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Polymorphic study of OGG1 gene in gastric cancer patients of Kashmir valley

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ABSTRACT

Background: Gastric cancer is the fourth leading cause worldwide and leading cancer in several countries. Polymorphic changes in specific genes are a key event in the formation and progression of cancer. The study was carried out to check the polymorphism of OGG1 gene in gastric carcinoma in Kashmir valley, a recently reported prone area to gastric cancer. Material and method: In this study, the OGG1 genotype of 30 patients with Gastric cancer, and 20 healthy subjects were assessed by Polymerase chain reaction (PCR-RFLP) method. DNA was extracted by Sambrook and Russel method, quantified by UV spectrophotometer (Hitachi U-1800), and visualised at Gel Doc system (BioRad). The DNA obtained was amplified by PCR (Roche) and restriction enzyme- Fnu4H1 (Fermentas) digestion was done on the amplified product. The primers of specific sequence were added to RFLP-PCR and polymorphism was studied. On genetic level the role of polymorphism of OGG1 gene has been widely studied across the world in different ethnic populations. Result: In this study genetic polymorphism of OGG1 gene showed 63% of gastric cancer was homozygous mutant, 26% of the cases showed heterozygous condition and 10% were homozygous normal. Almost 80% (16/20) of the normal samples showed heterozygous condition and 5% (1/20) was found to be homozygous mutant and 15% (3/20) homozygous normal. The allelic association of this polymorphism with gastric cancer and normal healthy individuals was evaluated by $\chi 2$ (chi square test) and was found to be significant ($p=0.000084$). In gastric cancer patients the homozygous mutant condition was found to be certainly higher in cases of above 60 years of age (81%) than the ages below 60 years (57%) and association was insignificant as $p=0.3947$ and in controls 14.28% was the distribution in comparison to below 60 years of age groups 0%. Conclusion: The present study found that OGG1 polymorphism was significantly associated with gastric cancer risk in Kashmiri population so it can be concluded that there is an important role of polymorphism of OGG1 gene and may act synergistically to increase the risk of gastric cancer in the patients of Kashmir valley.

Keywords- Gastric cancer, Kashmir valley, Mutation, OGG1 gene, Polymorphism

I.INTRODUCTION

Stomach cancer refers to any malignant neoplasm that arises from the region extending between the gastro esophageal junction and the pylorus. Approximately 95 per cent of stomach tumors are epithelial in origin and designated as adenocarcinomas. Adenocarcinoma of the stomach, a leading cause of cancer death worldwide is

the second and fourth most common cancer in males and females respectively [1,2]. In 2012, almost 952,000 new cases of gastric cancer were estimated to have occurred in the world (6.8% of all cancer localizations). Stomach cancer represents the third cause of cancer death in the world (723,000 deaths). It accounts for 8.8% of all cancer deaths [3]. Despite advances in diagnosis, the disease is usually detected after invasion of the muscularis propria, because most patients experience vague and nonspecific symptoms in the early stages and the classic triad of anemia, weight loss, and refusal of meat-based foods is seen only in advanced stages. Furthermore, surgery and chemotherapy have limited value in advanced disease and there is a paucity of molecular markers for targeted therapy. Since cancer of the stomach has a very poor prognosis and the 5-year survival rate is only around 20 per cent, a new look at the results of epidemiological and experimental studies is important to establish strategies for primary prevention.

Stomach cancer incidence is known to increase with age with the peak incidence occurring at 60-80 years. Cases in patients younger than 30 years are very rare [4]. In India, the age range for stomach cancer is 35-55 years in the South and 45-55 years in the North. The disease shows a male preponderance in almost all countries, with rates two to four times higher among males than females [5].

A number of polymorphisms in genes that encode DNA repair proteins have been described [6]. Given the known relationship of DNA repair to cancer, polymorphic, variants in the DNA repair enzymes have the potential to be population risk factors for cancer because of the large number of individuals affected. Polymorphism in several DNA repair genes have been described affecting DNA repair capacity and modulate cancer susceptibility by means of gene-environment interactions [7]. For that reason, SNPs in DNA repair genes are an important area of investigation for epidemiology of carcinogenesis.

1.1 DNA repair gene – OGG1: The human 8-oxoguanine glycosylase 1 (OGG1) gene (chromosome 3p26), as a member of the base-excision repair pathway, is regarded as a logical candidate for involvement in the underlying cause of cancer [8], with its expressed protein actively removing the directly 8-hydroxy-2-deoxyguanine (8-OHdG) from DNA. OGG1 is the primary enzyme responsible for the excision of 8-oxo guanine – a mutagenic base product that occurs as a result of exposure to reactive oxygen species [9].

1.2 OGG1 polymorphism and gastric cancer: The OGG1 polymorphism is the important risk factor for gastric carcinoma [10]. This has been evidenced that a variety of factors, including sodium chloride, Helicobacter pylori infection and smoking [10] induce inflammation in the stomach tissue. A considerable inflammatory cell infiltrate in the gastric mucosa causes the production of reactive oxygen species (ROS) [11], and ROS are thought to lead to 8-OHdG accumulation in the gastric mucosa [10], suggesting that the level of inflammatory cell infiltration in the stomach may be one of the factors that determine the 8-OHdG level in the stomach.

II.MATERIALS AND METHODS

2.1 Sample Collection:

The blood samples of Gastric cancer patients were collected in EDTA vials from the Biochemistry department of Shri Maharaja Hari Singh (S.M.H.S) hospital, an associated hospital of Government Medical College Srinagar. Half of the whole blood from each sample were collected in separate EDTA vials, transported to laboratory on ice and stored at -20^{o}C for molecular analysis; and rest of the blood, serum and plasma were separated in eppendorf tubes and stored at -20^{o}C for Biochemical analysis. The information regarding gender, age, cancer stage, occupation and residence were collected from the record file of patients present in the hospital.

2.2 Molecular Analysis:

2.2.1. Extraction of genomic DNA:

For the isolation of genomic DNA, phenol chloroform method was used. The protocol followed was as per Sambrook and Russel method. The DNA eluted was stored at -20^{o}C for further investigation.

2.2.2. Qualitative Analysis of genomic DNA :

The integrity of the genomic DNA was examined by gel electrophoresis using 1 % agarose gel, to which 10 µl /50ml (gel solution) of fluorescent dye ethidium bromide was added during its cooling, the gel was cast into gel tray and 15 µl wells were made into it by using suitable combs. 4 µl of each DNA sample was mixed with 2 µl of 1X DNA loading dye and then the mixture was loaded in the gel. Electric current was applied at 5volts/cm between two electrodes. The power supply was stopped when the dye travelled nearly 2/3 of length of gel. DNA in the gel was visualized with the help of Gel Doc system (BioRad) under UV light and picture was captured by using CCD camera system.

2.2.3. Quantitative Analysis of genomic DNA:

The quantity of DNA was determined by U.V. spectrophotometric method. An aliquot of 5µl of DNA was dissolved in 495µl of TAE buffer and loaded into 500 µl cuvette. Optical density (OD) was determined at 260 nm and 280 nm in UV spectrophotometer (Hitachi U-1800) against TAE buffer as blank sample. The quantity of DNA was calculated using the following formula:

Quantity of DNA (µg/ml) = (Absorbance$_{260}$ x 50 x Dilution factor) (1)

The ratio of absorbance 260/280 was calculated and DNA with ratio of 1.7 – 1.9 was considered for the future use. DNA was alliqouted into four tubes so as to protect damage from freeze thawing and stored in -20^{o}C freezer for longer duration of time.

2.2.3.1. Electrophoresis:

The integrity of the genomic DNA was examined by gel electrophoresis using 1 % agarose gel.

2.2.4. Polymerase Chain Reaction

Amplification of the promoter region of the OGG1 gene was carried out in Eppendorf Gradient Thermal cycler in a 25µl reaction mixture as shown in Table 1.

Table 1: Volume and concentrations of different reagents used in PCR

S.No	Reagent	Concentration	Volume
01	PCR Master mix	2X	12.5 µl
02	Forward primer	10 pmol/µl	1µl
03	Reverse primer	10 pmol/µl	1 µl
04	DNA sample	250 ng/µl	1µl
05	Nuclease free water		9.5 µl
			Total Volume= 25 µl

Information about primers is given below:

Table 2: Primer sequence

Gene	Nature of primers	Primer sequence	Amplicon size
	Forward primer	5′ACTGTCACTAGTCTCACCAG 3′	200 bp

OGG1	Reverse primer	5′ GGAAGGTGCTTGGGGAAT 3′	

Gradient thermal cycler (Eppendorf) was programmed as under, to carry out the PCR amplification of the OGG1 loci as under:

Table 3: Thermal Cycling conditions

S. No.	Steps	Temperature (ºC)	Time	Number of cycles
01	Initial Denaturation	95	10 min	1
02	Denaturation	95	30 sec	35
03	Annealing	58	30 sec	
04	Extension	72	45 sec	
05	Final extension	72	10 min	1
06	Hold	42	5 min	

After completion of PCR, the reaction products were run on 2% agarose gel, with the ladder of 100 bp as marker, to confirm the amplification of desired product. The reaction tubes were kept at -20ºC till further use.

2.2.5. Restriction Fragment Length Polymorphism (RFLP):

The PCR products were digested by the restriction endonuclease Fnu4H1 (Sat I) respectively and the samples were incubated at 37ºC for six hours. The volume of the different reagents used in RFLP is shown in Table 4.

Table 4: Volume of different reagents used in RFLP

S. No.	Reagents	Volume
1	DNA Amplified	10 µl

2	Nuclease Free Water	18 µl
3	Buffer G (1X)	2 µl
4	Fnu4HI	2 µl

The digested products were run on 1.5% agarose gel with ladder of 100 bp as a marker for the visualization of RFLP analysis.

III.RESULTS

The electrophoresis gel view of DNA samples on PCR amplification and restriction digestion was obtained as:

L1 L2 L3 L4 L5 L6 L7 L8 L9 L10

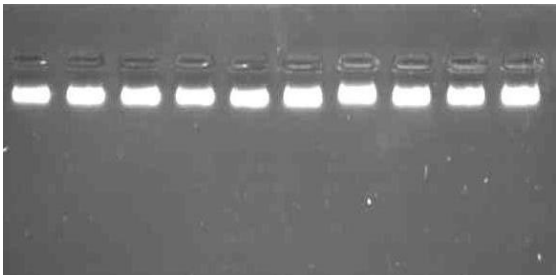

Figure 1: Lane 1-10 showing the isolated DNA of case samples, run on 1% agarose gel.

Ladder L1 L2 L3 L4 L5 L6 L7 L8

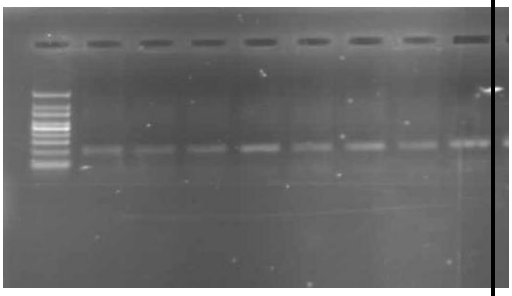

Figure 2: PCR Amplified product of gastric cancer DNA samples run on 2% agarose gel.
(Ladder: 100 bp, Product size: 200 bp)

Ladder L1 L2 L3 L4 L5 L6 L7 L8

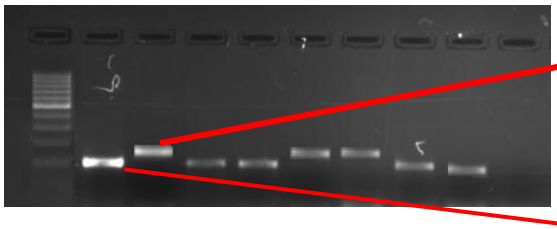

Figure 3: OGG1 Genotype Analysis using PCR-RFLP

200bp

100bp

Ladder– 100 bp

Lanes 2,5,6 - Homozygous mutant

Lanes 1,3,4,7,8 – Heterozygous normal

The genotypes were designated pp (200bp), Pp (100 bp) and PP (100 bp). The prevalence of gastric cancer was highest among homozygous pp patients and lowest in homozygous PP patients. The prevalence of gastric cancer in heterozygous subjects was intermediate between those of the two homozygous patient groups.

Restriction digestion was done to examine the genetic polymorphism in the OGG1 gene. As shown in Fig 3. The genetic polymorphism of OGG1 gene showed 66.0 % (20/30) of the Gastric cancer was homozygous mutant, 26 % (8/30) of the cases however showed heterozygous condition and 6.6 % (2/30) were homozygous normal. Almost all 80% (16/20) of the normal samples showed heterozygous condition except only in four cases where OGG1 gene was found to be Homozygous Mutant, 5% (1/20) & Homozygous normal, 15% (3/20). The allelic association of this polymorphism within Gastric cancer cases and normal controls was evaluated by $\chi 2$ (Chi square) test and the level of significance remained significant (P=0.005).

Frequency of Gastric cancer cases among different genotypes and their histogram representation is shown below:

Table 5: *OGG1* genotype analysis of gastric cancer cases and normal controls.

CASES – 30	**GENOTYPE**	**FREQUENCY**
20	Homozygous Mutant	66.6 % (20/30)
8	Heterozygous	26.6 % (8/30)
2	Homozygous normal	6.6 % (2/30)
CONTROLS – 20		
1	Homozygous Mutant	5 % (1/20)
16	Heterozygous normal	80 % (16/20)
3	Homozygous normal	15 % (3/20)

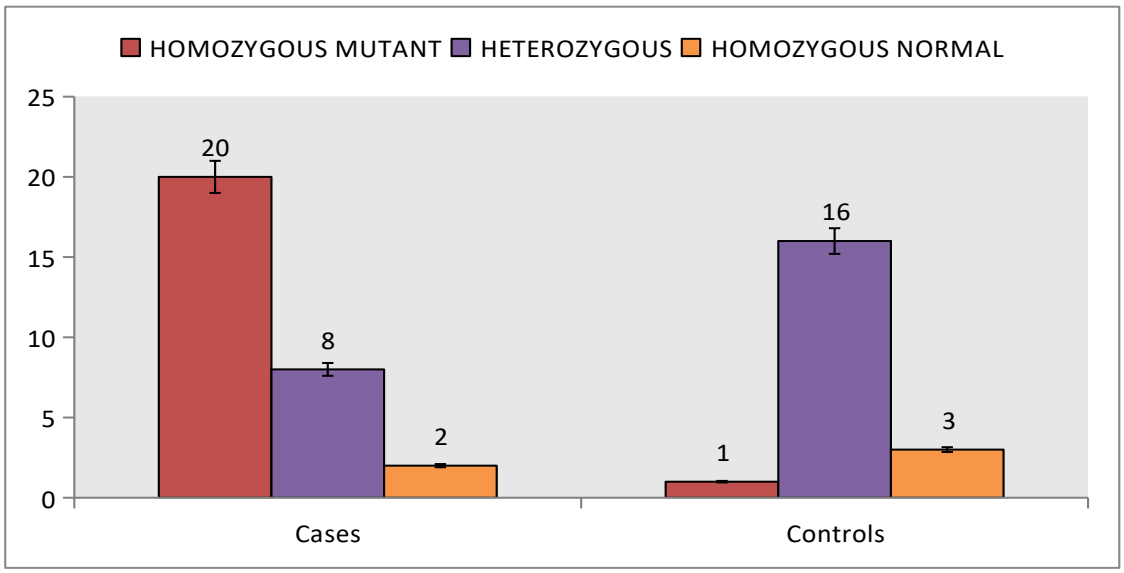

Figure 4: Histogram representing restriction conditions of cases of Gastric cancer and normal controls

The frequency of the polymorphism of OGG1 gene was also studied in relation to varying age-groups. The data obtained was as:

Table 6: Age group polymorphism of *OGG1* gene

TOTAL NUMBER OF CASES (30)			
AGE GROUP (years)	**CASES**	**GENOTYPE**	**FREQUENCY**
30-39	04	2- Homozygous Mutant	50 % (2/4)
		1- Heterozygous	25 % (1/4)
		1- Homozygous normal	25% (1/4)
40-49	06	3- Homozygous Mutant	50%(3/6)
		2- Heterozygous	33.3%(2/6)
		1- Homozygous normal	16.6%(1/6)
50-59	9	6- Homozygous Mutant	66.6%(6/9)
		3- Heterozygous	33%(3/9)

		0- Homozygous normal	0%(0/10)
Above-60	11	9- Homozygous Mutant	81%(9/11)
		2- Heterozygous	22%(2/11)
		0- Homozygous normal	0%(0/10)

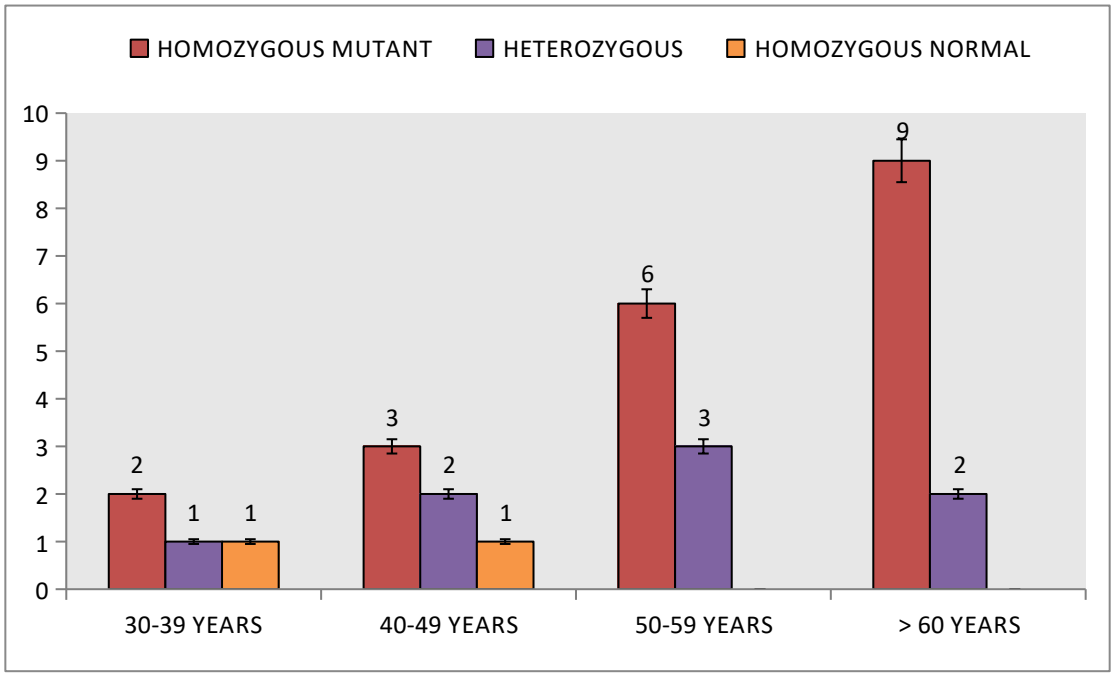

Figure 5: Histogram representing age group polymorphism of OGG1 gene

Homozygous mutant condition of *OGG1* gene was found to be certainly higher in Gastric cancer cases of above 60 years of age (81%) than ages below 60 years (57%) and association was insignificant as p=0.3947

IV.CONCLUSION

The present study found that OGG1 polymorphism is significantly associated with gastric cancer risk in Kashmiri population. So it can be concluded that there is an important role of gene polymorphism of OGG1 gene and may act synergistically to increase the risk of Gastric cancer patients in Kashmiri Valley.

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