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DIAGNOSTIC AND PREDICTIVE DNA MARKERS IN SUDANESE PATIENTS WITH COLORECTAL CANCER *"THE IMPACT OF ERCC1, XPD, KRAS AND APC GENE'S POLYMORPHISM ON SUDANESE PATIENTS WITH COLORECTAL CANCER"*

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ABSTRACT

Aim: The current study is to examine whether polymorphisms in (ERCC1, ERCC2 (XPD), KRAS, APC) genes have any impact on the risk of colorectal cancer in Sudanese population, also identify their role in clinical outcome and toxicity profile on patients treated with 5-Flurouracil (FU)/Oxaliplatin for colorectal cancer. **Method:** In total, 50 patients with CRC were recruited, 47 of them received (FOLFOX) treatment as a first- line chemotherapy. Polymorphism in one oncogene (KRAS), one tumor suppressor gene (APC) and two DNA-repairing genes (ERCC1, ERCC2) were assessed in these patients using polymerase chain reaction (PCR), and PCR-restriction fragment length polymorphism (PCR-RFLP) techniques. The correlation between these polymorphisms and risk of CRC also with the incidence of oxaliplatin toxicity were evaluated. **Result:** ERCC1 118, XPD 751 T,C alleles respectively and mutated KRAS genotypes associated with risk for developing colorectal cancer in Sudanese population with percentages (14%,12%,24%) respectively. Besides no significant association between APC I1307K variant and risk of CRC in Sudanese population, but results show a high prevalence of this variant in Sudanese population (patients and healthy individuals) which may predict an ethnic difference. Also our results

showed that patients with either ERCC1 118 T/T , XPD 751 C/C ,APC wild genotypes show lower incidence of oxaliplatin toxicity. A stastically siginicant association between the oxaliplatin toxicity and XPD 751 (p= 0.02) and APC I1307K (p=0.01) were identified. **Conclusion:** Overall, our findings suggest that mutated KRAS, ERCC1 C>T, and XPD A>C have a good impact on CRC risk in Sudanese patients, also mutated KRAS, ERCC1 C>T, XPD A>C and APC I1307K may predict oxaliplatin toxicity in patients treated with oxaliplatin based chemotherapy.

KEYWORDS: ERCC1, ERCC2 (XPD), KRAS, APC, Oxaliplatin.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cause of cancer death in the world.[1] A recent study showed that a total of 189 ”candidate” cancer genes can alter in sporadic. These new findings indicate that CRC, in addition to the environmental background, is a more complex genetic disease than what was anticipated in the past.[2] Thus studying and comparing CRCs from population with different epidemic features of the disease can help us to a better understanding to the underlying molecular mechanism of CRC carcinogens.

The Sudan appears to be experiencing a growing cancer epidemic, which carriers many challenges characteristics of developing countries.[3] Recently, CRC has been listed as one of the most common cancers in Sudan.[4] Rectal cancer was more frequent than colon cancer per se and was detected more often in males. The oldest affected patients were in the fourth to sixth decades of life, about 80% are under age 60. Colon cancer was more frequently observed in the caecum, it occurred in relatively young patients.[5] WHO statistics for year 2004 showed that the incidence of colorectal cancer in people under 40 years of age has been found to be relatively high in Sudan, with increased frequency being observed in Northern than Southern Sudan.

A major challenge to treatment of Cancer in Sudan, as in most developing countries is that most patients first present with advanced stage disease. A total of 78% of Sudanese patients have stage III or IV disease (TNM classification) where they first seek medical treatment (data from Sudan federal ministry of health). Therefore, there is an urgent need for better early detection of cancer in Sudan to make treatment more effective, less costly, less invasive and more accessible and acceptable to patients.[6]

The individualization of cancer chemotherapy has become a challenge in recent years. For antitumor therapy there are generally no such simple concentration- effect relationships and clinical efficacy depend upon a diversity of factors, including inherited and acquired drug resistance of human tissue or host body. In addition to classical therapeutic drug monitoring the newly evolving fields of pharmacogentics advocates drug choice taking into account genetic difference among patients and/or tumor.[7] Genetic variants and predictive markers and SNP- analysis can help us predict risk of cancer development also predict responsiveness in patients undergoing specific chemotherapy treatment.

Chemotherapy agents; Oxaliplatin -based regimens

There are many cytotoxic drugs and targeted biological agents for which evidence of efficacy exists for patients with early- and advanced-stage CRC. Although oxaliplatin as a single agent has limited efficacy when administered as first-line or second-line treatment for patients with metastatic colorectal cancer, clinical benefit has been shown when it is combined with bolus fluorouracil and leucovorin followed by a 46-hour infusion of fluorouracil — a treatment regimen known as FOLFOX.[8] Randomized clinical trials have consistently shown that FOLFOX results in response rates and times to disease progression that are superior to those achieved with fluorouracil and leucovorin when given as first-line or second line treatment for advanced colorectal cancer. There was a trend toward improvement in overall survival, but it did not reach statistical significance in these initial studies.[8]

Although grade 3 and 4 haematopoietic and gastrointestinal toxicity is limited for 5-FU mono therapy, combined administration with oxaliplatin significantly increase the incidence of thrombocytopenia, neutropenia, diarrhoea and nausea.[7] The toxicity profile of oxaliplatin is well documented. The toxicity of oxaliplatin is often responsible for the suspension of therapy and its harmful effects in patients overpower its benefits. Primarily, toxicities include severe peripheral neuropathy linked to acute and cumulative doses of oxaliplatin. Current evidence suggests an association between different polymorphisms and adverse drug responses in colorectal cancer .The resistance phenomenon may be induced by a number of cellular adjustments, including reduced uptake, inactivation by glutathione (and other antioxidants), and increased levels of DNA repair or DNA tolerance.[9]

Oxaliplatin forms adducts with DNA, thereby preventing further DNA repair and replication Some enzymes involved in DNA repair may influence the efficacy of oxaliplatin and cause

drug resistance. Enzymes that affect oxaliplatin efficacy and toxicity include the excision repair cross-complementation (ERCC) proteins, which are regulated by the genes ERCC1 and ERCC2 and are involved in nucleotide repair systems. Patients with metastatic CRC (mCRC) who have ERCC and xeroderma pigmentosum group D (XPD) gene polymorphisms causing low ERCC and XPD enzyme levels have better survival and response to folinic acid, 5-FU, and oxaliplatin (FOLFOX) chemotherapy than those with wild-type ERCC and XPD genes . Conversely, overexpression of the X-ray cross-complementation (XRCC) and ERCC genes (resulting in a higher degree of DNA repair) is associated with resistance to oxaliplatin-based therapy.[10]

KRAS and sensitivity to Oxaliplatin based chemotherapy

Several post hoc analyses of recent randomized trials on CRC suggested that the KRAS gene mutation status might predict the efficacy of cytotoxic chemotherapy, especially for oxaliplatin-based regimens.[12] Lin *et al*. (2012) showed that in KRAS wild- type CRC cells, KRAS over expression by mutant vectors caused excision repair cross-complementation group 1 (ERCC1) down regulation in protein and mRNA levels, and enhanced oxaliplatin sensitivity. In contrast, in KRAS mutant CRC cells, KRAS knocked-down by KRAS-siRNA led to ERCC1 up regulation and increased oxaliplatin resistance. So their findings suggested that KRAS mutation is a predictor of oxaliplatin sensitivity in colon cancer cells by the mechanism of ERCC1 down regulation.

The APC variant I1307K and association with colorectal tumors

In 1997.Laken *et al*. identified a novel APC gene polymorphism. A specific single base germ-line transition of T to A at nucleotide 3920 of the APC gene causes the substitution of Lysine for Isoleucine at codon 1307. This alteration produces a small unstable hyper mutable region of DNA (a sequence of eight adenines) that may be prone to somatic mutations and thus carcinogenic. Laken *et al.* estimated an overall carrier rate of 6% in Ashkenazi Jews in the United States; worldwide, the carrier rate ranges between 5 and 10%. They also found a prevalence rate of 28% in a small group of 25 Ashkenazi Jewish patients with a personal and family history of CRC.[13]

However, recently, a missense variant of APC (I1307K) was described that confers an increased risk of colorectal tumors, including multiple adenomas, in Ashkenazim.[14]

Frayling *et al.* (1998), There is an increasing evidence that there is an existing germ-line variants of the APC gene that predispose to the development of multiple colorectal adenomas and carcinoma, but without the florid phenotype of classical familial adenomatous polyposis (FAP), and possibly with importance for colorectal cancer risk in the general population.

Also another study confirmed that APC I1307K gene variant is an important risk factor for CRC in average risk Ashkenazi Jews and should be considered for screening in this population.[15]

J. Kim *et al*. (2009) studied the correlation of molecular changes in colorectal tumorigenesis with response to chemotherapy and found that in metastatic settings, the high disease-control rate of oxaliplatin and irinotecan regimens correlated significantly with wild type APC and intact MMR, respectively, relative to mutant APC and defective MMR.

MATERIALS AND METHOD

This study was conducted at Radiation and Isotopes Centre Khartoum (RICK), while the laboratory work was carried out in the Biotechnology Lab at Ahfad University for Women, Omdurman, Sudan. A total of 70 individuals have been involved in this study. Fifty patients with age >18 (treated for CRC at (RICK), Between March 2007 and January 2014, and twenty Control blood samples were selected entirely at random from normal healthy donors.
The study was started on 14 December 2013. Blood sampling and data collection at RICK took four months, and laboratory work at Ahfad Bio-Lab also took four months.

Chemotherapy treatments

Of 50 patients with CRC, 47 received(FOLFOX); Oxaliplatin, 5-fluorouracil and Leucovorin (Fresenius Kabi Oncology Ltd) as first- line chemotherapy .This regimen shown at table (1) which was repeated every 14 days to the maximum 12 cycles, unless there was no evidence of disease progression, unacceptable toxicity, or patient refusal to treatment.

Table 1: FOLFOX treatment regimen

Drug		**DRUG REGIMEN**	
OXALIPLATIN	85 mg/m2	IV in 500mL of D5W over 120 minutes	Day 1
LEUCOVORIN	200 mg/m2	IV diluted in D5W over 120 minutes concurrently with Oxaliplatin on Day 1	Days 1, 2
FLUOROURACIL	400 mg/m2	IV bolus, after Leucovorin, then	Days 1, 2
FLUOROURACIL	600 mg/m2	CIV over 22 hours	Days 1, 2

Toxicity evaluation

In this study determining toxicities was used for evaluating the objective response to the FOLFOX treatment. Toxicities were graded according to the following oxaliplatin- specific scale: grade1, toxicity of short duration, but resolving prior to the next cycle: grade 2, toxicity persisting between two cycles without functional impairment: grade 3, persistent toxicity interfering with function. For toxicity analysis, the worst data for each patient in all cycles of chemotherapy were used.

DNA extraction and analysis of polymorphisms

Blood samples have been collected in EDTA vacutainer tubes for DNA extraction and genotyping for candidate DNA genes. Purified genomic DNA was extracted from 50 patients and 20 controls blood samples, using standard techniques, employing QiAamp DNA Mini Kit (50) from blood and bloody fluids: (QIAGEN GmbH. D. 40724 Hiden). Then DNA concentration and purity of each sample have been detected using Biophotometer plus 6132 (Eppendorf AG 2007, Germany). Polymorphisms were analyzed by polymerase chain reaction (PCR), and PCR fragment length polymorphism (PCR-RFLP) techniques. Primer sequences and restriction enzymes of all genes examined are presented in Table (2).

Table 2: Characteristics of polymorphisms with primer sequences and restriction enzymes

Site	polymorphism	Genotype	Res-Enzymes	Primers
APC	Specific Allele	I1307K	__	5’-TGAGTGGGGTCTCCTGAACATA-3’ common 5’-CTAATACCCTGCAAATAGCAGAAGA-3’ wild 5’-CTAATACCCTGCAAATAGCAGAAGT-3’specific
ERCC1 (exon 4)	SNP	C/T, Asn118Asn	MboII	5'-GCAGAGCTCACCTGAGGAAC-3' 5'-GAGGTGCAAGAAGAGGTGGA-3'
XPD (exon 23)	SNP	A/C,Lys751Gln	MboII	5'-TCTGCAGGAGGATCAGCTG-3' 5'-GCAAGACTCAGGAGTCAC-3'
KRAS (codon 12)	SNP		BstN1	5’-ACTGAATATAAACTTGTGGTAGTTGGACCT-3’ 5’-TCAAAGAATGGTCCTGGACC-3’

PCR-RFLP

Genomic DNA from patients and controls were examined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach, to determine the genotypes of XPD, ERCC1, APC, and KRAS, following digestion with suitable restriction enzymes. The PCR reaction volume was 25 µL, using Illustra^{TM} puReTaq Ready-To-Go PCR Beads$^{!}$:Room temperature-stable beads containing stabilizers; BSA, dATP,dCTP,dGTP,dTTP, 2.5 units of puReTaq DNA polymerase and reaction buffer. Each bead was reconstitute by adding 18 µL deionized water, 1µL of each forward and reversed primer and 5µL DNA template to reach

25µL final volume for each 0.2 ml PCR bead. The PCR technique carried out using PCR thermal cycler (Flexcycler; Analytikjena AG 2007, Germany) as shown in table (3).

Each plate contains 96 0.2ml tubes ready for 96 reactions. GE healthcare,UK 407513-PLT, Lot 390495.

Table 3: presents the PCR conditions and PCR product for each gene

Gene/PCR product	Initial denaturation	Final denaturation	Annealing	Initial extension	Final extension	Cycles NO
APC/ wild and I1307K at 255bp	95°C/5min	94°C/1min	56°C/1min	72°C/1min	72°C/1min	30
ERCC1 208 bp	95°C/5min	94°C/1min	56°C/1min	72°C/1min	72°C/1min	30
KRAS 157 bp	95°C /3min	95°C /45sec	61°C /15sec	72°C /30sec	72°C /10min	30
XPD/132 bp	95°C /3min	95°C /45sec	61°C /15sec	72°C /30sec	72°C /10min	30

PCR products of ERCC1, XPD and KRAS genes were digested using suitable restriction enzymes, buffers. Mixtures were incubated at suitable temperature inside PCR Flexcycler for known period. To produce 20µL digestion reaction mixture in each tube, 7.5µL deionized water, 2µL buffer, 0.5µL restriction enzyme and finally 10µL PCR product was added, see table (4).

Table 4: presents restriction enzymes, buffers used and reaction condition applied for each gene

Gene site	Res. enzyme	Buffer	PCR product	Incubation period of reaction mixture	Digestion fragments		
					Homo-Wild	Hetero-wild	Homo-mutant
ERCC 1-Exon 4 - C/T, Asn118Asn	Mbo II	Neb buffer 1[I]	208bp	37°C for 3 hours	169 bp	169bp,208bp	208bp
ERCC2-XPD-Exon 23-A/C,Lys751Gln	Mbo II	Neb buffer 1[I]	132bp	37°C for 3 hours	67 bp	67bp, 132bp	132bp
KRAS-codon 12 Point mutation	BstN1	Neb buffer 3[II]	157bp	60°C for 1 hour	114bp		143bp

[I]NEBuffer 1: 1X Buffer Components;10mM Bis-Tris-Propane-HCl10mM $MgCl_2$ 1mM DTT pH 7.0@25°C. Storage temperature -20°C.
https://www.neb.com/products/b7001-nebuffer-1

[II]NEBuffer 3: 1X Buffer Components 100mM NaCl, 50mM Tris-HCl, 10mM MgCl2, 1mM DTT,pH 7.9@25°C.Storage temperature -20°C.
https://www.neb.com/products/b7003-nebuffer-3

All these fragments were separated in 2% agarose (Agarose-Vivantis for molecular biology grade, Vivantis technologies sdn-Bhd, Malaysia) and visualized after ethidium bromide staining. Visualization of each gel containing DNA fragments done using Gel documentation and analysis system (Uvitec Cambridge 2008, Uk).

Statistical analysis

SPSS (Statistical Package for Social Science) 'version 20' was used for analysis, chi square test performed to calculate p-value, (sig< 0.05). The analysis has been divided into three parts, part (I): Descriptive analysis for some variables of interest, part (II): Cross-Tabulation for some couples of variables, and part (III): Comparison between the patients and control group considering lab results of 4 types of genes.

Ethical Clearance

The study was approved by the institutional review board of Ahfad University for Women. The informed written consent was obtained from all subjects.

RESULTS

Patient Characteristics

The study was performed in total of 50 CRC patients. Of these 33 were male (66.0%) and 17 were female (34.0%). Most patients (32.0%) were within the range of range 50-59 years. The majority of patients came with rectal bleeding (54.0%) and with Duke C tumor stage (26.0%). Besides, high incidence of Irritable bowel syndrome and chronic diarrhoea in patients' medical history was observed (10%), as compared with haemorrhoids and chronic constipation (8%). The primary tumor locations of the patients were colon (28%) and rectum (42%). The number of patients who had tumor metastasis, regional recurrence and tumors histo-differentiation are shown in Table (5).

Table 5: Summary of clinopathological data for patients with colorectal Cancer

	Clinopathological features APC								APC		KRAS point mutation		ERCC1 allele frequency			XPD allele frequency		
	Tumor location		Tumor Mets		Hissto-diffrentiation			Tumor recuurence	Homo wild allele (-)	Hetro-specicif allele carrier (+)	NO	Yes	CC	CT	TT	AA	AC	CC
	Colon	Rectum	Yes	No	poor	well	moderate	Yes										
No of subjects	14 (28%)	21 (42%)	5 (10%)	45 (90%)	5	10	16	7	6	44	38	12	39	4	7	42	2	6
Males	11	13	2	31	3	7	10	5	5	28	24	9	25	4	4	29	0	4
Females	3	8	3	14	2	3	6	2	1	16	14	3	14	0	3	13	2	2

Genotype Frequency

The genotype frequencies of the four genetic polymorphisms in this study, and their distributions are shown in Table (2). For ERCC1 codon118, 39 (78.0%) were C/C, 4 (8.0%) were C/T and 7 (14.0%) were T/T genotype carriers. For XPD codon751, 42(84.0%) were A/A and 2 (4.0%) were A/C genotype carriers and 6 (12%) were a C/C genotype carrier. For KRAS, 12 (24.0%) were have point mutation at codon 12, 38 (76.0%) were wild allele carriers. For APC, specific allele carriers were 44 (88.0%) and homo wild carriers were 6 (12%).

As shown in table (6) analysis revealed that the ERCC1 C118T TT genotype , XPD A751C CC genotype and KRAS codon 12 point mutation (14%, 12%, 24% respectively) were associated with a significantly increased risk of CRC cancer patients as compared with the relevant controls (0.0%, 0.0%, 0.0%). No significant association was detected between APC positive and negative individuals ((88% and 12%) as compared to their relevant positive and negative controls (84.2% and 15.8%).

Table 6: Genotypic and allelic frequencies of (APC, KRAS, ERCC1, ERCC2) gene polymorphisms in cases and controls.

No of subjects	APC		KRAS		ERCC1			XPD		
Cases	I1307K negative individuals (Wild allele) (-)	I1307K positive individuals. Specific allele (+)	Wild type allele	Point mutated allele	CC	CT	TT	AA	AC	CC
N	6	44	38	12	39	4	7	42	2	6
%	(12%)	(88%)	(76%)	(24%)	(78%)	(8%)	(14%)	(84%)	(4%)	(12%)
Controls										
N	4	15	19	0	19	0	0	10	3	0
%	(21%)	(79%)	(100%)	(0.0%)	(100%)	(0.0%)	(0.0%)	(77%)	(23%)	(0.0%)

Toxicity Profile

Prolonged toxicity was recorded for each patient during treatment cycles. Significant association was detected between toxicity and Oxaliplatin- treated patients with ERCC1 (CT, TT) and XPD (AC, CC) genotypes as compared to the wild genotypes.

Table 7: Oxaliplatin related SE Vs (ERCC1) alleles

	ERCC1		
SE of (^{1st}) cycles of first line type of chemotherapy treatment	CC	CT	TT
	13 (72.2%)	2	3 (27.8%)
No side effects appear	24	2	3
P-Value	0.46		
SE of (2^{nd}) cycles of first line type of chemotherapy treatment	16 (100%)	0	0 (0.0%)
No side effects appear	21	4	6
P-Value	0.59		
SE of (3^{rd}) cycles of first line type of chemotherapy treatment	11 (91.7)	0	1 (8.3)
No side effects appear	26	4	5
P-Value	0.81		
SE of (4^{th}) cycles of first line type of chemotherapy treatment	10 (100%)	0	0 (0.0%)
No side effects appear	27	4	6
P-Value	0.90		

Table 8: Oxaliplatin related SE Vs (XPD) alleles

	XPD		
SE of (^{1st}) cycles of first line type of chemotherapy treatment	AA	AC	CC
	13 (72.2%)	1	4 (27.8%)
No side effects appear	26	1	2
P-Value	0.50		
SE of (2^{nd}) cycles of first line type of chemotherapy treatment	14 (87.5%)	0	2 (12.5%)
No side effects appear	25	2	4
P-Value	0.82		
SE of (3^{rd}) cycles of first line type of chemotherapy treatment	8 (66.7%)	1	3 (33.3%)
No side effects appear	31	1	3
P-Value	0.10		
SE of (4^{th}) cycles of first line type of chemotherapy treatment	8 (80%)	1	1 (20%)
No side effects appear	31	1	5
P-Value	0.02		

As shown in tables (7, 8), the incidence of hematological and GIT prolonged side effects in patients treated with Oxaliplatin- based chemotherapy, were much lower in patients with ERCC1 (CT, TT) and XPD (AC, CC) genotypes, during all treatment cycles, when compared with patients with wild type of both genes (ERCC1, CC) and (XPD, AA) indicating the significance of Oxaliplatin treatment for these genotypes.

As for KRAS, significant differences were identified between the KRAS genotype (mutated and wild) and oxaliplatin related toxicity (Table: 9).

Table 9: Oxaliplatin related SE Vs KRAS point mutatin

SE of (1^{st}) cycles of first line type of chemotherapy treatment	KRAS point mutation	
	NO	Yes
	14 (73.7)	5 (26.3%)
No side effects appear	24	6
P-Value	0.75	
SE of (2^{nd}) cycles of first line type of chemotherapy treatment	12 (75%)	4 (25%)
No side effects appear	23	8
P-Value	0.86	
SE of (3^{rd}) cycles of first line type of chemotherapy treatment	10 (83.3%)	2 (16.7%)
No side effects appear	25	10
P-Value	0.12	
SE of (4^{th}) cycles of first line type of chemotherapy treatment	8 (80%)	2 (20%)
No side effects appear	27	10
P-Value	0.81	

For APC I1307K, results demonstrate that patients with APC I1307K show high incidence of haematological and GIT related toxicities during treatment cycles compared with patients with wild genotype. As shown in following table (10).

Table 10: Oxaliplatin related SE Vs APC I1307K

SE of (1^{st}) cycles of first line type of chemotherapy treatment	Wild allele (-)	Specific allele (+)
	1 (5.6%)	17 (94.4%)
No side effects appear	4	25
P-Value	0.68	
SE of (2^{nd}) cycles of first line type of chemotherapy treatment	3 (18.8%)	13 (81.2%)
No side effects appear	2	29
P-Value	0.18	
SE of (3^{rd}) cycles of first line type of chemotherapy treatment	2 (16.7%)	10 (83.3%)
No side effects appear	3	32
P-Value	0.01	
SE of (4^{th}) cycles of first line type of chemotherapy treatment	2 (20%)	8 (80%)
No side effects appear	3	34
P-Value	0.41	

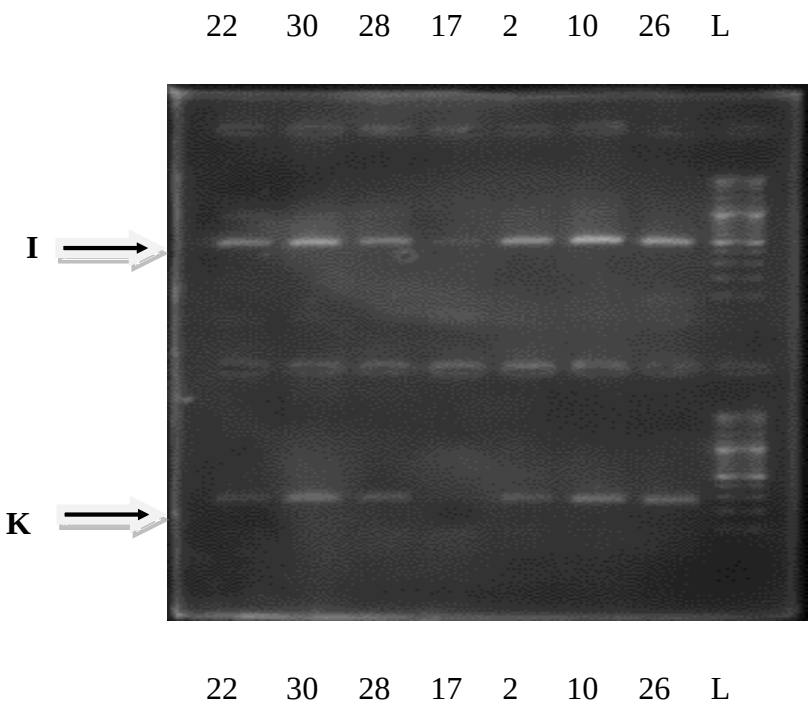

Fig. 1: PCR analysis for APC I1307K

Agarose gel electrophoresis of PCR products. The upper row (I) shows I1307- (wild-type) PCR products, and the lower row (K) shows K1307-specific products at 255 bp. In any given numbered lane the products in the two rows are from the same individual; lanes labelled ‘‘L’’ are 100-bp ladder DNA markers. Lane (17) is from I1307K- negative individuals; lanes 26, 10, 2, 28, 30 and 22 are from I1307K carrying patients.

DISCUSSION

Pharmacogenetic data concerning CRC patients treated with platinum-based chemotherapeutic regimens are scarce and sometimes contradictory. In an attempt to obtain clinically useful information, the present discussion is limited to data from similar studies concerning the diagnosis of the patient (CRC patients), and the type of treatment (OX/5-FU).Our results are subsequently discussed gene by gene.

ERCC1

Compared with reported frequencies in Chinese[16] and Taiwanese[10] populations with CRC. Our results showed a significantly higher percentage of the C/C genotype observed in CRC Sudanese patients. Ethnic differences regarding codon 118 C→T polymorphism of ERCC1 has been observed between Caucasian and African populations, and this polymorphic variant is seen most commonly in Americans of European descent and is associated with altered

NER function. In the present study, the percentage of C/C, C/T, and T/T genotypes were 78%, 8%, 14% respectively, in comparison with percentage of C/C. CT, and T/T genotypes were 54.9%, 38.5% and 6.6% respectively in Chinese population,[16] and were 55.3% (C/C), 38.9% (C/T), and 5.8% (T/T) in Taiwanese populations.[10] Previous studies showed that decreased efficiency of DNA repair is considered as a crucial role in carcinogenesis as such defects accelerate genetic instability and the rate of genetic change. As genes in NER pathway (ERCC1 and ERCC2) are essential to the repair of DNA adducts in colorectal cancer.[16]

Ethnic differences do exist between enzymes that are involved in the targeting and metabolism of certain chemotherapeutic drugs that may affect the efficacy and toxicity of treatment in patients of different ethnic origins. The resistance phenomenon to oxaliplatin based therapy may be induced by increased levels of DNA repair or DNA tolerance.[9] Our findings demonstrate that in a group of patients suffer from oxaliplatin related side effects; patients carrying C/C genotype for the ERCC1 show the highest percentage of side effect appearance during all FOLFOX chemotherapy cycles, when compared with C/T and T/T genotypes as shown in table (7).

Another study conducted in paients with advanced colorectal cancer confirmed that that protein expression of the ERCC1 T allele is lower than that of the C allele, and show that the response to chemotherapy was significantly better in the T/T genotype group ($P = 0.033$), with explanation came from the fact that although both the AAC and AAT codons encode asparagine, the AAT codon usage is significantly reduced, thereby decreasing ERCC1 translation efficiency and protein level.[17]

But there still exists a contrary report which indicates that the objective response to platinum-based chemotherapy is significantly better in patients with the C/C. As with results of a study conducted in Asian patients with metastatic colorectal carcinoma showed a marked increase of ERCC1 protein expression levels in patients with C/T or T/T genotypes (70% vs 20%; $P < 0.01$), which was associated with significantly lower response to FOLFOX-4, and shorter progression-free and overall survival times.[11] A result showed by (Yu *et al.* 2000) in an in-vitro study, cells carrying the T allele showed a poor capacity to repair the adducts induced by cisplatinum. These data support our findings and the pharmacogenetic role of the 118 C>T change and emphasise results that point to the T allele as a marker of a better outcome in Sudanese patients with CRC treated with OX/5-FU schemes.

XPD

The distribution of ERCC2-751 polymorphisms clearly differs between Asian and Western individuals. Among Asians, the frequencies of the ERCC2-751 A/A, A/C and C/C genotypes are 84-92, 8-16 and 0%, respectively, whereas among Americans and Europeans the frequencies are 25-38, 50-61 and 11-15%, respectively.[18] Whereas our results in Sudanese population were 84%, 4%,12% in patients and 75%, 25%, 0.0% in controls, of A/A,A/C, C/C respectively for each patients and controls.

Our results proposed that A>C transition may not be a significant marker for increasing risk of CRC when compared with ERCC1 118 C>T transition. Study on Chinese population although suggest that SNPs of ERCC2 do not directly contribute to the susceptibility to CRC, they may perhaps affect CRC risk by combining with additional polymorphisms in other genes or non- inherited risk factors.

When studying its prognostic role in predicting the efficacy of FOLFOX treatment in Sudanese patients. We found A/C and C/C genotypes were associated with lower incidence of oxaliplatin related toxicity during all treatment cycles compared with A/A genotype as shown in table (8).

Conflicting results are reported on whether the XPD Lys751-Gln (A751C) polymorphism causes an increase or decrease in NER activity. But several studies have demonstrated that the ERCC1-118 and ERCC2-751 genotypes are associated with the clinical outcome of MCRC patients receiving oxaliplatin-based treatment.[18] study conducted in 2008 by (L *et al.* 2008), showed that sixty seven percent of lys/lys patients responded compared with 45% of Lys/Gln patients and 40.9% of Gln/Gln patients (p= 0.047). Likewise, a better OS for Lys/Lys patients was observed in the univariant and multi variant analysis. Also (Kumamoto *et al.* 2013) found that PFS of the patients with the ERCC2-751 A/A genotype was longer than that of patients with the ERCC2-751 A/C genotype, and this was consistent with previous studies.

All the available data, including our present results, indicate that the ERCC1 Asn118Asn and XPD Lys751Gln polymorphisms are useful markers in predicting the clinical outcome and toxicity profile of platinum-containing chemotherapy.

K-RAS

In our current study KRAS point mutation at codon 12 account for 24.0%. This may be a good marker for CRC risk in Sudanese patients. Literature demonstrate that K-RAS mutations are closely associated with impairing GTPase activity and leaving G protein in its active form, what results in uncontrolled and excessive cell proliferation and disturbances in their differentiation [2]. Aberrations in RAS contribute to neoangiogenesis and to metastatic progression independently of proliferative hyperactivity of RAS proteins.[19]

The prognostic value of K-RAS mutation in colorectal cancer remains controversial. This abnormality was described to correlate with distant spread and worse survival .Two previous study showed that the negative prognostic value of K-RAS mutation at codon (12, 13) was found in 117 colorectal cancer patients with over 10 years of follow-up as well as in 98 colorectal cancer patients with median follow-up of 21 months.[19] Andreyev *et al*. (2001) found that prognostically independent association between only one mutation in codon 12 of K-RAS (glycine to valine) and increased risk of disease recurrence and death in patients with Dukes C. They did not observe such an association in Dukes B patients. Kruszewski *et al*. (2004) study performed were also conflicting, with some finding a prognostic value of mutated KRAS alone, others finding this value concomitantly with mutated TP53 or PIK3CA and some reporting no prognostic value of mutated KRAS at all.

However, in a predictive setting, mutated KRAS has shown differentiation resistance to anti-EGFR monoclonal antibodies and since then has been used in clinic for this purpose. Indeed, previous studies showed the ineffectiveness of cetuximab or other EGFR inhibitors for CRC patients bearing mutated KRAS. Therefore, treatment of CRC patients with cetuximab, with all its costs and toxicities, would be most appropriate for CRC patients bearing wild type KRAS only.[20]

Our study suggested that mutated KRAS to be associated with lower toxicity of oxaliplatin based treatment. These results may be in line with study by Lin *et al*. (2012) which demonstate that KRAS mutation is a predictor of oxaliplatin sensitivity in colon cancer cells by the mechanism of ERCC1 down regulation. And with Lin *et al.* (2013) which showed that with multivariate analyses, patients who had used oxaliplatin-based regimens remains an independent prognostic factor for longer OS in KRAS mutant mCRC patients.

In conclusion, our data suggested that KRAS mutation is a predictor of oxaliplatin sensitivity in Sudanese patients with colorectal cancer.

APC

We have found that the frequency of APC I1307K variant was in 44 (88%) of 50 CRC patients. These results are in close agreement with those reported in Sudanese healthy individuals with percentage of (84.2%) Thus, we did not suggest that APC I1307K variant will be a risk factor of CRC in Sudanese population.

In 1997, a Johns Hopkins research team found an inherited genetic mutation called APC 1I307K. This mutation is found primarily in people of Ashkenazi Jewish heritage. Researchers believe that 6% of the Ashkenazi Jewish population carries this gene mutation, which gives them a significantly increased risk of developing colorectal cancer.

Rozen *et al.* (2002) showed that I1307K is a founder genetic variant in Jews of different ethnic origin, mainly Ashkenazim, but it explains only partially their higher incidence of colorectal carcinoma.

Recent study conducted on Ashkenazi Jews (2014) demonstrate that The I1307K adenomatous polyposis coli gene variant is not an important marker for increased risk for CRC (Strul *et al*. 2014). Other results found to be in contrast with previous studies which confirmed that APC I1307K gene variant is an important risk factor for CRC in average risk Ashkenazi Jews and should be considered for screening in this population (Frayling *et al.* 1998). Also our study found that this variant is statically associated with higher incidence of hematopoietic and gastrointestinal toxicities during cycles of oxaliplatin-based chemotherapy treatment as shown in table (11). J. Kim *et al*. (2009) studied the correlation of molecular changes in colorectal tumorigenesis with response to chemotherapy and found that in metastatic settings, the high disease-control rate of oxaliplatin and irinotecan regimens correlated significantly with wild type APC and intact MMR, respectively, relative to mutant APC and defective MMR.

In conclusion high prevalence of APC I1307K variant in Sudanese population either patients or healthy subjects may predict an ethnic difference. Also our results suggest that APC I1307K variant may be a good predictive marker for Oxalipltin realted toxicity.

CONCLUSION AND FUTURE OUTLOOK

In conclusion, (combined) SNP-analysis may help us predict risk of cancer development also predict responsiveness in patients treated with oxaliplatin containing regimens. Genetic variants and predictive markers allow physicians to improve the efficacy of cancer therapy. In contrast, the clinical utility of the described polymorphisms involved in Oxaliplatin-based therapy is, in part, limited by the less frequent use of genotyping methods in routine clinical diagnostics in Sudan.

The usefulness of the described genetic variants in clinical practice will depend on whether they are capable of improving diagnostic prediction or fostering changes in the prevention or treatment strategies. Particularly, molecular testing for mutations in ERCC1, ERCC2, KRAS, APC and other genes could help Oncologists stratify patients on the basis of their ability to minimize oxaliplatin toxicity. To assess the basic profile of response (good/bad) in patients, a test of different SNPs in most important genes involved in metabolism of platinum-based therapy is recommended. Also the study recommend to study APC I1307K variant and its distribution through Sudanese population in a large population to examine its ethnic difference which proposed by this study.

One of our study limitations; these DNA markers need to be addressed experimentally in larger clinical trials. This could help the clinicians to stratify patients in two genomic profiles, (a) Cases with favorable prognosis showing lower toxicity for oxaliplatin because of a protective genetic profile; (b) Cases showing unfavourable prognosis and very high risk of toxicity to oxaliplatin, because of their genetic variants.

Hopefully, the development of methods for genotyping the variants influencing oxaliplatin-based therapy in the future will result in personalized treatment options. Therefore, it is fundamental that pharmaceutical and biotechnology companies work together to develop a standardization method to validate pharmacogenomic tests for oxaliplatin that are suitable for routine diagnostics.

In summary, with the increasing number of novel pharmacogenomic markers being identified and validated, Oncologists will have new means to make treatment decisions on the basis of individual genetic profiles and may eventually be able to personalize treatment for patients, thereby minimizing toxicity.

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