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Analysis of Genetic Alterations in Colorectal Cancer (CRC) Patients in South Indian Population

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ABSTRACT Colorectal cancer is the third most common type of cancer and third topmost cause of cancer death in the world. Majority of the colorectal cancer is sporadic (65-80%) with a family history of the disease (15-30%). Only five percent is due to hereditary mutations in major genes. Tumorigenesis of colorectal cancer is due to chromosome instability, microsatellite instability and CpG island methylator phenotype involve various tumor suppressor genes and proto-oncogenes in the deoxyribonucleic acid. Chromosome instability proceeds by two ways, aneuploidy through which loss/gain of whole chromosomes and gain or loss of regions of the chromosome. The loss of function of a gene occurs in the first stage of cancerogenesis, in addition a change of methylation pattern of many key genes can develop colorectal cancer. The paper depicts the incidence rate, mortality rate, risk factors and prevention of colorectal cancer.

INTRODUCTION

Colorectal cancer (CRC) develops in more than one million individuals every year with a specific mortality rate of approximately thirty-three percent worldwide (Testa et al. 2018). The gastrointestinal cancer mainly affects the colon diagnosed in 250,000 new cases each year and constitutes nine percent of all malignancies (Labianca et al. 2010; Ferlay et al. 2013; Alharbi 2017). Generally, CRC occurs sporadically in sixty-five to eighty percent of the affected, fifteen to thirty percent among patients with family history of the disease and only five percent due to hereditary mutations in major genes (Migliore et al. 2011; Giglia and Chu 2016). The risk of CRC increases with industrialization, urbanization and environment factors. Diet is also a well-known exogenous reason of cause of CRC (Mármol et al. 2017). CRC starts with a sequence of the clinical and histopathological stage from benign tumors to malignant cancers usually explained in tumor node metastasis as described by Ntagirabiri et al. (2016). Usually, the depth of tumor invasion defines stage and extends from an invasion of submucosa into serosa of the wall (Wolpin and Mayer 2008; Dukes 1932). CRC is a multi-step disorder (Vaiopoulos et al. 2014) or a multi-hit model (Fearon and Vogelstein 1990) that involves the growth of genetic mutations in suppressor genes and oncogenes for progression (Slattery et al. 2017). The inactivation of adenomatous polyposis coli (APC) gene is the most basic cause mutation in CRC pathway. Genetic alterations in other tumor suppressor genes (APC, SMAD2, SMAD4 and TP53), oncogenes (KRAS) and several other genes drive a tumor

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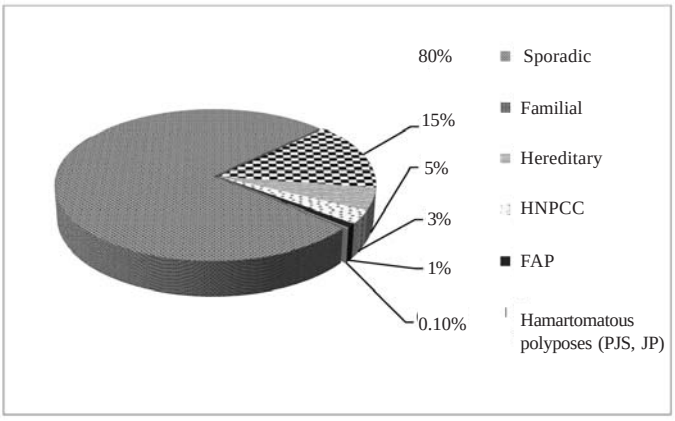

Fig. 1. Graph depicting the marked genotypic and phenotypic heterogeneity in colorectal cancer. Abbreviations: HNPCC, hereditary nonpolyposis colorectal cancer; FAP, familial adenomatous polyposis; PJS, Peutz-Jeghers syndrome; JP, juvenile polyposis

toward metastasis (Migliore et al. 2011; Carethers and Jung 2015; Jauhri et al. 2017; Qi and Ding 2017). Deregulation of gene-expression of tumor suppressor gene and oncogene can take place by epigenetic changes in their promoters (Kazanets et al. 2016).

Objectives

The aim of this paper is to investigate chromosomal and gene variants to identify the genes expression pattern in South Indian population mainly targeting the screening of APC, TP53 and MTHFR gene variants in CRC patients.

Genetics of Colorectal Cancer

Globally, colorectal cancer is third most diagnosed cancer in man and second in women (Marley and Nan 2016; Bhandari et al. 2017). A survey of the diagnoses reveals a two-three fold increase risk of CRC among the first degree relative above 50 years of age whereas three-six fold high risk below 45 years of age was observed (Jasperson et al. 2010; Samadder et al. 2015). The risk factors of CRC also involves the presence of serrated adenomas, serrated polyp and hyperplastic polyps. The dietary habits including red meat, fatty food, cigarette smoking, alcohol intake, anti-inflammatory drugs, sedentary lifestyle, abdominal obesity and body mass index are the major possibilities for CRC (Zhu et al. 2014; Shaw et al. 2017; Zhao et al. 2017; Nunez et al. 2018; Rossi et al. 2018). The genotypic and phenotypic heterogeneity in CRC shown in Figure 1.

Major Genes of Colorectal Cancer

Association of various major genes including APC, MUTYH, MLH1, MSH2, MSH6, MTHFR, PMS2, TP53, TACSTD1, STK11, SMAD2, 4, BMPR1A and PTEN were identified in CRC (Ngeow et al. 2013; Khan et al 2017; Hankey et al. 2018). The study of these genes has led to identification of several CRC syndromes (Table 1). The APC gene is (100%) dominant in FAP syndrome and PTEN showed rare lifetime risk for

Table 1: Major genes and syndromes in CRC

Genes	*Syndrome*	*Inheritance*	*Lifetime CRC risk*
APC	FAP	Autosomal dominant	100%
APC	AFAP	Autosomal dominant	69%
MUTYH	MAP	Autosomal recessive	80%
MLH1, MSH2, MSH6, PMS2	LS	Autosomal dominant	80%
STK11	PJS	Autosomal dominant	39%
SMAD4, BMPR1A	JPS	Autosomal dominant	39%
PTEN	CS	Autosomal dominant	Rare

CRC. APC gene is located on chromosome 5q at band 22 (5q22.2), consist of 21 exons, the transcript is 9.0 kb DNA and protein contains 2843 amino acids (Fearnhead et al. 2001). TP53 is a tumor suppressor gene located on short (p) arm of chromosome 17p (17p13.1), a mutation in this gene later transits from adenoma to carcinomas (Baker et al. 1989). SMAD4 is an important tumor suppressor gene, located on 18q 21.2, consist of 13 exons and 49.5 kb of deoxyribonucleic acid (DNA) reported by Wrana (2000). MTHFR gene converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and co-substrate for homocysteine remethylation to methionine may also increase the risk of CRC (Yu et al. 2010; Xu et al. 2017; Zhang et al. 2017).

Genetic Syndromes of Colorectal Cancer

There are FAP (familial adenomatous polyposis), AFAP (attenuated FAP), MAP (MUTYH-associated polyposis) and HNPCC (hereditary nonpolyposis colorectal cancer) as explained by Talseth-Palmer (2017). Some rare syndrome includes LS (Lynch syndrome), PJS (Peutz-Jeghers syndrome), JPS (Juvenile polyposis syndrome) and hyperplastic polyposis (Mishra and Hall 2012; Huiying et al. 2018). FAP is characterized by the development of a number of adenomas in the colon after first ten years of life and equally affected both male and female. In CRC cases, FAP accounts for less than one percent with the prevalence of 1/11,300-37600 in European countries (Half et al. 2009). AFAP is characterized by less than hundred colorectal adenomatous polyps and an average of sixty-nine percent risk of CRC (Jasperson et al. 2010; Roncucci et al. 2017). HNPCC is autosomal dominant inheritance patients regularly develop CRC below the age of 45 and one-third patients develop another tumor HNPCC within ten years (Steinke et al. 2013). Mutation in methyl mismatch repair genes caused HNPCC approximately three percent of the total CRC (Lynch et al. 2014). Table 2 has lists some examples of gene associated with increased risk of CRC.

Cytogenetic of Colorectal Cancer

Three major molecules of genomic instability included chromosomal instability (CIN), microsatellite instability (MIN) and the CpG island methylator phenotype (CIMP) in cytogenetics study of CRC (Pino and Chung 2010; Birgisson et al. 2015; Guerra et al. 2017). It was found that forty seven percent CIN and thirteen percent MIN is involved in instability of gene of CRC patients (Lengauer et al. 1998; Hamzehzadeh et al. 2017). CIN, MIN and CIMP pathway are not mutually exclusive, tumor shows the multiple pathways features and nature of overlap is still not determined (Muleris et al. 2008; Pino and Chung 2010; Kanthan et al. 2012; Mundade et al. 2014). In CRC, CIN is the most common type of chromosome instability nearly eighty five percent (Vargas-Rondón et al. 2018). The most recurrent alter-

Table 2: Some genes associated with CRC risk

Gene	*Type of study*	*Comment*
APC	Genetic association studies	The APC I1307K associated with increased risk of CRC (Leshno et al. 2016).
MTHFR	Meta-analysis of genetic association studies	The MTHFR 677C>T associated with increased risk of CRC (Teng et al. 2013).
SMAD7	Genetic association studies and GWAS	The SMAD7 variants associated with increased risk of CRC (Huang et al. 2016).
COX2	Meta-analysis of genetic association studies	The promoter polymorphisms associated with increased risk of CRC (Cossiolo et al. 2017).
MTR	Meta-analysis of genetic association studies	The MTR 2756A>G associated with increased risk of CRC (Morita et al. 2013).
GSTT1	Meta-analysis of genetic association studies	The GSTT1 null genotype associated with increased risk of CRC (Nissar et al. 2016).
GSTM1	Meta-analysis of genetic association studies	The GSTM1 null genotype associated with increased risk of CRC (Zhao et al. 2013).
NATs	Gene-environment interaction	The interaction between NATs polymorphisms and smoking status affect risk of CRC (Song et al. 2017).
IGF1	Genetic association studies	The IGF1 promoter polymorphisms associated with HNPCC age at onset of CRC (Reeves et al. 2008).

ations found in all cytogenetic studies performed in primary tumors and infixed CRC tissue blocks (Table 3). First classical cytogenetic study on cancer cells from colorectal adenocarcinomas was described by Dutrillaux (1988).

Epigenetic of Colorectal Cancer

An epigenetic change is the DNA methylation, an addition of a methyl (CH3) group to the fifth position of pyrimidine ring of cytosine without alterations in DNA sequence (Moore et al. 2013; Tse et al. 2017). In mammals, ninety to ninety eight percent DNA CpG sites are methylated but most of CpG not methylated are particularly in CpG rich areas studied by Jin et al. (2011). The high methylation of cytosine in CpG island of tumor suppressor gene promoters can provide a guideline to block transcription in the cytoplasm (Ashktorab and Brim 2014). Epigenetic alterations in CRC includes APC, MGMT, CDKN2A/P14/P15, TP16, P73, UNC5C, MLH1/2, HLTF, DCC, RUNX3, HACE1, ADAM23, DLEC1, RGC-32, miRNA124a, miR-34b/c, miR-9-1, miR-129-2, R137, miR-21, miR-143 and miR-135 (Goto et al. 2010; Li et al. 2011; Schee et al. 2012; Sefrioui et al. 2017; Wang et al. 2017).

METHODOLOGY

Subject Recruitment

In this paper, the researchers collected 65 samples from CRC patients. An equal number of normal and healthy individuals were selected as controls including those who have not exposed themselves to any kind of chemicals or radiation. The patients and the controls were divided into two groups based on age (Group I <50 years and group II > 50 years). Average patient age in group I was *n*=30 and in the group II was *n*=35 respectively. Peripheral blood samples of patients and control subjects were collected using the heparinized syringe for leucocyte culture. Chromosomal preparations obtained were processed and stained with Giemsa to obtain G-bands. The anatomical distribution of the tumor was as follows: right bowel (cecum, appendix, ascending colon, hepatic flexure, transverse colon) and left bowel (descending colon, sigmoid colon and sigmoid junction and rectum). Tumor grades were separated into three categories; well-differentiated, moderately differentiated and poorly differentiated tumor.

Sample Collection

For each study, 5 mL of blood was drawn from the participants by vein puncture and collected in sterile tubes containing EDTA and heparin to be used for cytogenetic and molecular assays.

Chromosome Aberration Assay

Cytogenetic techniques such as conventional chromosomal analysis (karyotyping) using Trypsin G-Banding were studied. Cultures of leucocytes obtained from peripheral blood were set-up as described in the protocol (Moorhead et al. 1960).

APC, TP53 and MTHFR Genotyping

DNA was isolated from the samples and the frequency of the genotypes was evaluated in 65 CRC patients along with an equal number of healthy controls. The APC, TP53 and MTHFR genotypes were determined by PCR-RFLP.

Table 3: Most frequent alterations found in CRC

Chromosome loss	*Chromosome gain*	*References*
18q	13q	Fensterer et al. 2007
18q	20q	De Angelis et al. 1999
18q21		Baker et al. 1989
	20q13	Korn et al. 1999
18, 17p, 8p, 1p3, Y	20, 13, 12, 7,6, X	Muleris et al. 2008
18p, 14q, 4	19, 17p, 17q, 12p, 1q11	Diep et al. 2006
18p21-pter, 18q12-21, 17p12-13, 15q11-q21	20q13, 13p14-31, 8q23-ter	Hermsen et al. 2002
18q, 18, 8p	20, 20q, 13, 8q, 7, 5, 5p, 5q, 3, 3q, X	Knutsen et al. 2010
18q, 15q21, 8p, 4q26, 1p22	20, 20q13, 16q24.3, 8q, 8q28	Camps et al. 2006
21, 18, 17p, 14, 5q, 4, 1p	12, 8,7, 5, X	Dutrillaux 1988

RESULTS

A total of 130 subjects including 65 CRC patients and 65 controls were recruited. The study represents the lifestyle characteristics, location site, tumor grade, family history, follow-up and clinical pathological analysis of the subjects. The CRC patients and controls were divided into two groups based on their age as the group I < 50 years and group II > 50 years. Average patient age of group I was *n*=30 (45.76 ± 2.17) and group II was *n*=35 (61.77 ± 6.98). The subjects recruited includes male *n*=37 (56.92%) and female *n*=28 (43.07%), smokers *n*=44 (67.69%) and non–smokers *n*=21 (32.30%), alcoholic *n*=42 (64.61%) and non-alcoholic *n*=23 (38.46%), and sporadic *n*=40 (64.61%) and hereditary *n*=25 (38.46%) respectively. The demographic characteristics such as age, sex and lifestyle factors including alcohol consumption and smoking status, tumor stage, tumor grade, location site and patient history were taken into account. All the subjects were recruited consecutively with controls being matched to the respective CRC subjects in terms of age with ±2 years relaxed.

Chromosomal damages of CRC patients and controls are divided into Chromatid-type aberrations (CTAs) and Chromosomal type aberrations (CSAs). CTAs in group I and group II CRC subjects were found to be significant when compared to their group I and II controls. The CSAs of group I and II CRC were showed highly significant compared to their group I and II controls respectively. The values of total Chromosomal alterations (CAs) in group I and II CRC showed statistical significance when compared to their controls respectively. All the CRC subjects showed significant values by ANOVA at $p<0.05$ level. The results of this paper depict the detailed karyotype finding in CRC patients. The deletions were observed in 17p, 5p, 21(p), 18q, 22p, 18q, 15p and 1p. The higher percentage of deletions found was 46, XY, del 18p- and translocation was 46, XY. The chromosomal alterations were observed in Stage I CTAs, Stage II CTAs, Stage III CTAs and Stage IV CTAs in CRC patients. In CRC patients, the group II subjects, especially in stage III and IV showed statistically significant values in CA, compared to other groups.

Genotype distributions among control groups were in agreement with Hardy-Weinberg equilibrium with the exception of APC, TP53 and MTHFR polymorphism. The frequency of APC, TP53 and MTHFR genotype were measured among controls and CRC patients. The genotype distribution patterns were followed by Hardy-Weinberg equilibrium.

DISCUSSION

CRC is a disease in which normal cells in the lining of the colon or rectum begin to change, start to grow uncontrollably and no longer die. Genetic and environmental factors including diet and lifestyle may play a major role in the carcinogenesis of CRC (Hughes et al. 2017). The heterogeneous prototypes of tumor mutations suggest the presence of multiple alternative genetic pathways for CRC and it was also speculated that the widely accepted genetic model of cancer development is not a representative of the majority of CRC (Malhotra et al. 2013). Tumor markers are antigens and bioactive substances produced by tumor cells because of the irregular expression of correlated genes. The MAP is an autonomous recessive disorder considered by adenomatous polyps of the colon and high risk of CRC (Aretz et al. 2013; Ma et al. 2018). The MAP is caused by biallelic mutation of MUTYH gene. PJS is distinguished by the presence of hamartomatous polyps in the colon, involved in various CRC patients and prevalence is 1 in 8300 to 280,000 (Kopacova et al. 2009; Beggs et al. 2010). JPS a rare disorder identified by the presence of hamartomatous polyp throughout colon estimated thirty-nine percent risk of CRC (Campos et al. 2015; Ahmed and Alsaleem 2017).

CEA is a soluble glycoprotein, which has been reported to be markedly elevated in patients with digestive tract cancer, most conspicuously those involving the colon and rectum (Ren et al. 2006; Thomas et al. 2015). CRCs are characterized by multiple chromosomal abnormalities. Recent studies addressing the characterization and identification of distinct pathways of tumor progression suggests that there are several important correlations between the selection of any specific type of genetic pathway and variations of the clinical outcome in stage I to IV CRC patients.

These broadly defined alterations are in perfect agreement with chromosome specific trends in researchers expression data, especially the exclusive presence of alterations on chromosome 1, 4, 5, 8, 13, 17, 18 and 20. Deletions observed

were 17p, 5p, 21(p), 18q, 22p, 18q22.q23, 15p and 1p 36. The higher percentage of deletions found was 46, XY, del 18p- and translocations were 46, XY t (1; 21p) and t (4; 6) which confirm that researchers results support the previous findings and most of the studies reported frequent gains of chromosome 7, 8q, 13q, 20q and losses of 4 and 18q in CRC (Knosel et al. 2004). Dutrillaux (1988) observed two different patterns of the chromosomal abnormality monosomic type loss of several chromosome 17p, 18q, 1p, 4q, 5q, 14q and 21. In trisomic type, there is the gain of multiple chromosomes X, 5, 7, 8 and 12. The mechanism of chromosomal instability by causing the loss or gain of copy genes, such as in CRC; APC, TP53, SAMD4, KRAS normal activity oppose to the malignant phenotype (Markowitz and Bertagnolli 2009; Drost et al. 2015). The first cytogenetic studies on CRC tumor were limited due to inadequate quality of preparations. Molecular cytogenetics studies were performed consequently with the use of fluorescence in-situ hybridization, comparative genomic hybridization and spectral karyotyping. Several groups of a disease have been categorized on the bases of the pattern of chromosomal alterations from benign to the malignant stage (Hermsen et al. 2002; Vargas-Rondón et al. 2018).

Polymorphism of APC, TP53 and MTHFR gene in South Indian population were reported in researchers study. Earlier epidemiology studies report approximately sixty percent mutations in the APC gene, involving codons 1286 and 1513 of exon 15. A study represented the patients having FAP with a mutation rate of sixty-one percent of APC which is lower than the rate of eighty percent reported in Caucasians (Gavert et al. 2002; Siegel et al. 2017). Thus, the mutation frequency of APC and TP53 in researchers study fell within the range reported in the literature. MTHFR polymorphisms have been the focus of many studies and in exacting for investigations into CRC where fluctuations in folate levels caused by the 677 C4T and 1298 A4C variants potentially lead to an altered risk of cancer by subsequent variation of deoxynucleotide pool (Pande et al. 2007; Zhang et al. 2017). An insufficiency of folate in tissues with rapidly replicating cells results in ineffective DNA synthesis, which reduces cell proliferation, impairs cellular physiology and alters cell morphology. However, several studies observed positive associations between the MTHFR 677TT genotypes and an increased risk of CRC; but in contrast, researchers results have no significant association. Guerreiro et al. (2008) in Portugal demonstrated that the MTHFR 677TT presented an increased risk of CRC. In researchers analysis of the MTHFR polymorphism, the genotype distribution in the controls deviated from the Hardy–Weinberg equilibrium but exhibited insignificant results. Genotype distributions among control groups were in agreement with Hardy-Weinberg equilibrium with the exception of C677T MTHFR polymorphism. The rate for the C677T polymorphisms showed insignificant results in association with CRC risks.

Finally, there is an evidence for significant association between CRC and other risk factors, including diet habits, physical activity, obesity, alcohol consumption, cigarette smoking and non-steroidal anti-inflammatory drugs (NSAIDs) consumption. Hormone replacement therapy is capable and rising (Mármol et al. 2017). The loss of function of the gene occurs in the first step of CRC, in addition a change of methylation pattern of many genes can develop colorectal cancer. It's not accurately clear that the abnormal CpG island methylation is involved in CRC growth. CRC could be influenced by the epigenetic changes. Genetic polymorphism and epigenetic methylation may be identified in future. Colorectal cancer develops over the course of twenty five years due to genetic alterations of APC, TP53 and MTHFR pathways.

CONCLUSION

The process of colorectal tumorigenesis arise due to genetic instability but whether it is first event and drives from neoplastic transformation are still considered unresolved. Different tumors show many patterns of aneuploidy. Chromosome alterations observed in the tumor of dissimilar individuals are not random, a side effect of aneuploidy is not considered. Cancer can take different paths, it's very important to collect data including sex, age, diets and tumor site during the investigation of a genetic and epigenetic risk factor for CRC. Utilization of biomarkers, such as, APC, TP53 and MTHFR gene mutation is a preventive approach for appropriate screening of CRC.

RECOMMENDATIONS

According to the paper results, the researchers of this paper recommend using the non-invasive strategy along with genetic analysis to detect the APC, TP53 and MTHFR gene in CRC patients.

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