www.jmscr.igmpublication.org Impact Factor 3.79
ISSN (e)-2347-176x
crossref DOI: http://dx.doi.org/10.18535/jmscr/v3i8.03

IGM Publication Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Association of BER Genes Polymorhism, XRCC3 and XRCC1 with Risk of Gastric Carcinogenesis: A Case Control Study from Eastern India

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Abstract
The aim of the study was to investigate the association between XRCC3 Thr241Met and XRCC1 Arg194Trp polymorphism and risk of gastric carcinogenesis in eastern Indian population. A total of 508 subjects (168 patients with gastric cancer, 170 with dyspepsia and 170 controls; age and gender matched) were prospectively investigated for this case control study. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was performed to distinguish XRCC3Thr241Met and XRCC1 Arg194Trp polymorphism and risk of Gastric Carcinoma (GC). Statistical analysis was done by two-sample t-test for continuous variables and χ^2test for categorical variables. Logistic regression models were used to find the risk factors for Gastric cancer. Subjects were matched for age and gender in all three groups except GC Vs. HC group. XRCC1 Arg194Trp genotypes were comparable among three group XRCC3Thr241Met Trp/Met has protective role and Met/Met mutant allele does not show any risk of gastric cancer
Key Words: *Gatsric Cancer, XRCC1, XRCC3, polymorphism*

Introduction

Gastric cancer (GC) still represents the fourth most common cancer in the world and the second leading cause of cancer death[1]. It is a multistep and multi-factorial disease [2] and therefore involves different mechanisms resulting in DNA damage [3]. Damaged DNA leads to production of defective amino acids hence lead to atypical cellular functions. Helicobacter pylori, categorized by IARC as a Group 1 human carcinogen[4] is strongly associated with both types of gastric cancer, irrespective of other underlying unique carcinogenic mechanisms. Risk of carinogenesis in individuals varies according to strain-specific virulence factors, host responses and specific host-microbe interactions.[5] Reduction in DNA repair capacity is associated with polymorphisms of certain genes[6,] Molecular studies have established that genomic instability is a major key step for development of carcinogenesis.[7] Recognition of replication errors (RERs) at microsatellite loci is also a sign of genomic instability.[8] Out of the three variations of genomic instability microsatellite instability (MSI) is thought to be evolved in DNA

mismatch repair pathway,[9] X-ray cross complementing group 1 protein (XRCC1) which acts as a scaffolding protein forms enzyme complexes optimized for single-strand break repair.[10] The XRCC1 gene is 33 kb in length, and is located on chromosome 19q13.2-13.3 [11] .Being a component of BER multi protein complex XRCC1share a strong association with PARP 12 polymorphisms and cancer susceptibility[13]. XRCC3 is located on chromosome 14q32.3 which is a member of Rec A / Rad 51 related protein complex mainly responsible for double stranded break repair[15,16,17]. The present study was aimed at determining the allele and genotype frequencies of the SNPs, XRCC1 Arg194 Trp and, XRCC3Thr241Met, in the Eastern Indian population, residing in and around Kolkata.

Materials and Methods

Subject Selection: During a period of 4.5 years we conducted a case-control study for gastric cancer after the approval of Institutional ethical committee. Subjects were divided in three groups GC, DC&HC respectively. GC group included subjects suggestive of gastric carcinoma on endoscopic examination and subsequently histologically confirmed (Lauren's system) 168 cases of gastric cancer were investigated. Disease control (DC) group (n=170) individuals suggestive of dyspepsia and same number (n=170) of healthy control (HC) who never had any complain of disease were studied.

Sample Collection and Processing: Endoscopic biopsies were collected from GC & DC group, these biopsies were subjected to RUT and histopathological study. Blood sample were collected collected in EDTA vials with informed consent from GC and DC group patients, sera was separated and transferred to eppendroff and stored at -40^{0}C. DNA for genotyping was extacted from EDTA blood by using commercially available kit (DNA sure Blood Minikit, Genetix Brand, Nucleopore NP-61105).

Laboratory Assays

Genotyping: The basic method followed for detecting polymorphism was based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primers used for identification of the XRCC3 Trp241Met polymorphism (eurofins genomics India Pvt. Ltd.). PCR products were then subjected to restriction digestion by NlaIII (New England Biolabs) and PvuII for at 370C for XRCC3 and XRCC1 respectively overnight on a water bath. The enzyme which recognizes 239 and 313 bp for XRCC3 wild type (Thr/Thr) identification and mutant allele (Met/Met) 105, 208, and 239bp bands19. The wild-type allele for XRCC1Arg/Arg was identified by the presence of 485 bp band, while the mutant allele Thr /Thr was represented by a396 and 89 bp bands.

Results

Genotyping XRCC1 Arg194Trp

The observed distribution for XRCC1 mutant (Trp/Tr p) typein 22.0% of GC group where as for the same genotype in healthy control(HC) showed 26.5% and 16.5% in DC (Dsease Control) group respectively. The p value for GC Vs. DC group has shown 0.106, with OR(Odds Ratio) 1.64 (95% CI: 0.90 -2.99) but there was a rise in OR when we compared the data with healthy controls, p value was 0.694, and OR 0.89 (0.51 - 1.57) respectively. We did not found any significant difference in distribution of heterozygous allele; the GC group (43.5%) showed higher distribution than DC and HC showed (41.2%) and (36.5%) respectively. But when it was compared between GC and DC p value was 0.288, with OR=1.29 (95% CI: 0.80 - 2.08). No significant differences were observed while comparing with HC as p value was 0.327, 1.28(95% CI: 0.78 - 2.09).

The XRCC3 Trp241Met distribution for mutant allele (Met/Met) was highest in GC among three groups (8.9%) upon comparison with DC (7.1%) and HC (6.5%) respectively. For mutant allele, GC Vs. DC we found p value was 0.371, with OR=1.44 (95% CI: 0.65 - 3.21) and GC Vs. HC

the p value was 0.695, OR=1.18 (95% CI: 0.52-2.27).

on comparison of GC Vs. DC for heterozygous allele p value obtained was 0.078, with OR=1.61 (95% CI: 0.95 -2.75) and GC Vs. HC exhibited a significant p value of 0.024, =0.58 (95% CI: 0.36 - 0.93) suggesting a increased risk for GC.

Table: 1 XRCC3 Trp241Met distribution and risk evaluation for GC

XRCC3 Trp241Met	GC (n = 168)	DC (n = 170)	HC (n = 170)	GC vs. DC P value, OR (95% CI)	GC vs. HC P value, OR (95% CI)
CC (Thr/Thr) Wild	111 (66.1%)	128 (75.3%)	96 (56.5%)	1(Reference)	1(Reference)
CT (Thr/Met) Hetero	42 (25.0%)	30 (17.6%)	83 (37.1%)	0.078, 1.61 (0.95 -2.75)	0.024, 0.58 (0.36 - 0.93)
TT (Met/Met) Mutant	15 (8.9%)	12(7.1%)	11 (6.5%)	0.371, 1.44 (0.65 - 3.21)	0.695, 1.18 (0.52-2.27)

Table: 2 XRCC1 Arg194Trp distribution and risk evaluation for GC

XRCC1 Arg194Trp	GC (n = 168)	DC (n = 170)	HC (n = 170)	GC vs. DC P value, OR (95% CI)	GC vs. HC P value, OR (95% CI)
TT (Arg/Arg) Wild	58 (34.5%)	72(42.4%)	63(37.1%)	1(Reference)	1(Reference)
TC (Arg/Trp) Hetero	73 (43.5%)	70(41.2%)	62(36.5%)	0.288, 1.29 (0.80 -2.08)	0.327, 1.28 (0.78 - 2.09)
CC (Trp/Trp) Mutant	37 (22.0%)	28(16.5%)	4(26.5%)	0.106, 1.64 (0.90 -2.99)	0.694, 0.89 (0.51 - 1.57)

Discussion

In our study we investigated the effect of XRCC3 Trp241Met and XRCC1 Arg194Trp polymorph-hism on increased risk of gastric cancer in eastern part of India which is reported to have higher incidence of GC. XRCC3 Trp241Met Trp/Met exhibited a higher risk in DC to develop GC. On Comparison with HC there was a significant protective nature seen in Trp/Met allele 0.024, OR=0.58 (95% CI: 0.36 - 0.93).The mutant allele (Met/Met) showed no association of developing GC in DC group [p value 0.371, with OR=1.44 (95% CI: 0.65 - 3.21)], as well as HC group 0.695, OR=1.18 (95% CI: 0.52-2.27). This might be a reason of increased risk of GC in eastern Indian population, and few more factors must be associated to play a role. In this study statistical analysis revealed gene – gene interactions and increased risk of GC in both the cases. Their carriers showed an increased risk to develop the disease. These defects lead to impairment in ability to DNA repair therefore resulting in formation of defective bases and generating neoplastic conditions and finally carcinogenesis. Many studies have been conducted on their effect on different cancers as well as gastric cancers. XRCC3 helps to correct impaired Rad 51 focus formation and elevated chromosome aberrations and protect from DSBR[20]. A study from Turkey showed two fold higher frequency of T allele in GC patients[18]. Chinese study concluded no association of Thr/Met or Met/Met allele and GC [Adjusted ratio (OR(a)), 1.06; 95% confidence interval (CI), 0.52-2.16).][21] GC and control group showed a higher risk along with smoking and alcoholism (2.70, 95%CI = 1.38-5.26 and 4.34, 95%CI = 2.17-9.09) [23].

Being a predominant mechanism for DNA repair mechanism BER consist of scaffolding proteins XRCC1 responsible of SSB. Two polymorphisms of this gene are extensively studied worldwide, Arg194Trp and Arg399Gln respectively. In XRCC1 Arg/Trp allele showed a higher risk for GC OR=1.28 (95% CI: 0.78 - 2.09) even the same association reflected in DC group OR=1.29 (0.80 -2.08). But it is noticeable that mutant allele has shown decreased risk of GC. Korean population reported no significant risk of XRCC1 and GC but

haplotyping showed D for XRCC1 had a risk type for gastric cancer involving gastric cardia (adjusted OR=1.57, 95% CI=0.93-2.65)[25].Shen et al. revealed wild-genotype Arg194Arg is associated with gastric cardia cancer.[14] Ratnasinghe, LD et al. reported no association of XRCC1 Arg 194Trp with cardia cancer in high risk population in China.[23]

Conclusion

From the present study it can be concluded that neither XRCC1 mutant allele nor the heterozygous allele showed a significant association risk of GC. However XRCC3 heterozygous allele (Arg/Trp) allele showed a higher risk. Therefore it might be a risk factor for GC development in this population.

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