# Arg399Gln polymorphism of *XRCC1* gene and risk of colorectal cancer in Kashmir: A case control study

SANIYA NISSAR[1], TUFAIL AHMAD LONE[2], MUJEEB ZAFAR BANDAY[3], ROOHI RASOOL[1], NISSAR A. CHOWDRI[2], FAZL Q. PARRAY[2], SAFIYA ABDULLAH[1] and AGA SYED SAMEER[1,4]

Departments of [1]Immunology and Molecular Medicine and [2]General Surgery, Sher-I-Kashmir Institute of Medical Sciences, Bemina; [3]Department of Biotechnology, Kashmir University, Hazratbal; [4]Department of Biochemistry, Medical College, Sher-I-Kashmir Institute of Medical Sciences, Bemina, Srinagar, Kashmir, India

**Abstract.** The aim of this study was to investigate the role of the *XRCC1* Arg399Gln polymorphism in the susceptibility of a Kashmiri population to colorectal cancer (CRC). We investigated the genotype distribution of the *XRCC1* gene in 130 CRC cases in comparison with that of 150 healthy subjects. There was no direct significant association between the *XRCC1* genotypes and CRC; however, the Arg/Gln genotype was associated with an elevated risk of CRC (OR>1.47) and the Gln/Gln variant genotype was associated with an increased risk of CRC in various clinicopathological parameters. This study suggests that the *XRCC1* polymorphism is associated with an increased risk of CRC.

## Introduction

Colorectal cancer (CRC) is the third most common cancer in males and the second most common cancer in females worldwide (1). In the Kashmir valley, CRC represents the third most common gastrointestinal cancer following esophageal and gastric cancer (2-4).

The Kashmiri population is exposed to a particular set of environmental and dietary risks, including exposure to nitroso compounds, amines and nitrates reported to be present in local foodstuffs, the majority of which have been shown to contain significant irritants and carcinogens (2,3,5,6).

Genetic polymorphisms in DNA repair genes, which lead to amino acid substitution, may influence individual capacity to repair DNA damage, which may be associated with increased genetic instability and carcinogenesis (7). In mammalian cells, four major DNA repair pathways have been identified: base excision repair (BER), nucleotide excision repair (NER), double-strand break repair and mismatch repair (8,9).

The DNA repair gene *XRCC1*, located at 19q13.2, codes for a scaffolding protein physically associated with DNA polymerase β, DNA ligase III, human AP endonuclease, polynucleotide kinase and poly (ADP-ribose) polymerase (8,10,11), which function in a complex to facilitate BER and single-strand break repair processes. The BER pathway mainly removes non-bulky base adducts produced by methylation, oxidation or reduction by ionizing radiation or oxidative damage (12). The *XRCC1* protein is capable of binding directly to both gapped and nicked DNA, as well as to gapped DNA associated with DNA polymerase β, suggesting that this protein may be independently involved in DNA damage recognition (13).

Three polymorphisms occurring at conserved sequences in the *XRCC1* gene have been reported, and amino acid substitutions were detected at codons 194 (Arg>Trp), 280 (Arg>His) and 399 (Arg>Gln) (14). Out of the 3 polymorphisms, the *XRCC1* codon 399 polymorphism has been studied most widely in a number of cancers but with varied results (15-23). While an increased risk has been reported in lung cancer (22) and breast cancer (17,18), there is no definite correlation between *XRCC1* 339 status and the risk of CRC. Abdel-Rahman *et al* (15) were the first to report that the *XRCC1* 399Gln allele, similarly to the *XRCC1* 399Arg/Arg genotype, was associated with an increased risk of developing CRC, particularly amongst young urban residents; however, these results were not reproducible and have not been demonstrated in any other population since. Stern *et al* (24) reported that the 399SNP of the *XRCC1* gene plays a role in modifying the risk of CRC by interactions with fatty acids of the diet. There are also some contradictory reports such as that of Mort *et al* (20), who reported no correlation between CRC risk and polymorphisms in any of the 4 NER genes or in *XRCC1*. Yeh *et al* (21) investigated the involvement of the DNA repair pathway genes in modulating the risk of CRC in a Taiwanese population and found the Arg 399 form to cause an increased cancer risk. Similarly, Skjelbred *et al* (2006) (23) reported a decreased risk of CRC with the Gln399 genotype. More recently, in a study by Wang *et al* (2010) in a

Table I. Genotype frequencies of the *XRCC1* gene polymorphism in CRC cases and controls.

| *XRCC1* genotype | CRC cases (n=130) | Controls (n=150) | OR (95% CI); P¥; Fψ | $\chi^2$; P-value (overall) |
|---|---|---|---|---|
| GG (wild) | 63 (48.5%) | 75 (50.0%) | 1 | 0.40; 0.81 |
| AG (heterozygous) | 37 (28.5%) | 30 (20.0%) | 1.47 (0.82-2.64); 0.19; 0.23 | |
| AA (variant) | 30 (23.0%) | 45 (30.0%) | 0.79 (0.44-1.4); 0.43; 0.47 | |

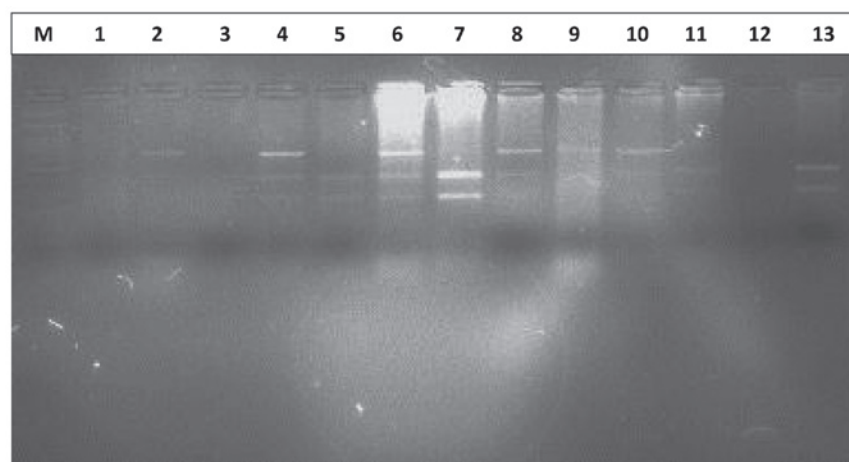CRC, colorectal cancer; ¥, Pearson's P-value; ψ, Fisher's exact P-value.



Figure 1. Representative gel of *XRCC1* Arg399Gln polymorphism, showing MspI digested amplicons. The Arg allele is represented by 374 and 241-bp fragments, while the Gln allele is represented by a 615-bp band. Lane M, 100-bp ladder; lanes 2, 9 and 10, homozygous (Gln/Gln) genotype (615 bp); lanes 5, 7, 11 and 13, homozygous (Arg/Arg) genotype (374 and 241 bp); lanes 4, 6 and 8, homozygous (CC) genotype (615, 374 and 241 bp).

South East Indian population (25), the *XRCC1* Gln399 allele was found to significantly increase the risk of rectal cancer. In the present study, we conducted a hospital-based case-controlled investigation to evaluate the potential impact of the *XRCC1* Arg399Gln gene polymorphism on the risk of CRC in a Kashmiri population. We also investigated whether there was a correlation between the clinicopathological variables and the *XRCC1* variant genotype (Gln/Gln), and hence its role in modulating the risk of CRC.

**Materials and methods**

*Study population*. This study included 130 CRC cases. All patients were recruited from the Department of General Surgery, Sher-I-Kashmir Institute of Medical Sciences, Kashmir, India. Blood samples were collected from 160 age- and gender-matched individuals with no signs of any malignancy to serve as external controls. The mean age in the patient and control groups was 53 years (Table I).

Data on all CRC patients were obtained from personal interviews with patients and/or their guardians, and their medical records. All patients and/or guardians were informed about the study and their willingness to participate was recorded on a predesigned questionnaire (available on request). The collection and use of blood samples (from patients and controls) for this study had been previously approved by the appropriate institutional ethics committee.

*DNA extraction and polymerase chain reaction*. DNA extraction was performed using the ammonium precipitation method. Genotyping for the *XRCC1 R399Q* polymorphism was determined using a method described previously (25). The oligonucleotide primers used for the amplification of the target region were: forward F-5'-TTG TGC TTT CTC TGT GTC CA-3' and reverse R-5'-TCC TCC AGC CTT TTC TGA TA-3', which generated a 615-bp fragment.

The polymerase chain reaction (PCR) was carried out in a final volume of 20 µl, containing 50 ng genomic DNA template, 1X PCR buffer (Fermentas, Glen Burnie, MD, USA), with 2 mM MgCl$_2$, 0.5 µM of each primer (Sigma-Aldrich, Bangalore, India), 50 µM deoxynucleotide triphosphates (dNTPs; Cinnagen, Tehran, Iran) and 0.25 units of DNA polymerase (Invitrogen, Bangalore, India). For PCR amplification, the standard programme was used as follows: one initial denaturation step at 94°C for 7 min, followed by 40 denaturation cycles of 30 sec at 94°C, 30 sec of annealing at 57°C and 30 sec of extension at 72°C for 40 cycles, followed by a final elongation cycle at 72°C for 7 min.

The PCR product of *XRCC1* was then digested with 2 units of *MspI* in a reaction mixture of 20 µl for 3 h at 37°C. The Arg allele revealed 374 and 241-bp fragments, while the Gln allele was not digested (indicative of the absence of the *MspI* cutting site; Fig. 1).

DNA amplicons, as well as the digestion products, were electrophoresed through a 2-3% agarose gel (Genie, Bangalore,

Table II. Association between the *XRCC1* polymorphism and clinicopathological characteristics.

| Variables | No. (%) | GG (n=63) | AG (n=37) | AA (n=30) | $\chi^2$; P-value |
|---|---|---|---|---|---|
| **Age group** | | | | | |
| ≤50 | 48 (36.9) | 14 | 20 | 14 | **11.73; 0.002** |
| >50 | 82 (63.1) | 49 | 17 | 16 | |
| **Gender** | | | | | |
| Female | 54 (41.54) | 22 | 12 | 20 | **10.20; 0.006** |
| Male | 76 (58.46) | 41 | 25 | 10 | |
| **Dwelling** | | | | | |
| Rural | 91 (70.0) | 36 | 33 | 22 | **11.61; 0.003** |
| Urban | 39 (30.0) | 27 | 4 | 8 | |
| **Smoking status** | | | | | |
| Ever | 81 (62.3) | 41 | 23 | 17 | 0.61; 0.737 |
| Never | 49 (37.7) | 22 | 14 | 13 | |
| **Tumor location** | | | | | |
| Colon | 52 (40.0) | 17 | 22 | 13 | **10.42; 0.005** |
| Rectum | 78 (60.0) | 46 | 15 | 17 | |
| **Nodal status** | | | | | |
| Involved | 88 (67.7) | 50 | 18 | 20 | **10.08; 0.006** |
| Not involved | 42 (32.3) | 13 | 19 | 10 | |
| **Tumor grade** | | | | | |
| WD | 98 (75.4) | 48 | 28 | 22 | **0.09; 0.956** |
| MD+PD | 32 (24.6) | 15 | 9 | 8 | |

The header "No. of cases (n=130)[a]" spans the GG, AG, AA columns.

[a]One was squamous cell carcinoma. Significant P-values are shown in bold. WD, well-differentiated; MD, moderately differentiated; PD, poorly differentiated.

India) for resolution. The genotypes of >20% of the samples were reassessed in a double-blind manner by two independent researchers, to confirm the results. A random sample of 10% of each genotype was re-checked with sequencing to confirm the results.

*Statistical analysis.* The observed frequencies of genotypes in CRC patients were compared with controls using the Chi-square test, or Fisher's exact test when the expected frequencies were small. The Chi-square test was used to verify whether the genotype distributions were in Hardy-Weinberg equilibrium. P≤0.05 was considered to indicate a statistically significant result. Statistical analyses were performed using PASW version 18 software.

**Results**

A total of 130 CRC cases and 150 control subjects were included in this study. The CRC patients consisted of 76 males and 54 females (M/F ratio, 1.41) and the control subjects consisted of 88 males and 72 females (M/F ratio, 1.2; data not shown). The mean age in both the patient and control groups was 53 years. No significant gender- or age-related differences were observed between the groups (P>0.05).

Furthermore, out of 130 confirmed cases of CRC, 125 cases were sporadic, 4 were familial adenomatous polyposis and one case was hereditary non-polyposis (Lynch syndrome) CRC. All but one CRC case had adenocarcinoma and one had squamous cell carcinoma of the basal cell type. Fifty-two cases had carcinoma in the colon and 78 had carcinoma in the rectum; 78 were rural and 39 urban, and 81 were smokers and 49 non-smokers (Table II).

Among the CRC cases, we found the frequency of the *XRCC1* genotype to be 48.5% GG (63/130), 28.5% AG (37/130) and 23.0% AA (30/130), while the frequency in the general control population was 50.0% GG (75/150), 20.0% AG (30/150) and 30.0% AA (45/150). The overall association between the *XRCC1* polymorphism and the CRC cases was found to be non-significant (P>0.05; Table II). Furthermore, an independent analysis for the AG and AA genotypes revealed a significant correlation with the risk of CRC (P<0.05). The overall hazard ratio of the *XRCC1* A allele in CRC was 0.79 (95% CI, 0.44-1.4).

The correlation of the *XRCC1* polymorphic status with the clinicopathological characteristics was also carefully analyzed. We found a significant association (P<0.05) of the A allele with the age, gender, dwelling, tumor location, nodal status and tumor grade of the patients (P<0.05; Table II).

## Discussion

In this hospital-based case-control study of CRC patients in Kashmir, we analyzed the polymorphism of the *XRCC1 R399Q* gene, a DNA repair gene, and its concomitant role in modulating the risk of CRC.

The *XRCC1* gene belongs to a DNA repair gene family and encodes for a protein which plays a role in the repair of single-strand breaks (SSB) and in base excision repair (BER) (23). Shen *et al* (14) first of all reported the three common polymorphisms for the *XRCC1* gene occurring at its conserved sequences, all of which affect the coding region of the gene. These coding polymorphisms result in amino acid substitutions affecting the overall activity of the synthesized proteins, which are reported to affect codons 194 (Arg>Trp), 280 (Arg>His) and 399 (Arg>Gln) (23).

We found noteworthy results in our population comparable to those of Abdel-Rahman *et al* (15), since we found the frequency of the *XRCC1* genotype to be 48.5% GG (63/130), 28.5% AG (37/130) and 23.0% AA (30/130) in CRC cases. This frequency distribution is also comparable to that observed in the study of Wang *et al* (25).

In the case of the *XRCC1* Arg194Trp polymorphism, a few studies reported a reduced risk of cancer associated with the 194Trp variant form (16) while another study by Skjelbred *et al* (23) reported no association of the codon 194 polymorphism with the risk of CRC. In one of the studies, which involved the investigation of the *XRCC1* Arg280His allele and the risk of cancer, no association was observed (19). The *XRCC1* Arg399Gln polymorphism has been well studied in many cancers and positive correlations have been established; however, the results from these studies are not consistent (15,17,19-21,26).

The Arg399Gln polymorphism of the *XRCC1* gene resides at the C-terminal side of the poly (ADP-ribose) polymerase interacting domain which has been indicated as a protein-protein interaction module in many proteins involved in DNA repair mechanisms in the cell (27).

The majority of epidemiological case-control studies did not find any significant correlation between the *XRCC1* 399Gln variant and the risk of CRC (23,24,28,29), However, a case-control study carried out on a Taiwanese population found that an increased risk of CRC correlated with the *XRCC1* 399Arg/Arg genotype when compared with the *XRCC1* 399Gln in younger subjects (21). Although a study on a Norwegian population by Skjelbred *et al* (23) reported the *XRCC1* 399Gln allele to be related to a reduction in the incidence of high-risk adenomas, there was no association with any risk of carcinomas. In contrast to these reports, Abdel-Rahman *et al* (15) observed a significantly increased risk of CRC with the *XRCC1* 399Gln allele in their study on an Egyptian population and this effect was shown to be more significant among urban residents. In addition, Hong *et al* (19) demonstrated a positive association for this polymorphism in their study on a South Korean population.

Although our study on a Kashmiri population did not find any significant correlation between the *XRCC1* Arg399Gln polymorphism and an increased risk of CRC, similarly to many of the already reported studies, we did find an increased risk of CRC among Arg/Gln heterozygous cases compared

to the controls (OR=1.47; 95% CI, 0.82-2.64). We also found a significant correlation between the 399Gln allele and the various clinicopathological parameters (Table II), particularly for gender (females), dwelling (rural) and tumor location (rectum). As already noted by Wang *et al* (25), differences due to gender may arise either due to physiologically different effects of the *XRCC1* 399Gln allele or due to different dietary habits, lifestyles and other genetic factors.

The interaction of the polymorphism with various environmental factors causes an increase in the overall susceptibility to CRC in any population (30,31). Therefore, we also hypothesize that since our population is exposed to certain environmental and dietary risks, including the consumption of sun-dried and smoked fish and meat, dried and pickled vegetables, red chilli, hakh (a leafy vegetable of the Brassica family), hot noon chai (salted tea) and hukka (water pipe) smoke (2,3,5), these may play a significant role in modulating the effect of the polymorphism in a dominant model of inheritance. As previously reported, the etiology and incidence of various gastrointestinal cancers in this population has been attributed to probable exposure to nitroso compounds, amines and nitrates, reported to be present in local foodstuffs, the majority of which have been shown to contain significant irritants and carcinogens (6).

The effect of 399Gln is also increasingly amplified in the carriers due to the impairment (or reduction) of the repair pathways, which in turn is reflected in significantly higher DNA adduct levels and an increased sister chromosome exchange frequency when compared with 399Arg (32).

We conclude that there is a significant correlation between the *XRCC1* A870G polymorphism and the risk of CRC in the ethnic Kashmiri population. These correlations now need to be authenticated in a large sample study, in order to discern racial differences and determine the aggressiveness of CRC.

## Acknowledgements

## References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, *et al*: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
2. Sameer AS, Nissar S, Abdullah S, Chowdri NA and Siddiqi MA: DNA repair gene 8-oxoguanine DNA glycosylase Ser326Cys polymorphism and colorectal cancer risk in a Kashmiri population. DNA Cell Biol 31: 541-546, 2012.
3. Sameer AS, Shah ZA, Nissar S, Mudassar S and Siddiqi MA: Risk of colorectal cancer associated with the methylenetetra-hydrofolate reductase (MTHFR) C677T polymorphism in the Kashmiri population. Genet Mol Res 10: 1200-1210, 2011.
4. Javid G, Zargar SA, Rather S, Khan AR, Khan BA, *et al*: Incidence of colorectal cancer in Kashmir valley, India. Indian J Gastroenterol 30, 7-11, 2011.

5. Rasool S, Ganai BA, Kadla SA, Ahanger AG, Qazi F, et al: The ECRG1 290Arg/Gln polymorphism is related to risk of esophageal squamous cell carcinoma in Kashmir. Asian Pac J Cancer Prev 12: 265-269, 2011.
6. Siddiqi M, Kumar R, Fazili Z, Spiegelhalder B and Preussmann R: Increased exposure to dietary amines and nitrate in a population at high risk of oesophageal and gastric cancer in Kashmir (India). Carcinogenesis 13: 1331-1335, 1992.
7. de Boer JG: Polymorphisms in DNA repair and environmental interactions. Mutat Res 509: 201-210, 2002.
8. Yu Z, Chen J, Ford BN, Brackley ME and Glickman BW: Human DNA repair systems: an overview. Environ Mol Mutagen 33: 3-20, 1999.
9. Christmann M, Tomicic MT, Roos WP and Kaina B: Mechanisms of human DNA repair: an update. Toxicology 193: 3-34, 2003.
10. Wood RD, Mitchell M, Sgouros J and Lindahl T: Human DNA repair genes. Science 291: 1284-1289, 2001.
11. Caldecott KW, Aoufouchi S, Johnson P and Shall S: XRCC1 polypeptide interacts with DNA polymerase beta and possibly poly (ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick-sensor' in vitro. Nucleic Acids Res 24: 4387-4394, 1996.
12. Duarte MC, Colombo J, Rossit AR, Caetano A, Borim AA, Wornrath D and Silva AE: Polymorphisms of DNA repair genes XRCC1 and XRCC3, interaction with environmental exposure and risk of chronic gastritis and gastric cancer. World J Gastroenterol 11: 6593-6600, 2005.
13. Marintchev A, Mullen MA, Maciejewski MW, Pan B, Gryk MR and Mullen GP: Solution structure of the single-strand break repair protein XRCC1 N-terminal domain. Nat Struct Biol 6: 884-893, 1999.
14. Shen MR, Jones IM and Mohrenweiser H: Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res 58: 604-608, 1998.
15. Abdel-Rahman SZ, Soliman AS, Bondy ML, Omar S, El-Badawy SA, Khaled HM, Seifeldin IA and Levin B: Inheritance of the 194Trp and the 399Gln variant alleles of the DNA repair gene XRCC1 are associated with increased risk of early-onset colorectal carcinoma in Egypt. Cancer Lett 159: 79-86, 2000.
16. Goode EL, Ulrich CM and Potter JD: Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 11: 1513-1530, 2002.
17. Nexo BA, Vogel U, Olsen A, Ketelsen T, Bukowy Z, Thomsen BL, Wallin H, Overvad K and Tjonneland A: A specific haplotype of single nucleotide polymorphisms on chromosome 19q13.2-3 encompassing the gene RAI is indicative of post-menopausal breast cancer before age 55. Carcinogenesis 24: 899-904, 2003.
18. Moullan N, Cox DG, Angele S, Romestaing P, Gerard JP and Hall J: Polymorphisms in the DNA repair gene XRCC1, breast cancer risk, and response to radiotherapy. Cancer Epidemiol Biomarkers Prev 12: 1168-1174, 2003.
19. Hong YC, Lee KH, Kim WC, Choi SK, Woo ZH, Shin SK and Kim H: Polymorphisms of XRCC1 gene, alcohol consumption and colorectal cancer. Int J Cancer 116: 428-432, 2005.
20. Mort R, Mo L, McEwan C and Melton DW: Lack of involvement of nucleotide excision repair gene polymorphisms in colorectal cancer. Br J Cancer 89: 333-337, 2003.
21. Yeh C-C, Sung F-C, Tang R, Chang-Chieh C and Hsieh L-L: Polymorphisms of the XRCC1, XRCC3, & XPD genes, and colorectal cancer risk: a case-control study in Taiwan. BMC Cancer 5: 12, 2005.
22. Ratnasinghe D, Yao SX, Tangrea JA, Qiao YL, Andersen MR, Barrett MJ, Giffen CA, Erozan Y, Tockman MS and Taylor PR: Polymorphisms of the DNA repair gene XRCC1 and lung cancer risk. Cancer Epidemiol Biomarkers Prev 10: 119-123, 2001.
23. Skjelbred CF, Saebø M, Wallin H, Nexø BA, Hagen PC, Lothe IM, Aase S, Johnson E, Hansteen IL, Vogel U and Kure EH: Polymorphisms of the XRCC1, XRCC3 and XPD genes and risk of colorectal adenoma and carcinoma, in a Norwegian cohort: a case control study. BMC Cancer 6: 67, 2006.
24. Stern MC, Siegmund KD, Corral R and Haile RW: XRCC1 and XRCC3 polymorphisms and their role as effect modifiers of unsaturated fatty acids and antioxidant intake on colorectal adenomas risk. Cancer Epidemiol Biomarkers Prev 14: 609-615, 2005.
25. Wang J, Zhao Y, Jiang J, Gajalakshmi V, Kuriki K, Nakamura S, Akasaka S, Ishikawa H, Suzuki S, Nagaya T and Tokudome S: Polymorphisms in DNA repair genes XRCC1, XRCC3 and XPD, and colorectal cancer risk: a case-control study in an Indian population. J Cancer Res Clin Oncol 136: 1517-1525, 2010.
26. Vogel U, Nexo BA, Wallin H, Overvad K, Tjonneland A and Raaschou-Nielsen O: No association between base excision repair gene polymorphisms and risk of lung cancer. Biochem Genet 42: 453-460, 2004.
27. Masson M, Niedergang C, Schreiber V, Muller S, Menissier-de Murcia J and de Murcia G: XRCC1 is specifically associated with poly (ADP-ribose) polymerase and negatively regulates its activity following DNA damage. Mol Cell Biol 18: 3563-3571, 1998.
28. Sliwinski T, Krupa R, Wisniewska-Jarosinska M, Lech J, Morawiec Z, Chojnacki J and Blasiak J: No association between the Arg194Trp and Arg399Gln polymorphisms of the XRCC1 gene and colorectal cancer risk and progression in a Polish population. Exp Oncol 30: 253-254, 2008.
29. Improta G, Sgambato A, Bianchino G, Zupa A, Grieco V, La Torre G, Traficante A and Cittadini A: Polymorphisms of the DNA repair genes XRCC1 and XRCC3 and risk of lung and colorectal cancer: a case-control study in a Southern Italian population. Anticancer Res 28: 2941-2946, 2008.
30. Zhang LQ, Huang X, Wang J, Shang JQ, Bai J, et al: The cyclin D1 G870A polymorphism and colorectal cancer susceptibility: a meta-analysis of 20 populations. Asian Pacific J Cancer Prev 12: 81-85, 2011.
31. Palmqvist R, Stenling R, Oberg A, et al: Expression of cyclin D1 and retinoblastoma protein in colorectal cancer. Eur J Cancer 34: 1575-1581, 1998.
32. Lunn RM, Langlois RG, Hsieh LL, Thompson CL and Bell DA: XRCC1 polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res 59: 2557-2561, 1999.