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Association of Adipor1 SNP Rs 2275738 and risk of colon cancer

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Abstract
Adiponectin acts its anticancer activity by binding to its receptors. Studies have shown that polymorphisms in the adiponectin receptors are cause in insulin resistance, type 2 diabetes, and colon cancer. The aim of this study is to estimate the prevalence of allele polymorphism of adiponectin receptor 1 rs 2275738 in a population of india and investigate the role of this polymorphism with increase risk of colon cancer.
Methods: The study type is a case - control. 53 patients with positive colon cancer as case group and 53 people as controls group were determined genotyping by using PCR-RFLP.
Results: we observe that the frequencies of CC,CT,TT genotypes for ADIPOR1 rs 2275738 was 22/6, 50/9, 26/5 for patients group and the frequencies of CC,CT,TT genotypes for ADIPOR1 rs 2275738 was 32, 39/6, 28/4 for control group. The allele frequency of C, T was determined 59/4%, 40/6% for patients group and 47/1%, 52/9% for controls. Allele frequency in the two populations showed significant differences between patients and controls groups (The odds ratio = 1.49, CI 95% = 1.09- 2/33, P = 0.01). The significant association was observed between adiponectin receptor polymorphisms and increased risk of colon cancer.
Conclusion: The results of this study indicate that the polymorphisms in the ADIPOR1 could be a risk factor for colon cancer.

Keywords: Polymorphism, Adiponectin Receptor, SNP, Obesity, Colon Cancer.

1. Introduction
Colon cancer is a disease which is malignant cells (cancer cells) is formed in the large intestine. Several factors are involved in the creation and development of colon cancer; age, diet, family history, smoking, drinking and obesity [1]. In addition, the role of genetic factors cannot be neglected in the development of colon cancer. Colorectal carcinoma pathogenesis is complex and multifactorial, and it is evident that multiple genes and genetic pathways involved in the development of cancer [2, 3]. One of the proteins that may be considered as anticancer is adiponectin. Adiponectin with binding to its receptors causes of, Activation of caspases 3,8,9 which is involved in death cell programmed, Prevents of growth and proliferation, Adiponectin is increased the expression levels of BCL2 (a protein that is involved in cell death programmed)[4,5]. Polymorphisms in the adiponectin receptors have been correlated with insulin resistance and colon cancer which is cause of decreased expression of this receptor [6, 7, 8]. Adiponectin receptor 1 is located on large arm of chromosome 1(1q 32.1) and is allocated 17kb [7]. In adiponectin receprot rs2275738 (Nm_ 015999/3) intron 1, Cytosine is replaced by thymidine; the heterozygosity of adiponectin receptor polymorphisms, 0.49 has been reported [9]. Since the decreased expression of adiponectin receptor 1 are associated with insulin resistance, therefore, the study was performed with the aim of evaluate role of adiponectin receptor gene polymorphisms rs 2275738 in increased risk for colon cancer and to measure the prevalence of this polymorphism[10,11,12].

2. Materials and Methods
2.1 Sample Collection:
In this study, 5 ml blood samples were collected in EDTA vials from 53 northeast women patient with colon cancer diagnosed at the Northeast Cancer Hospital, Assam, India and 53 healthy Northeast women from Health Screening Center of Northeast Cancer Hospital, Assam, India between the period February 2013 to December 2014, Information regarding sex, current age, age at colon cancer diagnosis, and ethnic status was recorded. The study has been approved by Gauhati University ethical committee, and the written informed consent to participate (i.e., case and controls) in the study was obtained from all subjects.

2.2 DNA Isolation and PCR-RFLP
Phenol–chloroform method was used to isolate genomic DNA from the collected blood, and the quantity of DNA was determined by spectrophotometer. Amplification of the gene polymorphism regions was carried out by Polymerase Chain Reaction (PCR) and the Restriction Fragment Length Polymorphism (RFLP) was used to identify the Single nucleotide polymorphism region (SNP) of AdipoR1 (RS 2275738). The PCR primer sequences (Invitrogen, India) used for AdipoR1 SNP (RS 2275738) are 5-TTTGTGGGAAGACTCTGGCTGGT-3(forward) and 5-

TTAGTGAGGTTCTGGGTAAAGGTT-3 (reverse). PCR was standardized and carried out for about 34 cycles. The PCR products were separated on 1.5 % agarose gel, visualized with ethidium bromide. The different restriction enzymes (genome diagnostics private limited, India) used to study the respective gene polymorphisms by RFLP method was MSL I. The restriction enzyme digestion was carried out for 37C (overnight; 16 hours), and the products were visualized on 2% agarose gel stained with ethidium bromide "Figure 1".

2.3 Statistical analysis

The allele frequency differences between case and control groups were obtained using used X^2 (Chi- square) test. For each SNP the odds ratios (ORs) with confidence intervals (CIs 95%) were calculated, and the identification of genotypes risk was performed using logistic regression analysis. Pearson $\chi 2$ statistics with threshold of $P<0.05$ for each SNP was used to test the Hardy-Weinberg equilibrium. SPSS 16 software was used to perform the statistical analysis and the possibility less than 0.05, was considered significant. Power and Sample Size Calculations was used to estimate the Power (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize)

3. Results

Genotype groups of patients and control subjects in terms of gender and shown in "Table 1".

The frequencies of CC,CT,TT genotypes for ADIPOR1 rs 2275738 was 22/6, 50/9, 26/5 for patients group and the frequencies of CC,CT,TT genotypes for ADIPOR1 rs 2275738 was 32, 39/6, 28/4 for control group ("Table 2").

The allele frequency of C, T was determined 59/4%, 40/6% for controls group and 47/1%, 52/9% for patients. In addition, it determined, the CT genotype is significantly associated with an increased risk of colorectal cancer (OR = 2/73, CI = 1/36-5/46, P = 0/04), and based on this study, no significant correlation found between mutant genotype TT and colon cancer(OR = 1/95, CI = 0/89- 4/25, P = 0/09), and allele frequency in the two populations showed significant differences between patients and controls group (P = 0/019).In this study male to female ratio in the patients group was 0/89 and in the control group was0/96. Most people in both patients and controls were non-smokers ("Table 3").

4. Discussion

Several published studies have shown an association between obesity and risk of developing prostate, breast and colon cancers, Additionally, there has been evidence that the link between obesity and risk of cancer is due, in part, to obesity-associated reduction in circulating adiponectin levels, but a few studies have been reported for association of adiponectin receptor 1 gene polymorphism and colon cancer. In the present study we demonstrated that adiponectin receptor 1 gene polymorphism is considered to predisposing factor for increased risk of colon cancer, and since the decrease in gene expression of adiponectin receptor 1 are associated with insulin resistance and obesity, we expected the association between these polymorphisms and colon cancer [12, 13]. There is a Hypothesis, on correlation between colon cancer and polymorphisms in the adiponectin receptor 1 gene, which is the polymorphisms in adiponectin receptor 1 gene cause of decreased expression of this protein and thus decrease induce of the anticancer effect of adiponectin on the cells [6,14].

According to the result of this study, polymorphism in ADIPOR1 has increase risk of colon cancer. Furthermore, this study showed that environmental factors such as smoking, cancer family history, alcohol consumption and BMI with adiponectin receptor polymorphisms has influence the increase risk of colon cancer [15]. Kaklamani and his colleagues have shown that decreased expression levels of adiponectin receptor 1 gene polymorphism is associated with breast cancer, prostate cancer and colon cancer [8, 16, 17]. Otani and his colleagues showed that levels of adiponectin receptors in cancer cells is less than in normal cells Whereas, Byun and colleagues showed that high expression of receptors of adiponectin has effection in the progression of colon cancer [18, 19]. In a study in China in 2011, proved that ADIPOR1 gene polymorphism has protective effect against colon cancer.

As we seen in various studies have obtained different results. This difference in findings may relate to racial differences or different environmental factors faced by the population. However, in biochemical pathway may yield similar protein with protein impair compensate for deficiencies its activity, or at least the function of the defective protein is sufficient to promote the route. In this study we were faced with some limitations, including of these limitations could pointed out to the small number of samples due to practical limitations, therefore, we recommended similar studies with larger samples.

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Table 1: Distribution of study subjects according to gender and genotype

	Controls genotype			Patients genotype		
	CC	CT	TT	CC	CT	TT
Male	13 (24/5)	8 (15/1)	7 (13/3)	4 (7/5)	13 (24/5)	8 (15/1)
Female	4 (7/5)	13 (24/5)	8 (15/1)	8 (15/1)	14 (26/4)	6 (11/4)

TT: homozypous mutant, CT: heterozypous, CC: wild homozygous

Table 2: Final results from a polymorphism in adiponectin rs2275738 and colon cancer in patients and controls groups

Genotype	polymorphism adiponectin receptor gene rs2275738				
	Control No %	*Patient No %*	*OR*	*CI (95%)*	*P Value*
CC	17 (32)	12 (22/6)	1		
CT	21 (39/6)	27 (50/9)	2/73	1/36- 5/46	0.04
TT	15 (28/4)	14 (26/5)	1/95	0/8- 4/25	0.09
CT & TT	36 (68)	41 (77/4)	2.33	1.28- 4.23	0.05

Allele frequencies					
C	63 (59/4)	50 (47/1)	1		
T	43 (40/6)	56 (52/9)	1/57	1/073	0.019

In Chi-square test P≤0.05 was statistically significant.

Table 3: Characteristics of patients and control groups

Percent (%)	Control No	Patient No
Age	44/07 ± 17/55	54/59± 12/73
BMI(Kg/m2)	26.0 ±3	27.9 ± 6
Male No	28 (52/8)	25 (47/2)
Female No	25 (47/2)	28 (52/8)
Nonsmoking people	44 (83)	45 (84/9)
people with previous smoking	1 (1/9)	1 (1/9)
Smoking people	8(15/1)	7(13/2)

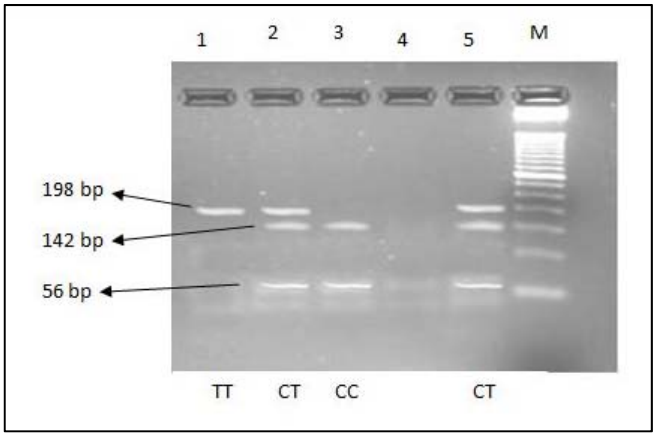

Fig 1: Identification of AdipoR1 polymorphism RS 2275738: MSLI restriction fragments. Lane no. 3 was homozygous wild type (genotyped CC) (142 bp, 56 bp), lanes no. 2 & 5 were heterozygous mutant (genotyped CT) (198 bp, 142 bp, 56 bp) and lanes no. 1 was homozygous mutant (genotyped TT) (198 bp). M, 50 bp DNA ladder.

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