

Does hogg1 Ser 326 Cys Polymorphism Play Any Role in Gastric Carcinogenesis: A Case Control Study from Eastern India, Kolkata

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Abstract

Aim: To investigate the association between Ser326Cys human oxoguanine glycosylase1 (hOGG1) polymorphism with gastric carcinogenesis and it's relation with *H.pylori* in eastern Indian population.

In the present prospective study, 168 patients with gastric cancer (GC), 170 subjects with dyspepsia were taken as disease control (DC) and 170 healthy controls (HC) were analyzed by PCR-RFLP method for genotyping of hOGG1 Ser326Cys to detect hOGG1 Ser326Cys (rs1052133) polymorphism. Endoscopic biopsy samples were subjected to Rapid urease test (RUT) and histopathological examination. Statistical analysis was conducted by two-sample t-test for continuous variables and χ^2 test for categorical variables. Logistic regression models were used to find the risk factors for gastric cancer. hOGG1 Ser326Cys genotypes were comparable among three groups. Neither heterozygous (Ser/Cys) nor mutant (Cys/Cys) allele was associated with gastric cancer upon comparison with the Ser/Ser genotype. P value for GC vs. DC was 0.148 and odds ratio (OR) for Ser/Cys was 1.39, (95% CI: 0.89-2.17) and p value for Cys/Cys was 0.436 and OR was 1.42 (95% CI: 0.58-3.47).

Conclusion: hOGG1 Ser326Cys polymorphism was not significantly associated with gastric cancer. No association was found in disease control group.

Keywords: Gastric Cancer, hOGG1, polymorphism, RUT

I. Introduction

Gastric cancer remains one of the most serious health burdens throughout the world, however its incidence is declining¹. Gastric cancer is the fourth most frequent cancer, almost 2/3 cases occur in developing countries². The disease is a multistep and multi factorial process¹. Maintenance of genomic integrity is an important process by which DNA can be guarded by injurious effects of endogenous and exogenous agents where hOGG1 plays an important role. *H.pylori* being regarded as group 1 carcinogen is responsible for inducing gastritis and liberation of cytotoxins, lipase, or phospholipase, or the urease mediated release of toxic ammonia resulting in development of ROS^{3,4}. Among major five types of DNA repair process of which SSBR is widely studied polymorphism among all BER process. The DNA repair enzyme OGG1 is a DNA glycosylase/AP lyase that has been hypothesized to play an important role in preventing carcinogenesis by repairing oxidative damage to DNA⁵. 8-OH-G is highly mutagenic and, if not excised on DNA replication, can cause GC to TA transversions⁶. In human, the OGG1 gene encodes two forms of hOGG1 protein alpha and beta-hOGG, ⁵. It is a major form of DNA damage, which is produced by reactive free radicals. It is suggested that reactive oxygen species (ROS) could induce both base lesions and single strand breaks in DNA⁷. hOGG1 gene encodes a DNA glycosylase/AP lyase which has the ability to suppress the mutagenic effects of 8-hydroxyguanine by catalyzing its removal from oxidized DNA⁸. The hOGG1 protein encoded by the wild-type 326Ser allele exhibited substantially higher DNA repair activity than the 326Cys and if polymorphism of C/G found at position 1245 in the 1 α -specific exon 7 of the hOGG1 results in an amino acid substitution from serine to cysteine in codon 326 therefore resulting altered expression of cell repair⁹. hOGG1 Ser326Cys SNP have suggested conflicting results in different types of cancers around the world. This particular polymorphism has reported conflicting association in till date in many countries. Asian population is found to be susceptible to hOGG1 Ser326Cys polymorphism in most cases of cancer risks¹⁰.

Chinese esophageal cancer study has suggested hOGG1 326 allele might have a role in carcinogenesis¹¹. In Indian scenario GB carcinoma was reported to be significantly associated with Ser326Cys polymorphism (P=.020; OR, 3.6; 95% CI, 1.2-10.7 and P=.015; OR, 7.7; 95% CI, 1.5-40.2, respectively)¹².

II. Materials And Methods

Subject Selection: The study was carried out during a period of 2 years and six months at IPGMER - SSKM Hospital Kolkata and Department of Pathology STM Kolkata. Cases were divided in three groups, gastric cancer GC (n= 168), cases of disease control group DC (n=170) individuals suggestive of dyspepsia and same number (n=170) of healthy control (HC) who never had any complain of disease. Written consent was obtained GC and DC group and healthy controls for the study. The Institutional Ethics Committee approved the study protocol. Endoscopic biopsies were subjected to In house rapid urease test (RUT) and histopathology.

Rapid Urease Test: (In house RUT solution) In house rapid Urease test was performed using 10% Urea solution Phenol red indicator. A change in colour of the urea solution from yellow to pink during considered positive from 4 to 24 hours.

Genomic DNA extraction: Genomic DNA from patients and controls was extracted from 1ml aliquot of buffy coat, which had been kept frozen since blood extraction and processing at -40°C. The entire DNA was extracted by using DNA sure Minikit, Genetix Brand. Before use, dried DNAs were reconstituted with water to a final concentration of 20 ng /ml.

Genotyping: The basic method followed for detecting polymorphism was based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The generated PCR product was detected running the product on 2% agarose gel against a 100bp ladder. Generated products were treated with restriction digestion by Fnu4HI (New England Biolabs) overnight on a water bath. Obtained digested product was run on 1% agarose gel.

Statistical Analysis: The strengths of associations between gastric cancer and the hOGG1 polymorphism were measured as ORs. The ORs were obtained using unconditional logistic regression analysis¹³. Each polymorphism was tested in controls to ensure the fitting with HWE.¹⁴ Statistical analysis was performed using SPSS 15.0 for Windows (SPSS, Chicago, IL, USA) Discrete variables were analyzed by the Pearson χ^2 test and continuous variables by the Student's t test or generalized regression models. Logistic regression models were fitted to find the risk factors for gastric cancer. For all analyses, significance was determined at a level of $P < 0.05$.

III. Results: hOGG1 genotype distribution

Samples were age and sex matched for GC and DC group. The demographic characteristics of the contributors of the study are presented in Table: 1. The male distribution for GC group is 121 (72.0%) and 118 (69.8%), 137 (80.1%) respectively for DC and HC group. The female distribution type also followed the same pattern, where their involvement was 47 (28.0%) for GC group, 52 (30.6%) and 34 (19.9%) for DC and HC group respectively. Mean age calculated in GC was (mean \pm S.D) 50.04 \pm 11.8 years which was comparable to DC 47.06 \pm 11.2 years. Upon comparison neither the hetero type (Ser/Cys) nor mutant type (Cys/Cys) of hOGG1 was associated with the development of GC. No association was found even with the disease control group so that we could consider if there were any significant association for the SNP to develop premalignancy stages. Both the groups were comparable. P value for GC vs. DC was 0.148 and odds ratio (OR) for heterotype (Ser/Cys) was 1.39, (95% CI: 0.89-2.17) and for mutant type p value was 0.436, OR=1.42 (95% CI: 0.58 -3.47). p value for GC vs. HC was Ser/Cys was 0.070 and OR was 1.51 (95% CI: 0.97 – 2.35) and p value 0.191, OR= 1.86 (95% CI: 0.73 – 4.81) for the mutant genotype. The distribution of the wild type allele was 80/168 (47.6%) in GC, 76/168 (45.2%) and 12/168 (7.1%) respectively for heterotype and mutant allele. In disease control group the distribution of wild type was a bit higher than the GC group; 95/170 (55.9%), 65/170 (38.2%) for heterozygous allele and 10 /170(5.9%) for mutant allele. In healthy control group distribution of wild type allele was 100/170 (58.5%), 63/170 (36.8%) and 8/170 (4.7%) for heterozygous and mutant allele respectively.

No association was detected between hOGG1 polymorphism and gastric cancer ($P = 0.61$) either. We found no significant association of hOGG1Ser326Cys with GC risk. p value is higher in GC Vs. DC group whereas GC Vs. HC was lower as .070 than the other group. Both the heterozygous and mutant allele showed the same distribution for the hOGG1 Ser326Cys SNP.

Table 1: Demographical Data—Age and Gender distribution.

Disease Group	Age in years (mean±S.D)	P value
GC (n=168)	50.04±11.8	GC vs DC = 0.018
DC (n=170)	47.06±11.2	
HC (n=170)	36.30±9.1	GC vs HC < 0.001

Table 2: Gender distribution

Disease group	Male	Female	P value	P value
GC (n=168)	121 (72.0%)	47 (28.0%)	GC vs. DC =0.598	GC vs. HC = 0.080
DC (n=170)	118 (69.8%)	52 (30.6%)		
	136 (80.1%)	34 (19.9%)		

Table 3 Genotype hOGG1 distribution and risk evaluation for GC

SNP	GC (n = 168)	DC (n = 170)	HC (n = 170)	GC vs. DC (95% CI)	P value, OR	GC vs. HC (95% CI)	P value, OR
OGG1 Ser326Cys							
Ser/ser (Wild)	80 (47.6%)	95 (55.9%)	100 (58.5%)	1(Reference)		1(Reference)	
Ser/Cys (Hetero)	76 (45.2%)	65 (38.2%)	63 (36.8%)	0.148, 1.39 (0.89-2.17)		0.070, 1.51 (0.97 – 2.35)	
Cys/Cys (Mutant)	12 (7.1%)	10(5.9%)	8 (4.7%)	0.436, 1.42 (0.58 -3.47)		0.191, 1.86 (0.73 – 4.81)	

IV. Discussion

Our study was undertaken to evaluate the effect of hOGG1 Ser326Cys on the development of GC. In comparison of DC and HC no report has been documented till date from this region. There were so many studies conducted worldwide till date on this polymorphism emphasizing on different types of cancers to evaluate the effect of the same. Overall the effect of hOGG1 Ser326Cys on cancer is still conflicting. Among all of them Sun LM et al. was the first to state that hetero (Ser/Cys) and mutant (Cys/Cys) allele had a positive association with atrophic gastritis but not with gastric cancer⁹. Turkish study reported no alliance of Ser326Cys polymorphism with the increased risk of gastric cancer¹⁵ and Japanese were also revealing the same pattern in GC¹⁶. OGG1-Ser326Cys (P=0.0138) showed with a reduced GC risk¹⁷. In India, a study from Kashmir reported no involvement of the Ser326Cys polymorphism with GC development where dietary factors (alcohol and high salted tea consumption) showed the probability towards the development of GC¹⁸. Meta analysis specifically performed on gastric cancer concluded significant association with GC (Cys/Cys) vs. Ser/Cys+Ser/Ser: odds ratio=1.31, 95% confidence interval: 1.03-1.67¹⁹. Li et al. found no relationship between the hOGG1 Ser326Cys polymorphism and increased risk of lung cancer susceptibility except in Asians (OR, 1.18; 95% CI, 1.01-1.38 for Cys/Cys+Ser/Cys versus Ser/Ser; P = 0.32)²⁰, but a significant association seen in Caucasian along with a dose-dependent effect with smoking²¹. But mutant genotype showed an increased risk of squamous cell carcinoma and non adenocarcinoma in lung cancer¹³. No association was reported even in colorectal cancer²², breast cancer²³ but a positive association with gallbladder cancer²⁴, prostate cancer⁸ was reported. A protective nature was exhibited by the variant allele in development of colorectal cancer.²⁵ Mutant (Cys/Cys) genotype showed significantly increased the risk of developing esophageal squamous-cell carcinoma, with OR 1.9 (95% confidence interval [CI] = 1.3-2.6).¹¹ A meta analysis on overall cancer risk and association of the above said polymorphism confirmed a strong association with lung cancer but no association with colorectal, breast, bladder, prostate, esophageal, and gastric cancer¹⁰. Distribution of mutant allele is different among the races, pattern ranges from 12% in Chinese, 27.7% in Japanese²⁶ where our study reflected only 7.1%.

DNA is assaulted on a daily basis by oxidative stress such as ROS, ultraviolet light, or genotoxic agents, guanine is easily oxidized into 8-oxo-7,8-dihydroguanine (8-oxo-Gua) which has the ability to lead transversion mutation such as G-T or G-A binding, accumulation of which can lead to detrimental consequences²⁷. Wild-type OGG1 has the capacity to correct and repair 8-OH-Gua, but OGG1 mutation leading to formation of heterozygous or mutant allele fall short of this capacity remove this harmful oxidized guanine, causing mutation and apoptosis. Alpha hOGG1 protein, having a nuclear localization is responsible for excises 8-OH-G and Fapy-G from gamma-irradiated DNA⁵. The exact scenario is still a puzzle. But in our study the GC vs. DC showed no statistical significance of hOGG1 Ser326Cys polymorphism in GC.

V. Conclusion

In the present study no evidence of association between of hOGG1 Ser326Cys polymorphism and gastric cancer risk was observed.

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