Journal of Cell and Tissue Research Vol. 23(2): 7413-7420 (2023)
(Available online at www.tcrjournals.com)
ISSN: 0973-0028; E-ISSN: 0974-0910

Orignal Article

ASSOCIATION OF ZIP14 GENE POLYMORPHISM IN COLORECTAL CANCER OF TELANGANA & ANDHRA PRADESH COHORT

BEESA, S.,[1] BODIGA, S.,[2] SOWJANYA, P.,[3] AVANCHA, R.,[4] LATHA, J.[4] AND BODIGA, V. L.[1]

[1]Department of Molecular Biology, Institute of Genetics and Hospital for Genetic Diseases (Osmania University), Begumpet, Hyderabad, Telangana. India-500016; [2]Laboratory of Biochemistry Forest College & Research Institute, Mulugu, Siddipet, Telangana; [3]Osmania Medical College, Koti, Hyderabad, Telangana. India-500095; [4] MNJ Institute of Oncology and Regional Cancer Centre, Red Hills, Hyderabad, Telangana, India-500004.
E. mail: suneethabeesa07@gmail.com, Cell: 7842120917

Received: August16, 2023

Abstract: ***Zinc is one of the essential trace elements and is involved in various functions in the body. Zinc dyshomeostasis is caused by the dysfunction of zinc transporters can contribute to the initiation or progression of various cancers including Colon cancer. Incidence rates of Colorectal cancers (CRC) are on high rise in developing countries like India. ZIP14, one of the Zinc transporter plays a major role in inflammation, a hallmark of Colorectal cancer. The mechanism involved in the role of ZIP14 in CRC is not fully understood but, single nucleotide polymorphisms (SNPs) can be used to predict its risk and prognosis. In the present case- control study, we investigated the relationship between ZIP14 SNP rs1051708 A>C, (Chromosome location 8p21.3), and the risk of CRC. The frequency of the polymorphism in the case and control groups was determined using the PCR-RFLP method. The association of age, gender, diet, and smokeless tobacco to CRC was calculated with different statistical parameters. The ZIP14 A>C rs1051708 variation was found to be significantly associated with CRC. However, the frequency of, the C allele independently and in the heterozygote state was significant in CRC association. Diet and usage of smokeless tobacco showed a significant association with CRC in subjects below 50 years of age. It is concluded that the occurrence 'C' allele of ZIP14 (rs1051708) SNP in genotype and Diet, and Tobacco consumption in adults below 50years are associated with CRC susceptibility alarming better awareness and diagnostic modalities leading to the detection of colon cancer among adults below 50 years given increasing incidence in India.***

Keywords: Zinc, Zip14, Polymorphism, PCR-RFLP

Ms. Sunitha Beesa a Research Scholar pursuing fer PhD in Human Genetics in the Department of Molecular Biology from the Institute of Genetics and Hospital for Genetic Diseases, Osmania University Begumpet, Hyderabad and Telangana, India. 500016. She has 5 years of Research experience in *in-vitro*, molecular and human Cancer studies.

Dedication: This work is dedicated to Dr. P.D. Gupta on his 85th birthday.

INTRODUCTION

Colorectal cancer (CRC) ranking as the third most common malignancy and the second most deadly cancer in both genders,is an intimidating health problem worldwide [1]. It reports 10% of all cancers, while the majority of them are from highly developed countries, frequency is growing in middle- and low-income countries. India has the lowest frequency compared to the Western and other countries in South Asia. However, with increasing urbanization, a steady rise in the incidence of CRC is witnessed in India [2]. The aetiology involved in the occurrence and development of CRC is a very complicated and multidimensional process involving Genetic and Environmental as important risk factors. Additionally, numerous epidemiological studies have found an association between unhealthy diets and lifestyles and the occurrence of CRC [3]. Intake of a diet deficient in micronutrients, especially of Zinc, an essential trace mineral, may contribute to an increasing risk of CRC [4]. Metabolism and mechanism of homeostasis of Zinc are regulated in a complex manner for normal cellular functions controlling oxidative stress and regulating inflammatory cytokines. Zinc deficiency may contribute to various health problems, including immune deficiencies and cancers. Various studies have indicated that zinc deficiency may lead to an impaired oxidant defence system, compromised DNA integrity, and damaged DNA repair enzymes, increasing the risk of cancer initiation and progression [5]. Inflammation and oxidative stress have been reported elsewhere to be involved in the initiation and development of CRC [6].

Ingested Zn through food is absorbed through several intestinal Zn transporters and is released into the bloodstream. Circulating Zn is taken up into cells and distributed within the cell. At each step, Zn transporters and metallothioneins play coordinated roles in the transport, distribution, and homeostatic maintenance of Zn. Based on the membrane topology, Zn transporters are divided into two major families, SLC39s/ZIPs(Zrt/Irt-like proteins) and SLC30s/ ZnTs(Zn transporters), which mediate the inward and outward transport of Zn through cell-surface membranes and intracellular organelles.There are 1-14 ZIP transporters encoded in the human genome and are identified at all phylogenetic levels. Their genes are designated SLC39A1-SLC39A14 and encode the proteins ZIP1–ZIP14 respectively[7].

Among the 1-14 ZIP transporters, ZIP14 is arguably the most robustly characterized in terms of function at the integrative level. This protein contains eight transmembrane domains, a histidine-rich motif, and a metalloprotease motif, and is expressed on the plasma membrane and the endocytic vesicle membrane.

Apart from mediating the cellular uptake of zinc and cadmium, it is an important transporter of non-transferrin-bound iron and a critical regulator of manganese homeostasis and plays a major role in glucose homeostasis. Various reports suggest that *ZIP14* is upregulated by proinflammatory conditions, particularly increased interleukin 6 (IL-6) and nitric oxide and inflammation is the hallmark of CRC[8]. Therefore, we hypothesize that variations in the Zip14 gene may limit the availability of intracellular zinc, yielding the unique phenotype of inflammation coupled with cancer.While various reports are available on variations in the ZIP14 gene in different cancers[9]. Reports on ZIP14 gene variations in colon cancer are scarce. In this study, we aim to study the association between the ZIP14 gene (3'UTR variant, rs1051708 A>C) polymorphism to understand the role of Zinc inthe progression of colon cancer.

MATERIALS AND METHODS

Study population: The sample collection for the study extended over four years. Subjects were recruited at the Department of Pathology, MNJ Institute of Oncology & Regional Cancer Centre, Hyderabad, Telangana, India. The selected Hospital is a Government Cancer Hospital which is located in the centre of Hyderabad. According to the hospital records patients from in and around Telangana and Andhra Pradesh state visit the hospital and we consider that the samples collected represent the two states. The recruitment process was initiated following the approval from the institutional ethics committee for biomedical research (Project Approval No. ECR/227/Inst/AP/2013/RR-16).

Inclusion & exclusion criteria: Inclusion criteria for the healthy control group were the willingness to participate in this study, no history of malignant diseases, no condition affecting food and drug intake, and within the age range of 30 to 80 years.The diagnosis of CRC was based on the standard colonoscopy/sigmoidoscopy methods and

Fig 1: PCR product of *ZIP14 A>C* polymorphism

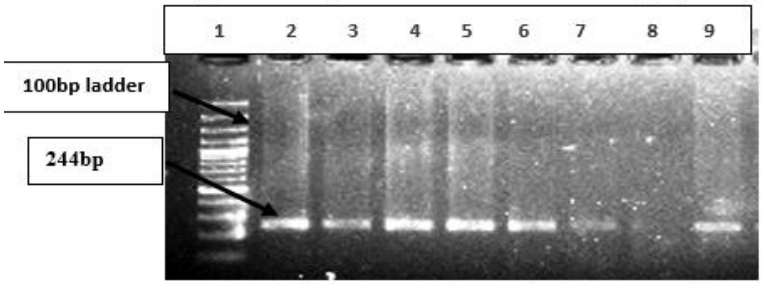


Fig.1: showing ethidium bromide-stained 1.5% agarose gel electrophoresis image of PCR amplified products using PCR primer sets spanning ZIP14 rs1051708. Lane 1 represents a 100bp size marker (GCC Biotech). Lanes 2-9 represent the PCR product of 244bp.

Fig 2: RFLP product of *ZIP14 A>C* Polymorphism digested using *MluCI* enzyme

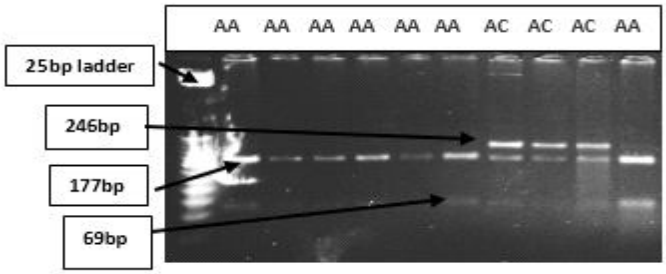
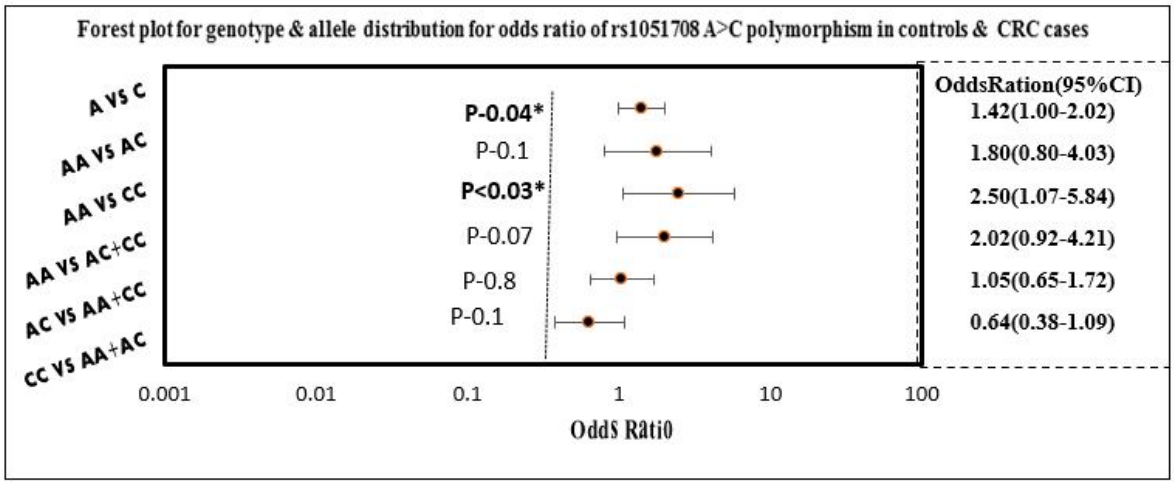


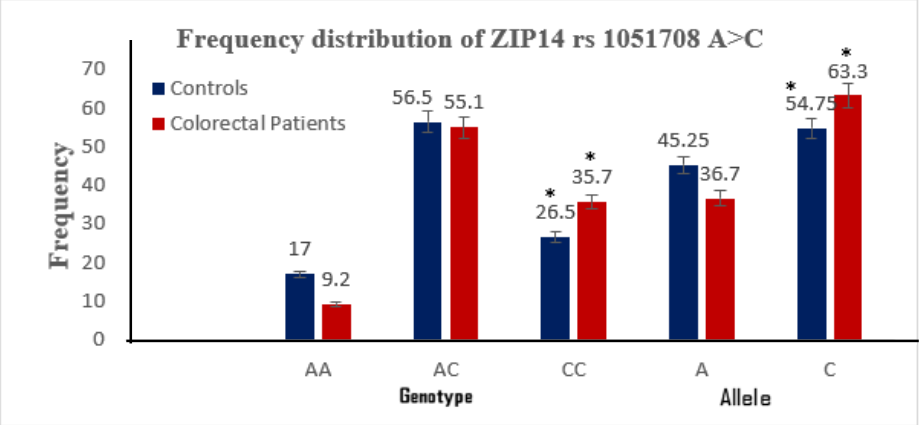
Fig. 2: showing ethidium bromide-stained 3% agarose gel electrophoresis image of RFLP (Restriction Length Polymorphism) products obtained using *MluCI* restriction enzyme (New England Bio labs Fast digest Cat#R0538S) on 244PCR product. Homozygous (AA)shows 175bp+ 69b pfragments, Heterozygous (AC) shows 244bp +175bp +69bp fragments, and Homozygous (CC) shows 244bp fragments. Lane 1 represents a 25bp size marker (Thermo Fisher Scientific). Lanes 2-7 and 11 are showing an AA band pattern. Lines 8-10 are showing the AC band pattern.

Figure: 3 Forest plot representing the odds ratio for ZIP14 A>C *rs1051708* gene polymorphism in CRC patients and controls



Forest plot showing the odds ratios, confidence intervals, and P-value for genotype and allele distribution of ZIP14 rs1051708 (A>C) in the Control and CRC groups

Graph 1: Distribution of ZIP14 rs1051708 A>C genotype and allele frequencies in controls and CRC Subjects



Bar diagramshowing Distribution of ZIP14 rs1051708 A>C genotype and allele frequencies in controls and CRCsubjects. The bar represents the number of subjects in each group(AA, AC, and CC) and the number of subjects with A and C alleles. * On the bar denotes the significance (P<0.05).

Table 1: Genotype distribution, allele frequency, chi-square, odds ratio, and 95% confidence interval (CI) of the ZIP14 rs1051708 A>C in controls and colorectal patients.

Genotype	Controls n=200 (%)	Patients n=98 (%)	χ2 Value	OR(95% CI)	p-Value
AA	34(17%)	9 (9.2%)			
AC	113(56.5%)	54 (55.1%)	2.1	1.80(0.80-4.03)	0.1
CC	53(26.5%)	35 (35.7%)	4.5	2.50(1.07-5.84)	<0.03*
	34(17%)	9 (9.2%)			
AA vs. AC+CC	166(83%)	89(90.8%)	3.24	2.02(0.92-4.21)	0.07
	113(56.5%)	54 (55.1%)			
AC vs. AA+CC	87(43.5%)	44(44.9%)	0.05	1.05(0.65-1.72)	0.8
	53(26.5%)	35 (35.7%)			
CC vs. AA+AC	147(73.5%)	63(64.2%)	2.67	0.64(0.38-1.09)	0.1
A	181(45.25%)	72(36.7%)			
C	219(54.75%)	124(63.3%)	3.89	1.42(1.00-2.02)	<0.04*
HWE	Controls		3.94(0.06)		
χ2 (p-Value)	**Patients**		3.37(0.07)		

AA refers is a wild genotype, and AC+CC refers to the variant; N represents the number of study subjects; the p-value in bold indicates statistical significance ($p< 0.05$); OR: odds ratio; conditional logistic regression models were used to get 95% confidence intervals (95% CI). Adjusted ORs (95% CI) were calculated by conditional logistic regression. P-values were analyzed using χ^2-tests.

histopathological criteria. Cancer cases with metabolic disorders and Genetic Diseases along with exposure to chemo and radiation therapy were excluded.

Sample Collection: All questionnaire data and samples of 90 tissues and 298 blood were collected during the initial recruitment of both the cases and controls. The informed written consent and a self-administered questionnaire regarding the socio-demographic character (e.g.age, family history of cancer, etc.) lifestyle (e.g. smoking habits and alcohol intake) and personal medical history were collected from all the participants. Cases and controls were frequency-matched by age and gender. Subjects in the study population ranged in age from 30 to 80 years, with mean ages ± SD of 39.41±5.45 years in subjects with colon cancer ≤50 years and 62.42±7.01 years in subjects with colon cancer ≥50 years. The mean age in controls was 40.17±7.35 and 64.15±8.65 in the group of ≤50 years and ≥50 years respectively. Among the colon cancer subjects, 59.2% were males and 40.8% were females; among the healthy controls, 68 % were malesand 32% were females, as shown in table 2. All

Table 2: Frequency Distribution Analysis of Demographic and Risk Factors, Age wise characterized in Colorectal Cancer patients and Controls Variable

Characteristic		Age-wise	Healthy Controls (N=200)	Patients (N=98)	p-value
Age		≤ 50(years)	40.17±7.35	39.41±5.45	0.3
		≥ 50(years)	64.15±8.65	62.42±7.01	0.07
Gender	Males	≤ 50	118(59%)	23(23.5%)	0.07
	Females		56(28%)	20(20.4%)	
	Males	≥50	18(9%)	35(35.7%)	0.6
	Females		8(4%)	20(20.4%)	
Diet	Veg	≥50	37(18.5%)	2(2.1%)	<0.01*
	Non-Veg		137(68.5%)	41(41.9%)	
	Veg	≥50	6(3%)	7(7%)	0.2
	Non-Veg		20(10%)	48(49%)	
Tobacco	Present	≥ 50	14(7%)	9(9.2%)	<0.01*
	Absent		16(8%)	34(34.7%)	
	Present	≥50	2(1%)	8(8%)	0.3
	Absent		24(12%)	47(48%)	

N represents the number of study subjects; the p-value in bold indicates statistical significance ($p < 0.05$); OR: odds ratio; conditional logistic regression models were used to get 95% confidence intervals (95% CI). Adjusted ORs (95% CI) were calculated by conditional logistic regression with adjustments for age and gender, diet, and Tobacco consumption. P-values were analyzed using χ^2-tests.SD and SEM stand for standard deviation and standard error of mean respectively.

the control subjects were healthy without any health problems. The non-tumorous tissue10cm away from the tumorous region of the same subject is considered a control.Precancerous tissue adjacent to the tumorous tissue was considered as peri-tumour samples. Each of the 30 tissues was collected for Non-tumorous, Peritumour, and Tumours cases. The tissue samples were used for DNA extraction. Blood samples were stored in ethylene diamine tetra acetic acid (EDTA)-containing tubes stored at -80°C until genomic DNA extraction.

DNA Extraction from blood and tissue samples: Genomic DNA from 5ml of whole blood was extracted by the Phenol chloroform method as mentioned elsewhere [10].Genomic DNA was extracted from Tissue using the QIAGEN DNeasy Tissue Kit (Cat. No./ID: 69581) according to the manufacturer's instructions.

Polymerase chain reaction (PCR): The primers required to perform the PCR reaction were designed by extracting the sequence of adjacent SNP regions. In addition, appropriate primers were designed using Primer Blast software by referring tothe dbSNP database located at http://www.ncbi.nlm.nih.gov/.A conventional polymerase chain reaction (PCR) was performed for the 244bp region by the forward 5'-GCAGCTTCGACCTCATTTTC-3' and reverse 5'-CGCTACCCAAACAGAGAAGC-3' primers for ZIP14 gene (3'UTR variant, rs1051708 A>C). A 25µl reaction mixture consisted of 100ng of genomic DNA, 50 Pico moles reverse and forward primers, 200µM of dNTPS and 1× PCR buffer. A Bio-Rad thermocycler (T100™ Thermal Cycler) was used for amplification with temperature conditions as follows: 5min 95°C, 30s 95°C, 40s 59°C, 40s 72°C repeat 40 cycles from step two,72°C for 5min and 4°C for hold. Subsequently, PCR products were subjected to 1.5% agarose gel electrophoresis. PCR amplicons were analyzed by visualizing gel stained with Ethidium bromide (2µg/ml) under a UV trans-illuminator, represented in figure 1. As a molecular standard, a low molecular weight marker (100 bp ladder 0.5µg/µlGCC Biotech) was used.

Restriction length polymorphism(RFLP): The amplified 244 PCR product was digested with *MluCI* restriction enzyme (New England Biolabs Fast digestCat#R0538S)which recognizes the target sequence and breaks the DNA phosphodiester bonds at the TCTAGTGTSA ¦AATT¦ GGAGCTATTC site. The reaction mixture was prepared using a 10µl PCR product and 5 units of restriction enzyme. The mixture was incubated at 37°C for 15min. The digested product was analyzed for genotyping on 3% agarose gel

and bands were viewed under UV gel documentation. A restriction fragment length of 175bp, 69bp indicates a genotype homozygous for AA, 244bp indicates homozygous for CC and the presence of 244bp, 175bp and 69bp indicates heterozygous for AC.A representative band cut pattern is shown in figure 2.

Statistical Analysis: Open EPI6 software (Open Epi Version 2.3.1, Department of Epidemiology, Rollins School of Public Health, Emory University, and Atlanta, GA 30322, USA) was used for data analysis. Mean and standard deviation was used to express quantitative descriptive findings and frequency and percentage for qualitative data. Independent T-test, ANOVA, and Chi-square were used to analyze normal data and their nonparametric counterparts Mann-Whitney, Kruskal-Wallis, and Fisher tests for non-normal ones. P <0.05 was considered statistically significant.

RESULTS

The present case-control study included 98 (58 male and 40 female) colorectal cancer patients and 200 (136 male and 64 female) healthy control subjects that closely matched the patients in terms of age and sex. The clinical and demographic data of the patients are presented in Table 2. The mean age calculated for cases was 53.40 years and that of controls was 51.06 years. Since no significant differences were observed between cases and controls concerning various characteristics (p>0.05), it suggested that frequency matching was adequate. The SNP rs1051708 A>C,**(**Chromosome location 8p21.3), followed Hardy Weinberg Equilibrium. Minor allele frequency and P value for controls in the study were 0.41(C) and 0.06 respectively.

Relationships of polymorphisms in ZIP14 gene and colorectal cancer susceptibility: The RFLP analysis in thestudy shows the percentage distribution of AA, AC & CC genotypeof ZIP 14 rs1051-708(A>C) was 9(9.2%) AA, 54(55.1%) AC, and 35(35.7%) CC in Cancer samples, and in control was 34 (17%) AA, 113(56.5%) AC and 53(26.5%) respectively as shown inTable 1 shows the obtained genotype and allele frequencies and the significance of the genotype and allele distribution of the tested SNP showed in Graph 1. The results showed that ZIP14 SNP rs1051708 had a statistically significant risk association with CRC patients. The homozygous mutant variant "CC" genotype was found to be predominant with two folds increased risk of CRC and show a significant association (OR: 2.50; 95% CI (1.07-5.84)P value<0.03). Table -1. Independently, the mutant allele "C" (OR: 1.42(1.00-2.02); χ2-3.89; p<0.04) is also significantly associated.However, with wild type (A) allele, "AC" (OR: 1.80(0.80-4.03); χ2-2.1; p:0.1) and in its combinations AA vs AC+CC"(OR: 2.02(0.92-4.21); χ2-3.24; p:0.07),AC vs. AA+CC (OR: 1.05(0.65-1.72); χ2-0.05; p:0.8),CC vs AA+AC (OR: 0.64(0.38-1.09); χ2-2.67; p:0.1) no significance. Fig3 shows A Forest plot depicting the allele and genotype frequency of ZIP14 A>C rs1051708 gene polymorphism.The HWE analysis showed no deviation in both CRCcases and controls.

Relationship of demographic parameters with colorectal cancer Susceptibility: To check the association of cancer susceptibility with age, gender diet, and tobacco consumption of the subjects', further stratified analysis was performed. To assess the association of cancer susceptibility with age, cancer, and control, samples were stratified into two groups as <50 years, and >50 years (split on median age). Frequency Distribution Analysis of Demographic and Risk Factors, Age wise characterized in Colorectal Cancer patients and Controls is shown in Table 2.

Out of 98 cancer samples,43(43.9%) were either below or 50 years of age, and 55(56.1%) were of above 50 years of age. The mean and std age in cases with the age <50 years was 39.41± 5.45 and in cases with age >50 years was 62.42± 7.01 (p-value: 0.3). There was no significant association found between age and cancer susceptibility. Among 98 cancer cases, 58(59.2%) were males and 40(40.8%) were females. Against controls males 136(68%) and females 64(32%), cases did not show a significant association with cancer susceptibility (p-value:0.1). Further males and females were categorized by age <50 years and >50 years. In the <50 years age category males with cancer, were 23(23.5%) and females with cancer were 20(20.4%). In >50 years age category males with 35(35.7%) and females were 20(20.4%). Out 98 cancer subjects 9(9.1%) were vegetarians and 89(90.8%) were non-vegetarians. Vegetarians and non-vegetarians were further stratified by age <50 years and >50 years and found that cancer susceptibility was significantly less among vegetarians <50 years (p-value : < 0.01). Similarly,

controls were also analyzed and the results were shown in the same Table 2. 18(18.3%) cancer subjects among 98 consumed tobacco,and 80 (81.6%) did not consume Tobacco in any form. Stratified data on tobacco consumption among <50 years and >50 years males and females as data shown in Table 2**.** Subjects <50 years of age who consumed tobacco in any form had significant susceptibility to CRC(p-value < 0.01).

DISCUSSION

Although various studies have reported polymorphisms of the ZIP family in different cancers. Very few reports are available on the ZIP14 gene and no studies from Telangana and Andhra Pradesh. While ZIP14 rs1051708 SNP studied elsewhere [11] reported a significant association to regulate Zinc levels in seminal plasmato our best knowledge, this study is the first of its kind to investigate the impact of the ZIP14 (rs1051708) SNP on colorectal cancer association in a Telangana and AndhraPradesh population.

In the overall case-control analysis and stratified analysis, the ZIP14 (rs1051708) SNP showed any significant association with cancer risk. There was a significant increase in the CC genotype of colorectal cancer patients compared to controls (OR: 2.5(1.07-5.84); p<0.03). The minor allele "C" (OR: 1.42(1.00-2.02); $\chi 2 = 3.89$; p<0.04) and additive genotype in the Co-dominant expression "AC+CC" showed lowered significant association.As stated,a single copy of the mutated allele is adequate to transform a proto-oncogene into an activated oncogene, while the non-mutated, wild-type (WT) alleleis involved in amplification[18]. We assume that homozygous mutant genotype "CC"of ZIP14 (rs1051708) in protooncogene to oncogene conversion andwith wild type (A) it is involved in the amplification of the gene.While this data is from the DNA of the blood samples. A parallel analysis was performed on tissue DNA which showed a similar frequency of polymorphism to that of genomic DNA isolated from blood. These results indicate that the polymorphism in the ZIP14 gene atrs1051708 is genetic and not a sporadic mutation.

CRC is a disease that has both biological sex differences and socio-cultural gender components. The stratified analysis of our study showed that Males above 50 years of age were more in number compared to females. However, the association was not significant. The association of gender to cancer may be statistically insignificant due to the low sample size (only 98 cancer samples), but the numbers that 58(59.2%) were males and 40(40.8%) were females suggesting that CRC varies between genders, which was consistent with GLOBICAN data and other recent reports[12] that suggested,there is a global trend for men to have both higher incidence and mortality for CRC.

Dietary factors have been implicated as important sources of modifiable risk for colorectal cancer [14]. In the present study analysis of Diet effect on CRC, it was found that non-vegetarians below 50 years who had cancer were more in number compared to those above 50 years of age and showed significant association with cancer susceptibility. The results are consistent with prior evidence made elsewhere [13] where it is reported that Vegetarian dietary patterns might be expected to be associated with a lower risk of colorectal cancer. The consumption of red meat, especially processed meats is often linked to an increased risk of colorectal cancers.Physical activity, healthy weight avoiding smoking, tobacco and alcohol consumption and daily usage of fruits, fibres, and vegetables such as Broccoli, Brussels Cauliflower, Cabbage, and Spouts reduces the risk of CRC.

Smoking or any form of Tobacco consumption was established as a causal factor for CRC a decade ago [15]. The most prevalent form of tobacco use in India is smokeless tobacco and commonly used products are khaini, gutkha, betel quid with tobacco, and zarda [16]. In our study,we found an association between CRC and tobacco consumption. 18.3% of cancer subjects were using smokeless tobaccowhile only 8% of controls were using it. A stratified analysis of our data found that tobacco consumption among subjects below 50 years of age had a significant risk to cause CRC. Similar data was found in the studies that are reported to date. Those using smokeless tobacco were 2.66 times more likely to have colorectal cancer compared to non-users. The association of diet and tobacco consumption to CRC among subjects <50 years of age documented in this study, probably reflects the actual increase in incidence in India and better awareness and diagnostic modalities leading to the detection of colon cancer in younger adults [17].

Although the current study is the first to report the ZIP14 gene (3'UTR variant, rs1051708 A>C) in CRC samplesit has some limitations. The sample size ofthe study could be very small to get stringent significancedespitecalculating the sample size as per thereported incidence of CRC. Secondly, other regions of the ZIP14 gene should be spanned for mutations to understand the exact role of ZIP14 in CRCs.Third, there were limited numbers of subjects in some subgroups when stratifying by risk to CRCs. This can be improved by future studies including largersample sizes.

Despite the a fore mentioned limitations, we conclude that the 'C' allele of ZIP14 (rs1051708) SNP, Diet, and Tobacco consumption are at risk and associated with CRC susceptibility. Future longitudinalstudies with larger sample sizes are needed to validate thesefindings.

Acknowledgement: The cooperation extended by theprincipals of MNJ Institute of Oncology & Regional Cancer Centre, Hyderabad, and staff is highly acknowledged.

REFERENCES

[1] Xi Y, Xu P.: Global colorectal cancer burden in 2020 and projections to 2040.Transl Oncol.,10:101174 (2021).

[2] N. Keum and E. Giovannucci.:"Global burden of colorectal cancer. emerging trends, risk factors and prevention strategies,"Nature Reviews Gastroenterology & Hepatology., 16, 12: 713–732 (2019).

[3] Willett WC. Micronutrients and cancer risk.:Am J ClinNutr., 5(1994).

[4] Wang J, Zhao H, Xu Z, Cheng X.: Zinc dysregulation in cancers and its potential as a therapeutic target. Cancer Biol Med., 17. 3:612-625 (2020).

[5] Hojyo S, Fukada T.: Roles of Zinc Signaling in the Immune System. J Immunol Res.,(2016)

[6] Bardelcikova A, Soltys J, Mojzis J.: Oxidative Stress, Inflammation and Colorectal Cancer: An Overview. Antioxidants (Basel). 12.4:901 (2023)

[7] Jeong J, Eide DJ.:The SLC39 family of zinc transporters. Mol Aspects Med. 34.2-3:612-9 (2013).

[8] Iyer AS, Shaik MR, Raufman JP, Xie G.: The Roles of Zinc Finger Proteins in Colorectal Cancer. Int J Mol Sci., 24:12(2023)

[9] Liu L, Yang J, Wang C.:Analysis of the prognostic significance of solute carrier (SLC) family 39 genes in breast cancer. Biosci Rep., 40:8(2020)

[10]Barker.: Phenol-Chloroform Isoamyl Alcohol (PCI) DNA Extraction. (1998).

[11]Deng P, Han X, Ma J, Huang X, Sun B, Geng Y, Zheng B, Wang S.:Association of SNPs in zinc transporter genes on seminal plasma zinc levels in humans.Biometals.,955-965 (2022).

[12] White A, Ironmonger L, Steele RJC, Ormiston-Smith N, Crawford C, Seims A.: A review of sex-related differences in colorectal cancer incidence, screening uptake, routes to diagnosis, cancer stage and survival in the UK. BMC Cancer.,18.1: 906 (2018)

[13] Continuous Update Project Report: Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer. Washington, DC. : American Institute for Cancer Research.: World Cancer Research Fund/ American Institute for Cancer Research (2021)

[14] Orlich MJ, Singh PN, Sabaté J, Fan J, Sveen L, Bennett H, Knutsen SF, Beeson WL, Jaceldo-Siegl K, Butler TL, Herring RP, Fraser GE.: Vegetarian dietary patterns and the risk of colorectal cancers. JAMA Intern Med. 5.5:767-76 (2015).

[15]Sharma, P, Cheah, N.P., Kaur, J.: Physical and chemical characterization of smokeless tobacco products in India. Sci Rep 13: 8901 (2023).

[16] Gram IT, Park SY, Wilkens LR, Haiman CA, Le Marchand L.: Smoking-Related Risks of Colorectal Cancer by Anatomical Subsite and Sex. Am J Epidemiol. 189.6:543-553 (2020).

[17] Haleshappa RA, Rao SA, Garg S, Kuntegowdanahalli CL, Kanakasetty GB, Dasappa L.: Is Colorectal Cancer in Young (<40 Years) Different from those in the Elderly (>40 Years): Experience from a Regional Care Centre. Indian J Med PaediatrOncol., 8.4:466-470(2017).

[18]Yu CC, Qiu W, Juang CS, Mansukhani MM, Halmos B, Su GH.: Mutant allele-specific imbalance in oncogenes with copy number alterations: Occurrence, mechanisms, and potential clinical implications. Cancer Lett.,384:86-93(2017)