

## Distribution of *XRCC1* genotypes in north Indian population

Manjula Kiran, Roli Saxena & Jyotdeep Kaur

*Department of Biochemistry, Postgraduate Institute of Medical Education & Research, Chandigarh, India*

Received August 26, 2008

**Background & objectives:** *XRCC1*, a major DNA repair gene, acts as a scaffold of different activities involved in repair by interacting with components of base excision repair (BER) at the site of damage. Polymorphisms in this gene are associated with variations in the repair efficiency which might predispose an individual to cancer risk. To associate a gene polymorphism with disease risk, it is imperative to have the data for its genotype distribution in normal population. The present study was therefore carried out to find distribution of *XRCC1* polymorphisms (codons 194, 280 and 399) in normal north Indian population.

**Methods:** Healthy volunteers hailing from north India (150) were enrolled in the study. DNA was isolated from blood samples and genotyping of codons 194, 280 and 399 of *XRCC1* gene was done by PCR-restriction fragment length polymorphism (RFLP), using specific primers.

**Results:** The frequencies obtained for heterozygous genotype of codons 194 and 399 were 45 and 49 per cent respectively and were higher than wild and variant genotypes. For codon 280, the highest frequency (59%) was obtained for the wild genotype. Frequencies of the variant genotypes of codons 194 and 399 were higher in males and females respectively. The allele frequencies also followed the similar trends.

**Interpretation & conclusions:** A significant distribution of variant and heterozygous *XRCC1* genotypes was noticed that warrants further studies on the association between these genotypes and disease risk in our study population.

**Key words** Base excision repair - DNA damage - genetic polymorphism - *XRCC1*

To counteract the deleterious consequences of the DNA damaging agents, the DNA repair system as a whole takes care of most of the insults inflicted on a cell's vital genetic information, thus repairing DNA damage and safeguarding genomic integrity<sup>1</sup>. Base excision repair (BER) pathway, one of the major defense mechanisms, is generally considered to constitute the primary defense against lesions generated by ionizing radiation and strong alkylating agents as well as by endogenous DNA-damaging agents. X-ray repair cross

complementing group 1 (*XRCC1*) is a major gene involved in DNA repair by BER<sup>2</sup>, out of more than 20 BER genes identified so far. It is located on chromosome 19q13.2, spans a genetic distance of 32 kb, comprises 17 exons and encodes a protein (XRCC1) of 633 amino acids having molecular weight of 70 kDa<sup>3</sup>. XRCC1 has no catalytic activity of its own but it acts both as a scaffold and a modulator of the different activities involved in base excision repair by interacting with and bringing together DNA polymerase  $\beta$ , DNA ligase III,

poly (ADP-ribose) polymerase in the N-terminal, C-terminal and central regions of XRCC1, respectively and polynucleotide kinase at the site of DNA damage<sup>4,5</sup>. Thus, XRCC1 protein provides a physical link between the incision and sealing steps of the BER process.

As mutations/polymorphisms in DNA repair genes are associated with variations in the repair efficiency of DNA damage, this repair deficit may eventually predispose an individual to cancer risk, birth defects and a reduced life span<sup>6,7</sup>. Till date, a total of eight non-synonymous single nucleotide polymorphisms (SNPs) have been reported in XRCC1 but the three most commonly identified<sup>8</sup> are in codon 194 (Arg to Trp), codon 280 (Arg to His) and codon 399 (Arg to Gln). These variations occurring at the evolutionarily conserved amino acid residues are thought to result in altered efficiency of the protein function and have been associated with cancer risk<sup>9</sup>. Available literature on genotype distribution of XRCC1 and its association with various cancers suggest that the results vary among different populations studied<sup>4,10</sup>. The present study was carried out to gather the preliminary data on three commonly occurring polymorphisms of XRCC1 in north Indian population as prior knowledge of XRCC1 genotype distribution in normal population is imperative to establish their association with risk of various diseases.

### Material & Methods

**Subjects:** All consecutive willing subjects showing no clinical signs to suggest any form of disease were included in this study after obtaining informed consent. These were the healthy relatives and attendants of the patients attending the Hepatology OPD, Postgraduate Institute of Medical Education and Research, Chandigarh, hailing from three northern States of India namely Punjab, Haryana and Himachal Pradesh. Blood samples (5 ml) were collected in acid citrate dextrose (ACD) from 150 healthy volunteers (103 males, 47 females; age range of 20-68 yr, median age of 35 yr) during January 2005 to September 2007. The protocol of the study was approved by the "Institute Ethical Committee". The number of subjects to be enrolled in this preliminary study was decided so that power of the study was more than 80 per cent.

Forward and reverse primers for PCR based genotyping of XRCC1 were procured from Sigma Aldrich Pvt. Ltd., Bangalore, India. All other reagents for PCR were purchased from Bangalore Genei Pvt. Ltd., Bangalore, India.

DNA was isolated from the blood samples by the method of Daly *et al*<sup>11</sup> and finally suspended in TE buffer (pH 8.0; 10 mM Tris, 1 mM EDTA). The genotypes for XRCC1 codons 194, 280 and 399 were studied by PCR-restriction fragment length polymorphism (PCR-RFLP)<sup>12</sup>. The sequence specific primers from the coding region designed by Primer3 Input (version 0.3.0)<sup>13</sup> were as follows:

Codon 194 forward primer: 5' GCCCCGTCCCAGGTA 3'; Codon 194 reverse primer: 5' AGCCCCAAGACCCTTTTCACT 3'; Codon 399 forward primer: 5' TTGTGCTTTCTCTGTGTCCA 3'; Codon 399 reverse primer: 5' TCCTCCAGCCTTTTCTGATA 3'; Codon 280 forward primer: 5' CCAGCTCCAACCTCGTACC 3'; Codon 280 reverse primer: 5' ATGAGGTGCGTGCTGTCC 3'.

PCR was carried out in 10 µl reaction mixture consisting of 1xPCR buffer (50 mM KCl; 2.5 mM MgCl<sub>2</sub>; 20 mM Tris HCl, pH 8.4), 200 µM each dNTP, 0.6 µg/ml of both codons 194 and 399 primers (or 0.8 µg/ml of codon 280 primers), 100 ng genomic DNA and 0.75U/ml Taq polymerase (Sigma Chemical Co., USA). Initial denaturation was carried out at 94°C for 30 sec, annealing at 64°C for codons 194 and 399 (61°C for codon 280) for 30 sec and synthesis at 72°C for 30 sec. Final elongation was carried out at 72°C for 10 min. The amplified products of codons 194 and 399 were then digested with 5U of Msp1 (New England Biolabs, Beverly, MA, USA), for 12-16 h at 37°C and resolved on 3 per cent agarose gel. Codon 280 amplification product was treated with 5U of Rsa1 (New England Biolabs, Beverly, MA, USA) under similar conditions and electrophoresed on 4 per cent agarose gel. 1U corresponds to the amount of enzyme required to digest 1 µg of lambda DNA in 1 h at 37°C in 50 µl of assay buffer.

**Data analysis:** The distribution of polymorphism in the normal subjects was expressed as allele frequency and genotype frequency. Chi square analysis was done by using SPSS v13 software to assess the significance of the distributions among different groups.

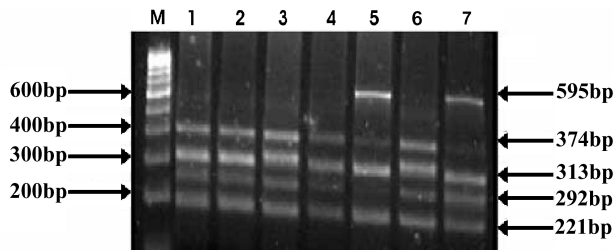
### Results

The amplification of codons 194 and 399 resulted in the product of 487 and 595bp respectively. For codon 194, the PCR amplified products upon treatment with Msp1, yielded the products of 292 and 313bp for wild and variant forms respectively. However, a product of 174bp was obtained in all samples due to the presence of an invariant Msp1 restriction site (Fig. 1). For codon 399, the PCR amplified products were digested to 374bp

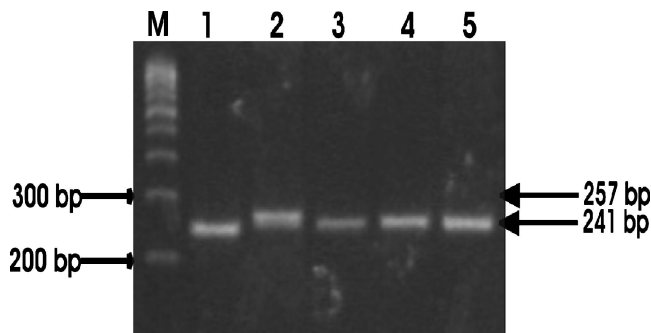
and 221bp with *Msp*I, showing the presence of wild genotype. However, the samples showing undigested product of 595bp denoted the presence of variant form (Fig. 1). The PCR amplified product of 257 bp for codon 280, showed the presence of 241 and 257 bp bands after *Rsa*I digestion depicting homozygous wild and variant genotypes respectively (Fig. 2).

The genotype frequency of codon 194 was 19 per cent for homozygous wild, 45 per cent for heterozygous and 36 per cent for homozygous variant genotype. However, in codon 280, genotype frequencies for wild, heterozygous and variant genotypes were 59, 19 and 22 per cent respectively. The genetic distribution for codon 399 was 32 per cent for wild and for heterozygous and variant it was 49 and 19 per cent respectively (Table I). The genotype distributions of *XRCCI* codons 194 and 399 were consistent with those predicted under conditions of Hardy-Weinberg equilibrium. Significant linkage disequilibrium was observed between codons 194 and 399 ( $P=0.00017$ ).

When the total allele frequency was calculated, it was revealed that in codons 194, 280 and 399, the frequencies for wild allele were 0.41, 0.68 and 0.56 respectively. However, for variant allele, the frequencies



**Fig. 1.** RFLP analysis of *XRCCI* codons 194 and 399. Lane M: 100 bp molecular marker; lanes 1-4, 6, 7: heterozygous variant genotype for codon 194 and homozygous wild genotype for codon 399; lane 5: homozygous variant for codon 194 and heterozygous variant for codon 399.



**Fig. 2.** RFLP analysis of *XRCCI* codon 280. Lane M: 100 bp DNA ladder; lanes 1, 3-5: homozygous wild genotype; lane 2: heterozygous variant genotype.

**Table I.** *XRCCI* genotype distribution (%) in the subjects

Codon	Wild	Heterozygous	Variant
194	28 (19)	68 (45) <sup>***</sup>	54 (36) <sup>***</sup>
280	88 (59)	28 (19) <sup>***</sup>	64 (22) <sup>***</sup>
399	48 (32)	74 (49) <sup>**</sup>	28 (19) <sup>***</sup>

Data represented as number of subjects showing respective genotype (%); \*\*  $P<0.01$  \*\*\*  $P<0.001$  compared to wild genotype

**Table II.** *XRCCI* allele frequencies\*

Codon	Wild allele	Variant allele
194	0.41	0.58
280	0.68	0.32
399	0.56	0.43

\*Specific allele/total alleles studied

were 0.58, 0.32 and 0.43 respectively for codons 194, 280 and 399 (Table II).

Hence, the results obtained in the present study revealed a significant distribution of frequency of heterozygous and homozygous variant *XRCCI* polymorphisms in North Indian population. This is particularly important in case of codon 194, for which the variant allele is observed to be widely distributed.

## Discussion

Amongst the DNA repair pathways, BER is generally believed to constitute the primary defense against single-strand breaks formed by various endogenous and exogenous DNA-damaging agents<sup>4</sup>. *XRCCI* gene has been found to play a pivotal role in the multistep BER pathway. Human *XRCCI* is the first mammalian gene isolated that affects cellular sensitivity to ionizing radiation<sup>14</sup>. The three potential functional polymorphisms reported in *XRCCI* have been implicated in cancer risk like squamous cell carcinoma of head and neck, lung cancer, oesophageal cancer, breast cancer and many more malignancies, although there is no concrete evidence to associate their incidence with *XRCCI* polymorphisms<sup>15,16</sup>.

The frequency of codon 194 observed in the present study was lower for the wild genotype and higher for the variant genotype as compared to that reported by Chen *et al*<sup>17</sup> in a case-control study conducted in Chinese population. The authors have reported the frequencies to be 55.90 and 4.90 per cent for *XRCCI* codon 194 homozygous wild and homozygous variant respectively and for codon 399, 52.5 per cent homozygous wild and 7.1 per cent homozygous variant genotype frequencies were reported. Various reports from Indian population reported that frequencies of homozygous wild genotype of codon 194 lie in the range of 81 per cent to 84 per cent

in controls<sup>18-22</sup>. Also, the frequency of the homozygous variant genotype of codon 194 was reported to be much lower (0.9-2%) in these studies than that observed in the present study (36%). The frequency of homozygous wild genotype of codon 399 as observed in the present study was comparable with earlier reports from Indian population<sup>18-23</sup>. Our results for codon 399 (32% and 19%) were comparable to the genotype frequencies in Caucasians which were 44 and 14 per cent for homozygous wild and variant genotypes respectively<sup>24</sup>. Two recent studies conducted in north Indian population, showed that 33.93 and 28.7 per cent controls were homozygous wild while 16.96 and 13.9 per cent were homozygous variant<sup>25,26</sup>. Similarly, a study in New Hampshire, revealed the existence of wild genotype in 40.6 per cent and variant genotype in 16.5 per cent of the normal subjects<sup>27</sup>; the values were 43 and 16 per cent for the two respective cases in a study conducted in Whites<sup>4</sup>. The frequencies were different from that reported in the Asians where as 57 and 10 per cent respectively for the wild and variant genotypes for codon 399<sup>28</sup>. Zhang *et al*<sup>29</sup> in a study conducted in China, showed that for *XRCC1* 399 codon, 53.1 per cent normal subjects had homozygous wild genotype while 8.9 per cent were homozygous variant. A study from Korea reported that 60 per cent of the subjects were homozygous wild while 4.4 per cent were homozygous variant for codon 399<sup>30</sup>. In another study in Korean population, 6.8 per cent subjects had homozygous variant genotype for codon 399 and a positive association of this polymorphism was established with the risk of breast cancer<sup>31</sup>.

The genotype distribution (22%) for the variant form of *XRCC1* codon 280 observed in our study was much higher as compared to its total absence in Americans<sup>28</sup>. However, the codon 280 heterozygous genotype (19%) in our study matched with that of the community control subjects (23%) in a study on nasopharyngeal carcinoma in Chinese population<sup>10</sup>. The frequency of the heterozygous genotype of codon 280 observed in the present study (19%) was comparable to that reported earlier (14-23%)<sup>20-22</sup>.

The allele frequency for codon 194 variant allele (0.58) was slightly higher than that observed in Chinese and Korean population (ranging between 0.3-0.5)<sup>31,32</sup>. However, the frequency observed for codon 399 variant allele in our study (0.43) was found to be comparable to that reported in the above mentioned studies. In a study from Egypt, *XRCC1* codon 194 and 399 homozygous variant allele frequencies were reported to be 0.05 and 0.14 respectively<sup>33</sup>. It was revealed that *XRCC1* 194

variant allele frequency was 0.27 in Taiwanese, 0.35 in Chinese and in the Koreans it was not defined, while the *XRCC1* 399 variant allele frequencies were 0.26, 0.26 and 0.22 for Taiwanese, Chinese and Korean population respectively<sup>34</sup>. In a study conducted in Polish, Danish and American populations, *XRCC1* allele frequency were 0.05, 0.05 and 0.35 for 194Trp, 280His and 399Gln alleles respectively<sup>35</sup>. A study in Taiwanese and North Carolinian populations reported the allele frequency of homozygous variant in codon 194 to be 0.06, 0.05 and 0.27 among Whites, Blacks and Taiwanese respectively. However, in codon 280, it was found to be 0.03, 0.02 and 0.11 respectively, while in codon 399 it was 0.37, 0.17 and 0.26 for the respective populations<sup>36</sup>. Hence, the distribution of allele frequencies varies among different populations.

In conclusion, our preliminary findings showed that the *XRCC1* genetic polymorphic forms are widely distributed in north Indian population. However, the small number of subjects studied here remains a limitation to be conclusive regarding the results obtained.

#### Acknowledgment

The first author (MK) acknowledges the Indian Council of Medical Research (ICMR), New Delhi, for awarding Senior Research Fellowship.

#### References

1. Smith TR, Miler MS, Lohman KK, Case LD, Hu JJ. DNA damage and breast cancer risk. *Carcinogenesis* 2003; 24 : 883-9.
2. Vidal AE, Boiteux S, Hickson ID, Radicella JP. *XRCC1* coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. *EMBO J* 2001; 20 : 6530-9.
3. Lindahl T, Wood RD. Quality control by DNA repair. *Science* 1999; 286 : 1897-905.
4. Duell EJ, Millikan RC, Pittman GS, Winkel S, Lunn RM, Tse C, *et al*. Polymorphisms in the DNA repair gene *XRCC1* and breast cancer. *Cancer Epimediol Biomark Prev* 2001; 10 : 217-22.
5. Whitehouse CJ, Taylor RM, Thistlethwaite A, Zhang H, Karimi-Busheri F, Lasko DD, *et al*. *XRCC1* stimulates human polynucleotide kinase activity at damaged DNA termini and accelerates DNA single-strand break repair. *Cell* 2001;104 : 107-17.
6. Hansen WK, Kelly MR. Review of mammalian DNA repair and translational implications. *J Pharmacol Exp Ther* 2000; 295 : 1-9.
7. Ronen A, Glickman BW. Human DNA repair genes. *Environ Mol Mutagen* 2001; 37 : 241-83.
8. Hu Z, Ma H, Chen F, Wei Q, Shen H. *XRCC1* polymorphisms and cancer risk: meta-analysis of 38 case-control studies. *Cancer Epimediol Biomark Prev* 2005; 14 : 1810-8.

9. Chacko P, Rajan B, Joseph T, Mathew BS, Pillai MR. Polymorphisms in DNA repair gene *XRCC1* and increased genetic susceptibility to breast cancer. *Breast Cancer Res Treat* 2005; 89 : 15-21.
10. Cho EY, Hildsheim A, Chen CJ, Hsu MM, Chen I, Mittl BF, *et al.* Nasopharyngeal carcinoma and genetic polymorphisms of DNA repair enzymes *XRCC1* and *hOGG1*. *Cancer Epimediol Biomark Prev* 2001; 12 : 1100-4.
11. Daly AK, Steen VM, Fairbrother KS, Idle JR. CYP2D6 multiallelism. *Methods Enzymol* 1996; 272 : 199-210.
12. Lunn RM, Langolis RG, Hsich LL, Thompson CL, Bell DA. *XRCC1* polymorphisms: effects on aflatoxin B1-DNA adducts and glycoporphin A variant frequency. *Cancer Res* 1999; 59 : 2557-61.
13. Rozen S, Skaletsky HJ. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S, editors. *Bioinformatics methods and protocols: Methods in molecular biology*. Totowa NJ: Humana Press; 2000. p. 365-86.
14. Thompson LH, Brookman KW, Jones NJ, Allen SA, Carrano AV. Molecular cloning of the human *XRCC1* gene. *Mol Cell Biol* 1990; 10 : 6160-71.
15. Han J, Hankinson SE, Colditz GA, Hunter DJ. Genetic variation in *XRCC1*, sun exposure and risk of skin cancer. *Br J Cancer* 2004; 91 : 1604-9.
16. Yu HP, Zhang XY, Wang XL, Shi LY, Li F, Su YH, *et al.* DNA repair gene *XRCC1* polymorphisms, smoking and esophageal cancer risk. *Cancer Detect Prev* 2004; 28 : 194-9.
17. Chen S, Tang D, Xue K, Xu L, Ma G, Hsu Y, *et al.* DNA repair gene *XRCC1* and *XPB* polymorphisms and risk of lung cancer in a Chinese population. *Carcinogenesis* 2002; 23 : 1321-5.
18. Syamala VS, Syamala V, Sreedharan H, Raveendran PB, Kuttan R, Ankathil R. Contribution of *XPB* (Lys751Gln) and *XRCC1* (Arg399Gln) polymorphisms in familial and sporadic breast cancer predisposition and survival: an Indian report. *Pathol Oncol Res* 2009; 15 : 389-97.
19. Gangwar R, Manchanda PK, Mittal RD. Implications of *XRCC1*, *XPB* and *APE1* gene polymorphism in North Indian population: a comparative approach in different ethnic groups worldwide. *Genetica* 2009; 136 : 163-9.
20. Mittal RD, Singh R, Manchanda PK, Ahirwar D, Gangwar R, Kesarwani P, *et al.* *XRCC1* codon 399 mutant allele: a risk factor for recurrence of urothelial bladder carcinoma in patients on BCG immunotherapy. *Cancer Biol Ther* 2008; 7 : 645-50.
21. Majumder M, Sikdar N, Ghosh S, Roy B. Polymorphisms at *XPB* and *XRCC1* DNA repair loci and increased risk of oral leukoplakia and cancer among NAT2 slow acetylators. *Int J Cancer* 2007; 120 : 2148-56.
22. Ramachandran S, Ramadas K, Hariharan R, Rejnish Kumar R, Radhakrishna Pillai M. Single nucleotide polymorphisms of DNA repair genes *XRCC1* and *XPB* and its molecular mapping in Indian oral cancer. *Oral Oncol* 2006; 42 : 350-62.
23. Sreeja L, Syamala VS, Syamala V, Hariharan S, Raveendran PB, Vijayalekshmi RV, *et al.* Prognostic importance of DNA repair gene polymorphisms of *XRCC1* Arg399Gln and *XPB* Lys751Gln in lung cancer patients from India. *J Cancer Res Clin Oncol* 2008; 134 : 645-52.
24. Duell EJ, Holly EA, Bracci PM, Wiencke JK, Kelsey KT. A population-based study of the Arg399Gln polymorphism in X-ray repair cross-complementing group 1 (*XRCC1*) and risk of pancreatic adenocarcinoma. *Cancer Res* 2002; 62 : 4630-6.
25. Sobti RC, Singh J, Kaur P, Pachouri S, Siddiqui EA, Bindra HS. *XRCC1* codon 399 and *ERCC2* codon 751 polymorphism, smoking, and drinking and risk of esophageal squamous cell carcinoma in a North Indian population. *Cancer Genet Cytogenet* 2007; 175 : 91-7.
26. Pachouri SS, Sobti RC, Kaur P, Singh J. Contrasting impact of DNA repair gene *XRCC1* polymorphisms Arg399Gln and Arg194Trp on the risk of lung cancer in North Indian population. *DNA Cell Biol* 2007; 26 : 186-91.
27. Nelson HH, Kelsey KT, Mott LA, Karagas MR. *XRCC1* Arg399Gln polymorphism, sunburn and non-melanoma skin cancer: evidence of gene-environment interaction. *Cancer Res* 2002; 62 : 152-5.
28. Ratnasinghe D, Yao S, Tangrea JA, Qiao YL, Andreson MR, Barrett MJ, *et al.* Polymorphisms of the DNA repair gene *XRCC1* and lung cancer risk. *Cancer Epimediol Biomark Prev* 2001; 10 : 119-23.
29. Zhang X, Miao X, Liang G, Hao B, Wang Y, Tan W, *et al.* Polymorphisms in DNA base excision repair genes *ADPRT* and *XRCC1* and risk of lung cancer. *Cancer Res* 2005; 65 : 722-6.
30. Park JY, Lee SY, Jeon HS, Bae NC, Chae SC, Joo S, *et al.* Polymorphism of the DNA repair gene *XRCC1* and risk of primary lung cancer. *Cancer Epimediol Biomark Prev* 2002; 11 : 23-7.
31. Kim SU, Park SK, Yoo KY, Yoon KS, Choi JY, Seo JS, *et al.* *XRCC1* genetic polymorphism and breast cancer risk. *Pharmacogenetics* 2002; 12 : 335-8.
32. Lee JM, Lee YC, Yang SY, Yang W, Luh SP, Lee J, *et al.* Genetic polymorphisms of *XRCC1* and risk of the esophageal cancer. *Int J Cancer* 2001; 95 : 240-6.
33. Abdel Rahman SZ, Soliman AS, Bondy ML, Omar S, El-Badawy SA, Khaled HM, *et al.* 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* 2000; 159 : 79-86.
34. Duarte MC, Colombo J, Rossit A, Silva A. Polymorphisms of the DNA repair genes *XRCC1* and *XRCC3* in Brazilian population. *Genet Mol Biol* 2005; 28 : 397-401.
35. Butkiewicz D, Rusin M, Enewold L, Shields PG, Chorazy M, Harris CC. Genetic polymorphisms in DNA repair genes and risk of lung cancer. *Carcinogenesis* 2001; 22 : 593-7.
36. Kiran M, Saxena R, Chawla YK, Kaur J. Polymorphism of DNA repair gene *XRCC1* and hepatitis-related hepatocellular carcinoma risk in Indian population. *Mol Cell Biol* 2009 (in press: PMID: 19194663).

Reprint requests: Dr Jyotdeep Kaur, Department of Biochemistry, Postgraduate Institute of Medical Education & Research Chandigarh 160 012, India  
e-mail: jyotdeep2001@yahoo.co.in