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Correlation between manganese superoxide dismutase and susceptibility to colorectal cancer

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Abstract : Oxidative stress is considerable in several kind of cancer, such as colorectal cancer (CRC) .free radical result from many CRC risk factors such as smoking, that is able to cause oxidative DNA damage. manganese superoxide dismutase (MnSOD) is an intracellular antioxidant enzyme that have an important role in limiting the harmful effects of oxidative stress .The present study aims to determine the concentration of manganese superoxide dismutase (MnSOD2) and investigation of SOD2 gene polymorphism in CRC cancer patients and the possibility of using this polymorphism as biomarker for CRC cancer. patients with clinically diagnosed CRC (n=70) and healthy control individuals (n=40) were investigated for determine SOD2 concentration and for single nucleotide polymorphism (SNP;rs4880) by polymerase chain reaction –restriction fragment length polymorphism .there were significant differences between patients and control in SOD2 concentration in sera of both patients and control ,since the P value was(0.007) . However, there was a significant difference between the patients and control groups(p=0.00398),patients were more likely to have C/C polymorphism than control group.

Introduction

Colorectal cancer (CRC) is the second explanation for cancer deaths worldwide(1). CRC characterized by several forms of sequence mutations, accompanied by order instability, including chromosomal instability and microsatellite sensitivity (2). CRC is a disease develop as a multistep and complicated process involving with the initiation of genetic and epigenetic alteration including the transforming of normal glandular epithelium into invasive adenocarcinoma (2,3) . millions of people develop colorectal cancer (CRC) annually (4,5). Globally colorectal cancer consider the third common diagnosed cancer since the highest incidence rates of CRC was distributed in Australia and New Zealand , North America and Europe , while the lowest rates are varies in Africa and South Asia (6). These different rates result from variation in dietary habits and the surrounding environment (7).

Iraq consider the sixth country prevalence rates of CRC within Asia as the Iraqi cancer registry (ICR) reported that in 2005; the maximum prevalence was at the mean age of 50 , (8,9) The development of the CRC occur over a period of 10 to 20 years (10). Usually started as precancerous growth called the polyps However males are usually affected with CRC more than females (11), Many of CRC patient develop a metastases in liver and lung (12).

Many risk factor associated with the development of CRC cancer ; these include family history of adenocarcinoma or polyps and history of chronic intestine disease , other behavioral risk factor for CRC cancer include smoking, meat consumption and obesity (13). Other factor decrease CRC risk such as diary consumption, fruit and vegetable consumption (14).

Free radical like reactive oxygen species (15)which include the superoxide (O2) and hydrogen peroxide (H2O2) and reactive nitrogen species (RNS) (16) are continuously formed through the metabolic process in the living organism . in the normal physiological situation , ROS production controlled by antioxidant enzymes and redox substances , superoxide dismutases are enzymes include of three isoforms that catalyze the conversion of superoxide into oxygen and hydrogen peroxide these isoforms classified according on the cofactor which's usually a metal as the following : homodimericcuznSod (SOD1) which's located in the nucleus ,cytoplasm and mitochondrial intermembrane space , homotetramericmnSod (SOD2) that's exclusively located in the mitochondrial matrix and homotetrameric extracellular cuznSod (SOD3) as its name refer its located in the extracellular matrix . MnSOD consider the main guard against mitochondrial ROS since MnSOD gene polymorphism is essential in Organization of free radical level in cell . numerous genetic variant of manganese superoxide dismutase were identified , for example rs4880 SNP in the mitochondria targeting sequence of MnSOD that result in amino acid subsituation from valin to alanin in the codon 16 (17).

Material and methods

Samples collection and practical work of this study extended from September 2015 to October 2016 . samples were collected from patient who are diagnosed with colorectal cancer and attended to Gastrointestinal Endoscopy Unit and chemotherapy centers at AL- Kahdimiah Hospital ,Imam Hussein Medical City , Mirjan Teaching Hospital , City of Medicine -Tumor Teaching Hospital , Gastrointestinal Tract Hospital and Tumor Center at Alnajaf governorate so as some of Iraqi governorates hospitals . a questionnaire format was filled including : age ,length, gender, canning food intake, smoking , as well as other data will be mentioned in the appendix .

Histopathologists and consultant physicians have been diagnosed the patient with specific diagnosis include :adenocarcinoma with acute chronic ulceration , colorectal mass, as well as carcinopolyps . Forty individuals were selected as control group with age ranging from 25- 75 having mean age (49.75±16.04) years old as well as The present study included (70) individuals as patient group with age ranging from 25 – 74 , having mean age (49.74±13.58) years old . Five milliliters of venous blood were taken from both patients and control then divided into two part ; (1-2) ml in EDTA tube for molecular tests and (1- 3) ml in dry – sterile tube for serological test . the sera were collected after clotting the blood by centrifuging for 10 min at 5000 rpm , then stored at -20 C .SOD2 concentration was determined byElisa kit uses competitive –Elisa as a teqnique . themicrotiter plate within this kit has been pre-coated with SOD2 . through the reaction , human SOD2 in the sample or standard competes with amount of SOD2 on the solid phase supporter for sites on the biotinylated finding Ab specific to human SOD2 . further conjugate and unbound sample or standard are washed from the plate , and avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well and incubated . then a TMB substrate solution was added to each well . the enzyme-substrate reaction was terminated by the addition of sulphuric acid solution and the color change was measured spectrophotometrically at wavelength of 450 . the concentration of human SOD2 in the samples was then determined by comparing the OD of the samples to the standard curve . Each frozen blood specimen was thawed, genomic DNA was then extracted directly, using the Bioneer genomic DNA extraction kit as mentioned by leaflet kit with some modification . By detecting the presence of the defect genes of DNA extracted from the blood , amplification conditions for every gene were optimized by the conventional PCR assay by using Applied-PCR System / USA company with specific primers sequences for the genes as mentioned in Table (1.1). The primers used for the detection of polymorphic regions of genes (SNPs) of the current study , so as analysis of these SNPs through RFLP- PCR technique .PCR product was digested with 3 units of specific restriction enzyme (BsaWI) for 4 hours at 60C .

Table (1.1) : The primers of detection genes used in colorectal cancer in blood and tissue extracted DNA.

DNA Primer with Reference	Sequence	Amplicon size (pb)
SOD2 gene /rs4880 SNP (19)	F- GCTGTGCTTTCTCGTCTTCAG R- TGGTACTTCTCCTCGGTGACG	208

Result and Discussion

The present study include some of socio-demographic characteristics were been assessed by questionnaire include about 20 question were been answered by an interview with both patients and control groups as mentioned in the appendix .The present study consist of patients group that comprises of (49%) males and (51%) females and control groups that comprises (55%) males and (45%) females. Table 1.2 shows the differences of colorectal patients and control by sex, residence and educational levels. There was no significant difference between colorectal patients and control by sex, residence and educational levels as show in table 1.2 **.**as well as Table 1.3 shows the differences of colorectal patients and control by family history, meat, soft drink, canned food intake as well as smoking habit. There were significant differences between colorectal patients and control by soft drink intake and smoking habit. Patients were three times more likely to drink soft drinks daily than control. Patients with colorectal cancers were three and five times more likely to be current and passive smokers, respectively.

Table 1.1: Differences of Patients with Colo-Rectal Cancer and Control Group by Socio-Demographic Characteristics

Variable	Study Group		χ^2	*P* values
	Patients (%)	**Control (%)**		
Sex				
Male	33 (47.1)	22 (55.0)	0.629	0.428
Female	37 (52.9)	18 (45.0)		
Residence				
Urban area	42 (60.0)	22 (55.0)	0.262	0.609
Rural area	28 (40.0)	18 (45.0)		
Educational levels	42 (60.0)	16 (40.0)		
Illiterate	3 (4.3)	6 (15.0)		
Primary school	7 (10.0)	6 (15.0)	6.212	0.102
Secondary school	18 (25.7)	12 (30.0)		
Higher education				

*p value ≤ 0.05 is significant

Table 1.2: Distribution of Patients with Colo-Rectal Cancer and Control Group by Medical History

Variable	**Study Group**		χ^2	***P* values**	**Odds Ratio**
	Patients (%)	**Control (%)**			**(95%) C.I.**
Family history					
Positive	22 (31.4)	18 (45.0)	2.026	0.155	0.56 (0.25-1.25)
Negative	48 (68.6)	22 (55.0)			
Meat intake					
Daily	21 (30.0)	18 (45.0)	1.281	0.258	0.62 (0.26-1.43)
Weekly	36 (51.4)	19 (47.5)	3.354	0.067	0.27 (0.06-1.09)
Monthly**	13 (18.6)	3 (7.5)			
Soft drink intake					
Daily	48 (68.6)	25 (62.5)	**3.354**	**0.050***	**3.71 (1.12-15.13)**
Weekly	12 (17.1)	15 (37.5)	1.395	0.238	2.28 (0.58-9.02)
Monthly**	10 (14.3)	0 (0.0)			
Canned food intake					
Daily	38 (54.3)	17 (42.5)	1.281	0.258	1.59 (0.71-3.59)
Weekly	28 (40.0)	20 (50.0)	0.399	0.527	1.67 (0.34-8.32)
Monthly**	4 (5.7)	3 (7.5)			

Smoking habit					
Non-smokers	6 (8.6)	12 (30.0)			
Current smokers	28 (40.0)	14 (35.0)	**5.381**	**0.020***	**3.50 (1.07-5.09)**
Passive smokers	36 (51.4)	14 (35.0)	**7.680**	**0.006***	**5.94 (1.60-7.19)**

* p value ≤ 0.05 is significant

In this study the concentration of SOD2 was measured by enzyme-linked immunosorbent assay ,We found significant differences in SOD2 level in sera of both patients and control , since the P value was(0.007) , many studies show that SOD2 concentration in the serum of cancer patients was higher than the control (Nalkiran *et al* ., 2015) , SOD2 levels were been also increased, particularly in progress tumor stages. Within colon and lung cancer both mRNA and protein SOD2 levels were been increased in patients compare to those in control samples. (18).

Table 1.3: Mean differences of concentration of SOD2, by colo-rectal patients and control

Parameter	**Group**	**N**	**Mean**	**S.D**	**t-test**	**p value**
CONSOD2	Patients	70	16.45	4.27	**2.757**	**0.007***
	Control	40	13.95	5.09		

As shown in table 1.4 the analysis of the polymorphisms located at sod2 gene in the stroke group shown that 9(12.86 %) were wild-type for TT genotype, 30(42.86%) variant homozygous for CC genotype, and 31(44.29%) heterozygous for CT genotype. There was a significant difference between the case and control groups(p=0.00398),patients were more likely to have C/C polymorphism than control group , and this deal with **(**19),that revealed C allele frequency of rs4880 was significantly higher in cancer patients compared with normal subjects

This polymorphic variant of SOD2 had been established to be factor that may enhance the risk of cancer development (20) **.**

Table 1.4: Differences of colo-rectal patients and control by SOD2 Polymorphism

Variable	**Study Group**		χ^2	***P* values**	**Odds Ratio**
	Patients (%)	**Control (%)**			**(95%) C.I.**
SOD2 Polymorphism					
C/C	30 (42.9)	10 (25.0)	**3.395**	**0.050***	**3.33 (1.04-11.70)**
C/T	31 (44.3)	21 (52.5)	0.502	0.479	0.67 (0.23-1.98)
T/T**	9 (12.9)	9 (22.5)			

* p value ≤ 0.05 is significant
**reference group

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