Study the polymorphism in DNA repair genes (XRCC1) and colorectal adenocarcinoma risk

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Abstract— XRCC1 gene has been extensively investigated both in its function and in its association with cancer risk. The presence of the variant Gln399 allele has been shown to be associated with measurable reduced DNA repair capacity. The present study aimed to study the association between XRCC1 Arg399Gln polymorphism and colorectal cancer risk, and to investigate their role as susceptibility markers for colorectal cancer. Twenty colorectal adenocarninoma patients attended Tanta cancer center during the period from December 2010 to May 2011 were enrolled in this study. Matching group of 20 healthy controls was used for comparison. Subgroup analyses based on age groups, sex, and smoking status were further performed. The overall data failed to indicate significant associations between XRCC1 Arg399Gln polymorphism and colorectal cancer risk (Arg/Arg odds ratio (OR) = 1.27; 95% CI = 0.34- 4.31; Arg/Gln: OR = 1; 95% CI = 0.27- 3.67; Gln/Gln: OR = 0.474; 95% CI = 0.04 - 5.69). In subgroup analyses stratified by age, gender and smoking status similar results were obtained. In conclusion, XRCC1 Arg399Gln polymorphism is not associated with colorectal adenocarinoma and is consistent with the results of a recently published meta-analysis

Key words: Colorectal cancer, DNR repair , polymorphisms, XRCC1 gene.

INTRODUCTION

COLORECTAL carcinoma (CRC) is one of the most frequent causes of cancer death for both men and women, accounting for 9.9% of all cancer incidences and 12.1% of cancer deaths in the world[1]. Genetic predisposition may contribute to the development of colorectal cancer through either the silencing of a tumor suppressor gene, the activation of an oncogene, or the suppression of DNA damage repair capacity[2].

Deficiency in the repair capacity due to mutations or polymorphisms in genes involved in DNA repair can lead to genomic instability that, in turn, is related to chromosomal instability syndromes and increased risk of developing various types of cancer[2-4]. Numerous polymorphisms mainly single nucleotide polymorphism (SNPs) have been indentified for DNA repair genes, although their functional outcome and phenotypic effect is often unknown[5]. Of particular relevance to the risk assessment of DNA damaging agents is the finding that DNA repair genes are polymorphic leading to altered ability for base excision-repair (BER) in response to radiation and chemical agents [6].

XRCC1 is a DNA repair gene whose protein product is involved in a number of repair pathways and therefore has been extensively studied in vitro (XRCC1 deficient cell lines) and in vivo (knockout mice) [7,8]. In addition, genetic polymorphisms have been characterized and implicated as a factor capable of modulating baseline and chemical-induced DNA damage [9]. However, this information has yet to be formally considered for incorporation into human health risk assessment.

XRCC1 is a 70 kDa protein essential to the repair of single strand breaks (SSBs) and BER. These functions are carried out in spite of the protein lacking any enzymatic activity of its own [10]. XRCC1 is thought to act as a scaffold protein facilitating the recruitment of DNA repair enzymes and acting as a loading platform for the repair process [11,12].

XRCC1 belongs to the X-ray repair cross complementing gene family of which greater than 20 genes have been identified[13,14]. The human XRCC1 gene is located on chromosome 19q13.2, spans 32 kb, and consists of 17 exons [15,16].

More than 60 single nucleotide polymorphisms (SNPs) have been identified [17] , two polymorphisms, more often found in XRCC1' conserved sites, lead to a C→T substitution at codon 194 in exon 6 and to a G→A substitution at codon 399 in exon 10 of the gene, leading to the amino acid alterations arginine (Arg) to tryptophan (Trp) and arginine (Arg) to glutamine (Gln), respectively. These changes in conserved protein sites may alter the BER capacity, increasing the chances of DNA damage [18]. The most extensively investigated is Arg399Gln on exon 10[19] due to its location within the region of the BRCT I binding domain.

The Arg399Gln variant is more frequent and has been associated mainly with head and neck [20] , colorectal [21], gastric [22], esophageal[23,24], breast[25] and lung[26,27] cancer.

This study aims to study the association between DNA repair-gene polymorphisms (XRCC1) and colorectal cancer risk, and to investigate their role as susceptibility markers for colorectal cancer.

PATIENTS AND METHODS:

Twenty patients (9 females and 11 males with age ranged between 28 -75 years old) were recruited in this study. They

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were previously diagnosed and histologically confirmed having colorectal adenocarcinoma. Patients suffering from familial adenomatous polyposis, hereditary non-polyposis colorectal cancer, or inflammatory bowel disease and other related malignancies were excluded from the study. All patients attended to Tanta Cancer Center for assessment and management in the period from December 2010 to May 2011. Twenty healthy volunteers (8 females and 12 males), with age ranged between 22 – 62 years old volunteered to participate in the study. All of them were apparently healthy with no history of any type of cancer or inflammatory bowel disease. They had no family history of cancer and / or colorectal disease. They were not receiving any form of medication between the start and conclusion of the study.

Genotyping

Genomic DNA was extracted from peripheral blood using the DNA modified salting out procedure (28). XRCC1 Arg399Gln polymorphism was determined by direct sequencing of PCR products obtained with the following gene specific primers(29).

Sense primer:

5'-TTGTGCTTTCTCTGTGTCCA-3'; Antisense primer: 5'-TCCTCCAGCCTTTTCTGATA-3'.

DNA amplification was performed in a Thermohybraied PCR Express Thermalcycler, with a total reaction volume of 25 µl containing:

Milli Q water: 12.5 µl
DNA: 300ng
Sense primer (Bioron): 20 Pico moles.
Antisense primer (Bioron): 20 Pico moles.
PCR master mix: 12.5 µl.

Amplification was performed using initial denaturation at 95 °C for 3 minutes followed by 35 cycles of 94 °C for 30 seconds, 66 °C for 30 seconds, and 72 °C for 30 seconds with final extension of 70 °C for 6 minutes. Following amplification, 9 µl of the PCR product were mixed with 2 µl of 6x loading buffer (0.09 % bromophenol blue, 0.09 xylene cyanol, 60 Mm EDTA in 60% glycerol) and loaded on agarose gel 3.5% (containing ethidium bromide 20 ng/µl).

5 µl of 20 bp ready to use DNA ladder (MBI Fermentas) was loaded in a separate lane. Products were visualized on UV Transilluminator. Restriction digestion was performed using the MSP1 (MBI Fermentas) for Arg399Gln(30). Overnight incubation at 37 °C was followed by gel electrophoresis on 3 % agarose gel.

Statistical analysis was performed using SPSS version 17 Chicago, IL, USA for windows.

Mean values for different variables of controls and colorectal adenocarcinoma patients were compared using the T test for independent samples. Alleles frequencies were estimated by counting. The genotype frequencies for each polymorphism were tested for deviation from the hardy-Weinberg equilibrium. Association between specific genotypes and controls/patients status were tested using 2x2 contingency tables comparing individual genotype against a pool of all other genotypes. Significance was measured using the chi square include odds ratios and 95% confidence intervals. Association between each genotype and other variables were tested using linear regression. In statistical tests alpha was set to 0.05. Results with $p \leq 0.05$ were considered to be significant.

RESULTS

General status of the 20 colorectal cancer patients and 20 cancer-free healthy control subjects are summarized in Table (1). These cases and controls were well matched for age and sex with no statistically significant differences between them (P= 0.058, and P= 0.757; respectively). In addition, there were no significant differences between the cases and controls as regards to tobacco smoking (P= 1.0).

Table(1): General status of colorectal cancer patients and controls.

Variable		Cases (n=20) N	(%)	Controls(n=20) N	(%)	p-value*
Age	≤ 60	11	55	16	80	0.058
	> 60	9	45	4	20	
Gender	Male	11	55	12	60	0.757
	Female	9	45	8	40	
Tobacco smoking	Yes	9	45	9	45	1.00
	No	11	55	11	55	

* Significance difference P < 0.05

The distribution of allele frequencies of XRCC1 gene among patients and controls groups were in Hardy-Weinberg equilibrium. No significant difference was observed in Arg and Gln alleles between colorectal cancer patients and control group (Table 2). The distribution of genotypes frequencies of XRCC1 gene at codon339 among patients and control groups showed that Gln-Gln, genotype had a higher frequencies in colorectal cancer patients (10 %) compared to control group (5 %), with no statistical significant difference between them.(OR= 0.474; 95% CI: 0.039 – 5.688).

Table (2): Genotype frequencies distribution of XRCC1 gene at codon399 among patients and control group**:**

Allele	Patients (n=20) No (%)	Controls (n=20) No (%)	P	OR	95% CI
Arg	29 (72.5%)	31 (77.5%)		1.307	0.473- 3.609
Gln	11(27.5%)	9 (22.7%)	0.606	0.765	0.277 – 2.114
Arg/Arg	11 (55%)	12 (60%)	0.749	1.227	0.34– 4.307
Arg/Gln	7(35%)	7 (35%)	1	1	0.273 – 3.667
Gln/Gln	2 (10%)	1 (5%)	0.548	0.474	0.039 – 5.688

* Significance difference P < 0.05

There was no significant difference (P > 0.05) in the distribution of XRCC1 genotypes at codon 339 among colorectal cancer patients and control groups when stratified by age; Table (3), gender ; Table (4)and tobacco smoking status Table (5).

Table (3):Genotype frequencies distribution of XRCC1 gene at codon 399 among patients and control group stratified by age:

Age	Genotype	Patients (n=20) No (%)	Controls (n=20) No (%)	χ^2	P
≤ 60	Arg/Arg	9 (81.8%)	10 (62.5%)	1.857	0.395
	Arg/Gln	1(9.1%)	5 (31.3%)		
	Gln/Gln	1 (9.1%)	1 (6.3%)		
> 60	Arg/Arg	2 (22.2%)	2 (50%)	1.264	0.532
	Arg/Gln	6 (66.7%)	2 (50%)		
	Gln/Gln	1 (11.1%)	0 (0%)		

*Significance difference P < 0.05

Table (4): Genotype frequencies distribution of XRCC1 gene at codon 399 among patients and control group stratified by gender:

Gender	Genotype	Patients (n=20) No (%)	Controls (n=20) No (%)	χ^2	P
Female	Arg/Arg	6 (66.7%)	5 (62.5%)	3.043	0.218
	Arg/Gln	1(11.1%)	3 (37.5%)		
	Gln/Gln	2 (22.2%)	0 (0%)		
Male	Arg/Arg	5 (45.5%)	7 (58.3%)	1.693	0.429
	Arg/Gln	6 (54.5%)	4 (33.3%)		
	Gln/Gln	0 (0%)	1 (8.3%)		

* Significance difference P < 0.05

Table (5): Genotype frequencies distribution of XRCC1 gene at codon 399 among patients and control group stratified by tobacco smoking status:

Tobacco smoking	Genotype	Patients (n=20) No (%)	Controls (n=20) No (%)	χ^2	P
Positive	Arg/Arg	2 (18.2%)	5 (55.6%)	3.086	0.143
	Arg/Gln	7 (81.8%)	3 (33.3%)		
	Gln/Gln	0 (0%)	1 (11.1%)		
Negative	Arg/Arg	9 (81.8%)	7 (63.6%)	3.280	0.094
	Arg/Gln	0 (0%)	4 (36.4%)		
	Gln/Gln	2 (18.2%)	0 (0%)		

* Significance difference P < 0.05

DISCUSSION:

The present study showed that the distribution of allelic frequencies of XRCC1 gene at codon 399 among colorectal cancer patients and cancer free controls were in Hardy-Weinberg equilibrium. The frequency of the XRCC1 399 Gln allele in the current study was comparable with several previous studies (2,21,22,31,-38) done one population from different ehtinc back grounds, these studies investigate the possible associations between colorectal cancer and polymorphisms in the coding region of XRCC1 gene yield conflicting results. The present study showed no significant association between XRCC1 gene at codon 399 among colorectal cancer patients and controls.

This study confirms another studies done on Mexican population from Western Mexico (39), and Caucasian population in Austria (40). It also agreed with many association studies from different ethnic and racial groups (41).

In contrast, other studies have reported association between different XRCC1 genotypes and colorectal cancer risk. Abdel Rahman et al (21), reported that the XRCC1 399Gln allele significantly increased the risk of colorectal cancer (OR = 3.98, 95% CI = 1.50–10.6). In addition; Mort et al. (42) noted that the risk of suffering colorectal cancer was significantly heightened for individuals who featured the XRCC3 241Thr allele (OR = 1.52, 95% CI = 1.04–2.22) and only slightly increased for those individuals who revealed the XRCC1 399Gln and XPD 751Gln alleles. Also; a further meta-analysis concerning XRCC1 and CRC was performed by Jiang et al (43).Suggested a protective effect of the polymorphic allele of XRCC1 399.

Although we didn't find any significant associations between these DNA-repair genes and colorectal cancer risk, the risk appeared to be slightly increased for individuals who featured the XRCC1 Gln allele and 399Gln/Gln genotype. The lack of association in the current study could be due to the small sample size of CRC cases that limit the statistical power, in addition the current study is considered to be preliminary as only one gene has been studied comparing with Mort et al (42). The result of the present study together with previous studies; suggested that the XRCC1 gene is not a risk factor for colorectal cancer and cannot be used as a susceptibility marker for the disease. The divergence in results from different studies on XRCC1 polymorphisms may be related to variation in carcinogenic exposure and ethnic origin of the studied populations. Too small sample size and/or the inadequate controlling for certain confounders such as age, gender, alcohol consumption and cigarette smoking may contribute to differing results. It is possible that some of the candidate genes only contribute to colorectal cancer development in combination with certain dietary and /or lifestyle factors. Interaction between various gene products may increase cancer risk. A combination of polymorphisms in these genes may have additive or synergistic effects (44).

Incidence of CRC varies considerably with age, gender, and race (45).The current study showed no significant difference in the distribution of XRCC1 genotypes at codon 399 among colorectal cancer patients and control groups when stratified by age, and this was in consistent with several studies (46).

In contrast, yeh et al(38), performed his study on 727 carcinomas case and has yield an association between XRCC1 allele and increased CRC risk among young individual. In addition, Abdel rahman et al (21) showed that XRCC1 allele is associated with increase CRC risk only in younger individuals less than 40 years old.

The conflicting results between the current study and the previous two studies might be due to the difference in the cut off age range, and selection of younger cases, as the cut off age range in the present study was 60 years old.

Colorectal cancer is the fourth most common cancer in men and the third most common cancer in women worldwide (47,48)., The present study failed to find a significant difference in the distribution of XRCC1 genotypes at codon 399 among colorectal cancer patients and control groups when stratified by gender, and this was in accordance with several studies (44). This may indicate that gender is not a risk factor for colorectal adenocarcinoma.

However; in contrast a meta-analysis provides strong evidence that men are at greater risk for advanced colorectal neoplasia across all age groups. Others (32) reported that genotype distribution of the XRCC1 and XRCC3 genes does not vary between sexes, but differences between ethnic groups have been suggested. In addition, Colorectal cancer incidence rates for both males and females were statistically significantly increased from 1983-87 to 1998-2002 for 27 of 51 cancer registries. (49) This might inform decisions to create sex-specific colorectal cancer screening recommendations, in addition, male gender and smoking have a larger impact on the prevalence of colorectal neoplasia than family history, suggesting an extensive evaluation of additional risk stratification in popula-

tion-based screening, particularly by sex.

Epidemiologic studies across different continents have consistently reported that cigarette smoking is strong adenoma risk factor (50-58) .The strength of the associations and the overall consistency of the results across different populations suggest that smoking is likely to be causal. A pooled analysis of various studies showed that smoking is more likely to be a risk factor for adenoma formation rather than adenoma growth or dysplasia (59). Whereas smoking for <20 years seems to be associated with smaller adenomas, smoking for >20 years tends to associate with larger adenomas (55, 57, 60). In addition, smoking was reported to increase the prevalence of multiple versus single adenomas, as well as adenoma recurrence (55,56,61). Altogether, these data support the hypothesis that smoking might be related to a small adenoma to large adenoma sequence. Given the known role of adenomas as precursors of carcinomas (62), these data support the hypothesis that smoking may contribute to the formation of lesions that can later progress to colon carcinomas (63).

The present study failed to find a significant difference in the distribution of XRCC1 genotypes at codon 399 among colorectal cancer patients and control groups when stratified by smoking status , however; Shin A et al found a Stronger associations were observed for high-risk adenomas. Two meta-analyses of cigarette smoking and colorectal cancer risk confirmed the increased colorectal cancer risk in smokers (64,65).

Also Shrubsole et al (66) found that cigarette smoking was associated with increased risk of adenomatous and hyperplastic colorectal polyps. The associations between cigarette smoking and increased polyp risk were particularly strong for current smoking and years of smoking and for cases with hyperplastic polyps or both types of polyps (66).

2 Procedure for Paper Submission

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