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RESEARCH ARTICLE

ALLELIC VARIATION OF P53 CODON 72 "EXON4" IN COLORECTAL CANCER IN SUDANESE PATIENTS

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ABSTRACT

Study Design and Objective: This was a prospective case control hospital based study aimed to identify the allelic variation of p53 codon 72 "exon 4" in colorectal cancer lesions in Sudanese patients.
Material and Methods: Endoscopic biopsies were obtained from 82 Sudanese patients with colorectal mucosal changes, DNA was extracted, and genotyping was performed using Allele Specific PCR (AS-PCR) to detect proline and arginine alleles.
Results: In the cancer-free group specimens, the genotype distribution for p53 polymorphism showed 8.6 %, 60 %, and 31.4 % for the Arg/Arg, Arg/Pro, and Pro/Pro genotypes, respectively. In the colorectal cancer group specimens, 12.8 % were Arg/Arg, 51 % were Arg/Pro, and 36.2 % of the cases were Pro/Pro.
Conclusion: Proline Homozygosity may be correlated with possible increased risk of colorectal cancer in Sudan.

INTRODUCTION

It is estimated that colorectal disease, especially cancer, has an increased incidence and remains an important cause of death in several parts of the world (**Max Parkin *et al*., 2005**). In Sudan, colorectal cancer has been reported the fifth in men and the seventh in women with overall rate of 7.1 per 100,000 (**Intisar E. Saeed *et al*., 2014**). Genetically, colorectal cancer is characterized by several certain gene alterations, such as mismatch repair gens (MMR), adenopolyposis coli oncogenes (APC), and p53 tumor suppressor gene (**Fearon and Vogelstein 1990**). The p53 tumor suppressor gene is a phosphoprotein made of 393 amino acids, located on the seventeenth chromosome (17p13.1) (**Olivier *et al*., 2002**). It is considered one of most mutated genes that affect many types of human cancer (**Khan *et al*., 2005; Borresen-Dale, 2003**). Inaddition to mutations, several polymorphismsin the wild-type p53 gene locus have been detected and investigated (**Soussi and Beroud, 2001**). Among these polymorphisms, the most commonly associated with cancer development is the codon 72 arginine (CGC Arg) to proline (CCC Pro) substitution at exon 4 (**Vannini *et al*., 2008**).

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The role of codon 72 polymorphism of p53gene has been noted in patients with colorectal cancer in several studies (**Lung *et al*., 2004, Gemignani *et al*., 2004, Zhu *et al*., 2007, Hamajima *et al*., 2002; Koushik *et al*., 2006**). The aim of this study was to investigate the association of codon 72 polymorphism of p53with colorectal cancer lesions in Sudanese patients attended endoscopy clinics at Khartoum general hospitals.

MATERIALS AND METHODS

This was a prospective case -control hospital- based study included 82 Sudanese patients attended the main endoscopic centers in Khartoum with colorectal mucosal changes during a period of about one year. The obtained endoscopic biopsies were collected into plain containers containing normal saline and then stored at -80 c°.

DNA Extraction

DNA was extracted from the tissue samples using promega wizard® SV genomic and DNA purification system and promega wizard® genomic DNA purification kits according to manufacturer's instructions. The yield was checked for quality

by running in 1% agarose gel electrophoresis, and for quantity by spectro -Nano drop (ANOVA).

Genotyping process

Allele specific PCR (AS-PCR) to detect either proline allele or arginine allele was applied. For p 53 arg specific allele, it was conducted by using:

Arg sequences forward 5'-TCC CCCTTG CCG TCC CAA-3'
Arg sequences reverse 5'-CTG GTG CAG GGG CCACGC-3'
For p 53 prospecific allele, it was conducted by using:
Prosequences forward 5'- GCC AGA GGCTGC TCC CCC-3'
Pro sequences reverse 5'-CGT GCA AGT CAC AGA CTT-3'

PCR mixture: 4µl genomic DNA (>100 ng) from each patient's sample was used in PCR reaction, then adding 1µl from forward primer, 1µl reverse primer, 4µl master mix (ready to load Solis Biodyne, Estonia), and 10µl double distilled water to reach 20µl total volume.

PCR running program: The thermo cycling condition was programmed as follows: denaturation at 94°C for 5 min, followed by amplification for 35cycles at 94°C for 30 sec, 54°C for the *p53Pro* allele and 60°C for the *p53Arg* allele for 30 sec each, with a final extension at 72°C for5 min. Then visualized using agarose gel electrophoresis (2.5%) and the products were run beside 100 bp ladder(r Solis Biodyne, Estonia), stained with save stain and identified by photographing under UV illumination of gel documentation system (Syngine, Japan).

***Ethical clearance**:* Written consent from each patient had been taken, and overall study was approved by Sudanese ministry of health and council of ethical clearance at Neelain University.

Statistical analysis: statistical package for social sciences (SPSS) version 20 was used to analyze data collected by structured questionnaire, and frequency of each genotype was collected using logistic regression and Chi square.

RESULTS

Depending on the gastrointestinal tract endoscopy reports, the colorectal changes were classified into two groups: tumor free group (inflammatory bowel disease) as control group contained 35 patients (42.7%) and colorectal cancer group contained 47 patients (57.3%); males were 51 (62.2%) and females were 31 (37.8%).The age of patients at the time of diagnosis ranged from 9 to 83 years with the mean age 47.7±1.62. The PCR product of the p53Arg primer was of 141bp band (lower row) and of the p53Pro primer was of 177bp band (upper row) as shown in Figure 1.

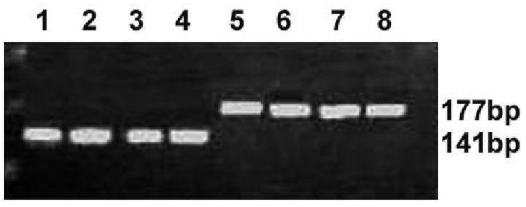

Figure 1. Arg and Pro bands in PCR

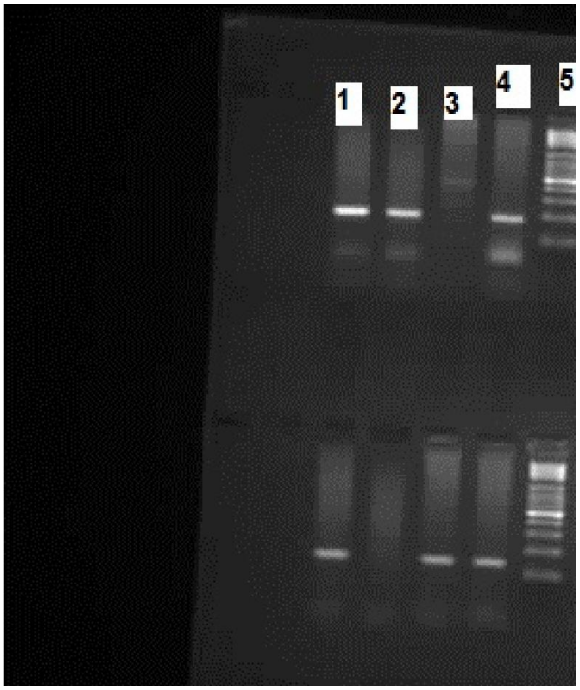

Figure 2. PCR Homozygous and Heterozygous products

Figure 2 One PCR product showing Arginine Homozygous (line 3), Proline Homozygous (line 2), and Heterozygous Arg/Pro (lines 1 and 4); reading against 100 bp ladder (line 5).
Genotype allele distribution among colorectal biopsies is shown in Table 1.

Table 1. genotype distribution among colorectal cancer and control

	p53 arg homo	p53 pro homo	p53 pro/arg hetero	Total
Control	(3)	(11)	(21)	(35)
tissue	8.6%	31.4%	60%	100
	33.3%	39.3%	46.7%	%
Cancer	(6)	(17)	(24)	(47)
tissue	12.8%	36.2%	51%	100
	66.7%	60.7%	53.3%	%
Total	(9)	(28)	(45)	(82)
	11%	34.1%	54.9%	100
	100%	100%	100%	%

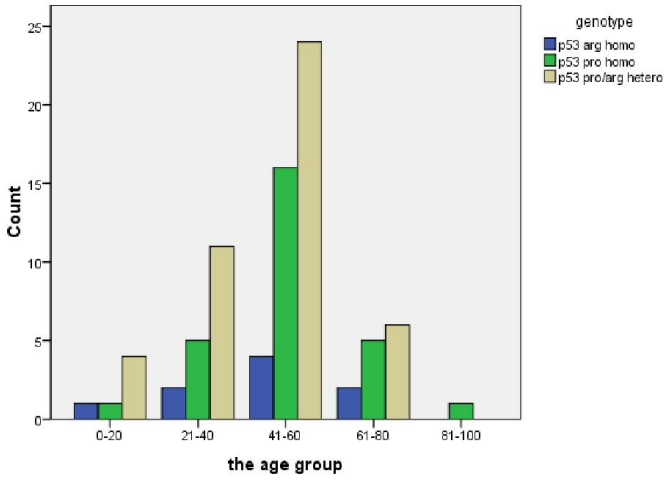

Figure 3. Genotype distribution of p53 arg72 pro among age groups

Genotype allele distribution among age groups revealed predominance between 40 and 60 years of age (Figure 3).

Genotype allele distribution among sex revealed male predominance in Pro/Arg and Arg/Arg and female predominance in Pro/ Pro (Figure 4)

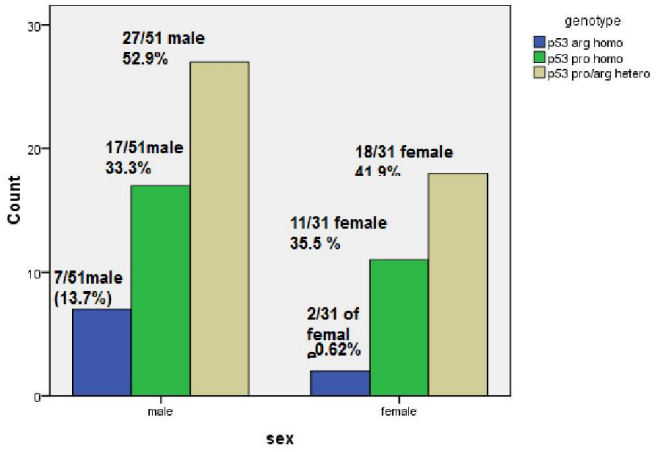

Figure 4. Genotype allele distribution among sex

DISCUSSION

To our knowledge, this study is the first in Sudan that investigated the association between p53 Arg 72 Propolymorphism and colorectal cancer among Sudanese patients. Several studies have been done to investigate the association of p53 arginine/72 proline polymorphism with various tumors in different populations. Results of this study are almost similar to most of these studies.

Lima *et al.* (2006) studied p53 arginine/ 72 proline polymorphism by PCR using specific primers in 100patients with colorectal cancer paired by sex and age to 100 patients without cancer. They reported no significant difference among both groups; frequency of homozygosis for arginine was 56% in the cancer group and 58% in the control group. In our study, there is significant difference in both groups; Arg/Arg was 66.7% in the cancer group and 33.3% in the control group.

Serap Tutgunorat *et al.* (2009) studied 27paraffin-embedded colon cancer tissue samples by PCR and reported genotype frequencies were 51.9% Arg/Arg, 40.7% Arg/Pro, and 7.4% Pro/Pro.In our study, genotype frequencies in cancer patients were 12.8% Arg/Arg, 51% Arg/Pro, and 36.2% Pro/Pro. Pérez *et al*. (2006) studied 109 controls and 53 patients with colon cancer from Argentina using allele-specific polymerase chain reaction. In control samples, the genotype distribution forp53 polymorphism showed 40.3%, 48.6% and 11.1% for the Arg/Arg, Arg/ Pro and Pro/Pro genotypes, respectively. In cancer cases, 58.5% were Arg/Arg, 37.7% were Arg/Pro and 3.8% were Pro/Pro. These findings indicated that arginine homozygous genotype may represent a genetic predisposing factor for colon cancer development in Argentinean patients. In our study, 12.8% of the cancer cases were Arg/Arg, 51% were Arg/Pro and 36.2% were Pro/Pro. However, Arg/Arg was higher in cancer patients (66.7%) compared to non-cancer patients (33.3%). **Abdul Aziz Ahmad Aizat *et al.* (2011)** studied 202 colorectal cancer patients and 201 normal controls using PCR and reported the frequency of homozygous (Pro/Pro) genotype was 21%in cancer cases and 13% in controls, concluding that Pro/Pro homozygous variant genotype showed significantly higher risk association with cancer susceptibility. This agrees with our study, in which Pro/Pro was higher in cancer patients (60.7%) than controls (39.3%).

Schneider-Stock *et al.* (2004) reported similar distribution between cancer patients and control group; Arg/Argwas 44.7% in control group and 47.3% in cancer group; Arg/Pro was 48.2% in controls and 44.1% in patients; Pro/Pro was 7.1% in control group and 8.6% in cancer group. These results did not reveal anenhanced risk of developing colorectal tumors by p53 polymorphism.

Abbas Doosti *et al.* (2011) investigated the status of p53 at codon 72 for Arg/Arg, Arg/Pro and Pro/Pro allele polymorphisms in blood samples from 145 colorectal cancer Iranian patients and 140 controls by PCR. Among cancer cases, 33.8% were Arg/Arg, 12.41 % werePro/Pro and 53.8%were Arg/Pro. The frequencies of the three P53 genotypes; Arg/Pro, Arg/Arg and Pro/Pro in controls were 60.7, 20 and19.3%, respectively. They concluded positive correlation between Arg/Arg and risk for colorectal cancer in Iranian patients.

Conclusion

The findings of this study indicate that codon 72 polymorphism of p53 may be a genetic predisposing factor for colorectal carcinoma in Sudanese patients. In addition, p53Pro72protein (Proline Homozygosity) may be correlated with possible increased risk of this type of cancer in Sudan.

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