. Investigating the predicted interactions can give hints to novel directions for future research.

In this case-control study the genotype distribution of *TP53* Arg72Pro SNP was examined, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, to evaluate its possible relevance in susceptibility to gastric cancer and to study the correlation of this SNP with the clinicopathological variables of gastric cancer cases.

Materials and Methods

Study population

This study included 120 primary gastric cancer patients. All gastric cancer patients were recruited from the Department of Surgical Gastroenterology, Sher-I-Kashmir Institute of Medical Sciences, from March 2009 to March 2011. Tumor types and stages were determined by two experienced pathologists. Blood samples of 170 age, gender, dwelling and smoking matched cases with no signs of any malignancy were collected for controls (Table 1). Data on all gastric

cancer patients were obtained from personal interviews with patients and/ or guardians, medical records and pathology reports. The data collected included gender, age, dwelling, tumor location, lymph node status, site of growth, EGD biopsy and Hot salt tea consumption. All patients and/or guardians were informed about the study, and their consent to participate in this study was obtained on a predesigned questionnaire (available on request). The collection and use of tumor and blood samples for this study were previously approved by the appropriate Institutional Ethics Committee.

DNA extraction and polymerase chain reaction-restriction fragment length polymorphism

DNA extraction was performed using any one of the previously described techniques. Previously reported primers[12] were used for the amplification of the target regions of the *TP53* Arg72Pro polymorphisms. PCR was carried out in a final volume of 25 µL containing 50 ng genomic DNA template, 1X PCR buffer (Biotools) with 2 mMMgCl$_2$, 0.4 µM of each primer (Genescript), 50 µMdNTPs (Biotools), and 0.5 U DNA polymerase (Biotools). For PCR amplification annealing at 54°C was used.

For RFLP, the PCR products of *TP53* Arg72Pro SNPs were digested with *Bst*UI (1 U at 37°C for 16 h) (Fermentas), respectively. In the case of *TP53* Arg72Pro polymorphism, the Arg/Arg wild produced two bands (160 and 119 bp); the Pro/Pro variant was identified by a single band (279 bp), and heterozygous Pro/Arg variant displayed three bands (279, 160, and 119 bp) DNA fragments were electrophoresed through a 2-3% agarose gel for resolution. The genotypes of >20% of the samples were double blindly reassessed to confirm the results by two independent researchers.

Statistical analysis

Statistical analyses were performed with the SPSS version 14 software. Observed frequencies of genotypes in gastric cancer patients were compared to controls using chi-square or Fisher exact tests when expected frequencies were small. The chi-square test was used to verify whether genotype distributions were in Hardy-Weinberg equilibrium. Statistical significance was set at $P < 0.05$.

Results

A total of 120 gastric cancer patients and 170 control subjects were included in this study. The patients comprised 90 males and 30 females (M/F ratio = 3), and the control subjects consisted of 122 males and 48 females (M/F ratio = 2.54). There were 86 rural and 34 urban cases. There were 89 smokers and 31 non-smokers. In this study, we found that the genotype frequencies in cases and controls were in Hardy-Weinberg equilibrium. The genotypic distribution and allelic frequencies of *TP53* Arg72Pro gene polymorphism in patients and controls are given in Table 2 and 3. There were statistically significant no differences in the genotypic distribution and allelic frequency between the patients and healthy controls [for PP vs. RR genotype: $X^2 = 0.74$; OR = 0.72 (95% CI: 0.35-1.51) and p 0.389; for PP vs. (RR + PR): $X^2 = 4.46$; OR = 0.49 (95% CI: 0.252–0.958) and (p =0.035]. The X^2 for A vs. G allele was 18.83; OR = 2.09 (95% CI: 1.49–2.93) and p =<0.0001 (Table 2-3).

The correlation of *TP53* Arg72Pro polymorphic status with the clinicopathological characteristics was carefully analyzed. It was found that the Pro/Pro mutant status was significantly related to clinical tumor stage (p=0.000164), lymph node involvement (p=0.00193), Age (p=0.00568) and EGD biopsy (p= 0.0097), but not to other variables (Table 4).

Discussion

The *TP53* codon 72 polymorphism results in either arginine or proline, there are many studies to clear the relationship between *TP53* codon 72 genotypes and specific cancer risk and susceptibility. Recently, the *TP53* codon 72 polymorphism has been extensively studied to

determine the risk factors responsible for carcinogenesis. The purpose of this study was to investigate the association of the genotype distribution of the *TP53* codon 72 polymorphism and gasric cancer susceptibility via in comparison of gastric cancer group and normal control genotypes.

Several studies have been carried out on the association of *TP53* polymorphism with increased risk for various cancers[12-18]. In this study, we assessed the SNP of *TP53* Arg72Pro, in an ethnic Kashmiri population for the first time in gastric cancer, since the role of *TP53* polymorphism in relation to gastric cancer risk had not yet been reported from this part of the world. The occurrence of *TP53* mutations in gastric adenocarcinoma patients in high and low incidence and racially diverse populations has been well established. 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 *TP53* mutations in invasive carcinomas ranges from a low of 0% to a high of 76.9%[19, 20]. It has been shown by some studies[21]. that *TP53* Pro72 variant induces transcription activation more efficiently than *TP53* Arg72 variant, and other studies have shown that *TP53* Arg72 variant is more efficient in inducing apoptosis[22]. As has been stated earlier,[23] in Asians, R allele is more activated during cancer development, but in our study we found the P allele is much involved in the development and progression of gastric cancer also reported by Niddaet al., 2010[13] in breastcancer. Control population has been studied by various authors in Kashmiri population and are according to the present study[12,13]. As the polymorphism is also affected by lifestyle, diet and/or environmental exposure, which vary according to race and ethnicity[24,25]. we suggest that as our population is exposed to a special set of environmental and dietary risks, which include the consumption of sun-dried and smoked fish and meat, dried and pickled vegetables, red chilli, hakh (a leafy vegetable of the Brassica family), hot noon chai (salted tea), and hukka (water pipe) smoke[13]. As previously reported, the etiology and incidence of various GIT cancers in this population has been attributed to probable exposure to nitroso compounds, amines and nitrates, reported to be present in local foodstuffs, most of which have been shown to contain important irritants and carcinogens[26][27].

Hence, in this study, which was carried out for the first time in Kashmir Valley, we observed a significant association between the Pro/Pro mutant status clinical tumor stage, lymph node involvement, age and EGD biopsy, but not with other clinicopathological variables. However, this correlation needs to be authenticated in a large sample study in the future, so as to help in the better discernment of racial differences and in determining the aggressiveness in gastric cancer.

In conclusion, the association between gastric cancer and Arg72Pro *TP53* polymorphism exists. We also found that the Pro form of codon 72 in *TP53* showed more abundance in advanced tumors and, thus, could become a useful marker to predict gastric cancer development. These correlations need to be authenticated in a large sample study in the future to help better discern racial differences and determine the aggressiveness of gastric cancer.

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