Risk effects of XRCC1 Arg399Gln and XPD Lys751Gln gene polymorphisms in de novo acute myeloid leukemia – A study from India

Santhi S1, Sangeetha Vijay1, Sureshkumar R1, Sreeja L4, Preethi Sara George2, Geetha N3 & Hariharan S1*

1Division of Cancer Research, 2Division of Epidemiology & Biostatistics, 3Division of Medical Oncology, Regional Cancer Centre, Medical College, Thiruvananthapuram, Kerala 695011, India, 4Genome Sciences Centre, BC Cancer Research Centre, 675 West 10th Avenue, Vancouver, BC V5Z 1L3, Canada

Received 28 July 2014; revised 22 July 2015; accepted 3 August 2015

Defects in the DNA repair pathway are connected to many malignancies including leukemia. Variability in the repair rates leading to genotoxic damage have been shown to be associated with polymorphisms of XRCC1 Arg399Gln and XPD Lys 751 Gln, involved in the base excision repair and the nucleotide excision repair pathway. The present study focused on the risk effects of these polymorphisms in acute myeloid leukemia (AML). Genotypes were analyzed in 211 AML patients and control samples using polymerase chain reaction and restriction fragment length polymorphism. Logistic regression analysis was carried out to find the association of various polymorphisms with susceptibility to AML. Kaplan-Meier survival analysis was carried out to find the influence of these polymorphism on patients overall survival. The carriers of XRCC1 399 GA genotype were at increased risk for AML than with carriers of GG genotype. Subjects carrying XPD 751 AC as well as CC genotype were at an increased risk for the onset of the disease. The CC genotype was found to be having the higher risk than other genotypes with an OR of 5.29 (P < 0.001). In the combination group XRCC1 399 GG/ XPD 751 AC + CC and XRCC1 399 GA+AA/ XPD 751 AA show an increased risk for AML with P value 0.010 and 0.004 respectively. While a 6.8 fold increased risk was shown by the both variant genotype combination. A significant association for the XRCC and XPD genotype with overall survival was not detected in the present study. Our findings suggests that genetic polymorphism in the DNA repair genes may modulate susceptibility to AML.

Keywords: DNA repair, XRCC1 399, XPD 751, Acute Myeloid Leukemia, genotypes, susceptibility

Introduction

Acute myeloid leukemia (AML) is a clonal hemopoietic disorder that is frequently associated with genetic instability which is characterized by diverse chromosomal and molecular changes. Most of the AML cases occur de novo, with no known exposure to leukemogenic substances1. The AML cells require more than one mutation, either as point mutations, gene rearrangements and/or chromosomal translocations for the disease to develop2. DNA is at constant risk from damage from both endogenous and exogenous sources. The genetic material is protected by means of many mechanisms, including DNA repair pathways and protection against oxidative stress which is made possible with the help of many genes that encode proteins that function to protect cells against genetic instability3,4. Among this DNA repair pathways play a vital role and various studies showed that defects in repair pathways are connected to many different types of diseases, including cancer5,6,7. The ability of an individual to prevent and repair damage is genetically determined and is the result of combinations of multiple genes that may display subtle differences in their activity8,9,10. Genetic polymorphisms have been identified in several DNA repair genes resulting in amino acid substitutions leading to alteration of the wild-type (WT) protein function, promoter activity, mRNA stability and splice variants, affects DNA adduct levels thus by decreasing the cellular ability to repair endogenous and exogenous DNA damage thereby contributing to disease susceptibility3,11,12.

Among the various DNA repair gene polymorphisms, the most commonly studied were the xeroderma pigmentosum group D (XPD, also known as ERCC2) and x-ray repair cross-complementing groups 1 and 3 (XRCC1 and XRCC3) genes13. Several variants of XRCC1 and XPD have been described of which XRCC1 codon 399 polymorphism in exon 10 that results in an arginine (Arg) to glutamine (Gln) substitution [399G→A substitution]
and the XPD lysine (Lys) 751– glutamine (Gln) polymorphism in exon 23 [751A→C] were important. The XPD gene located on chromosome 19q13. 3 consists of 23 exons, encodes a 5’ to 3’ DNA helicase protein (761 amino acids) that is a component of the transcription factor TFIIH involved in the nucleotide excision repair (NER) pathway and functions to remove bulky adducts and UV-induced DNA damage. The XPD lysine (Lys) 751 glutamine (Gln) polymorphism does not reside in a known functional domain of XPD and was initially thought to be unlikely to result in an altered DNA repair capacity, but later studies produced contrasting results with decreased and elevated nucleotide excision repair capacity. Individuals with XPD 751Gln/Gln have suboptimal DNA-repair capacity to remove UV photoproducts when compared to the XPD 751Lys/Lys and Lys/Gln genotypes according to one study. The association of XPD-751 polymorphisms with different cancers like breast, colorectal, bladder, lung, pancrease, esophageal cancer, haematological malignancy etc has been investigated in various studies and found to be associated with increased susceptibility for most of the cancers.

The XRCC1 gene (17 exons) located on chromosome 19q13.2 encodes the XRCC1 protein with 633 amino acids with no known enzymatic activity but act as a scaffolding protein directly associated with DNA polynuclease beta, DNA ligase III and poly ADP-ribose polymerase (PARP) and functions as a complex to facilitate the base excision repair (BER) and the repair of single strand breaks. DNA damage caused by a variety of internal and external factors, including ionizing radiation, alkylating agents, and oxidation, requires repair by the BER pathway. The XRCC codon 399 resides in the functionally important PARP-binding domain. Lunn et al. 1999 reported that individuals with XRCC1 399 Gln/Gln genotype were significantly associated with higher levels of aflatoxin B1-DNA adducts when compared to individuals with Arg/Arg genotype. Thus the functional DNA repair capacity was reported to be significantly deficient in this polymorphism. Polymorphisms in XRCC1 repair gene has been associated with an increased risk for colorectal cancer, breast cancers, pancreatic cancer, lung cancer, prostate cancer etc. Lack of association of polymorphism of this gene with bladder cancer were reported in some studies. Association of this polymorphism as susceptibility factor in leukemia were also studied. Several reports with respect to polymorphism of XRCC 399 and XPD 751 and association with various malignancies were available globally, but studies were not available from Indian population with regard to these gene polymorphisms and AML risk.

The aim of the present study was to investigate the relationships between XRCC 399, and XPD 751 polymorphisms and the risk for developing de novo AML, and to investigate the prognostic significance of these polymorphisms in AML patients from India.

Materials and Methods

Study Population

The study was carried out in 211 de novo AML cases and an equal number of random healthy unrelated individuals with no known malignant diseases selected from different areas of the local population which form the control group were subjected to polymorphism analysis. The control samples used for the study were age and sex matched. Blood (2 ml) or bone marrow samples (1 ml) from patients were obtained at the time of diagnosis of AML, who attended the Medical Oncology Department of the Regional Cancer Centre, Trivandrum between January 2009 and May 2011. Peripheral blood samples (2 ml) were collected from the controls. Samples were collected from the study subjects after getting the written informed consent. The diagnosis of AML was made by pathological and cytogenetic analysis and classification was based on the French-American-British criteria. Genomic DNA was extracted from cells by using phenol chloroform method. The study was approved by the Ethical Committee of Regional Cancer Centre. Patient’s clinical data like WBC count, blast percentage, platelet count, haemoglobin, LDH levels, etc were noted from the case file.

Genotyping

Genotypes of XRCC1 and XPD were determined by PCR followed by restriction fragment length polymorphism (PCR-RFLP). PCR was performed to amplify exon 10 of XRCC1 gene and exon 23 of XPD gene using specific primers, followed by RFLP using restriction enzyme MspI (10U) (Bangalore Genei) for XRCC1 and Pst I (10U) (Bangalore Genei) for XPD at 37°C for 16h. The PCR mixture 20 μl, consisting of 10 pmol of each primer, 0.2 mM each dNTP and 1 X
buffer, and 1U *Taq* polymerase were run at 94°C for 4 min for initial denaturation, followed by 37 cycles of 94°C for 30 s, 61°C for 30 s and 72°C for 30 s, followed by final extension for 4 min at 72°C for the amplification of XRCC1 399 gene. For the XPD 751 gene amplification the 20 μl PCR mixture containing 0.2 mM dNTP, 3% dimethyl sulfoxide, 8 pmol of each primers, 100 ng template DNA, and 1.5 units *Taq* polymerase in 1X PCR buffer was used. After an initial denaturation at 94°C for 7 min, there were 35 cycles of 50 s at 94°C, 50 s at 60°C, and 60 s at 72°C, and then a final extension step of 7 min at 72°C. After the restriction digestion of the PCR products they were subjected to 3% agarose gel electrophoresis, stained using ethidium bromide and analyzed in gel documentation system.

The genotypes were determined based on the band pattern. The *MspI* recognition site is present only in the wild type (Arg) GG genotype; hence, digestion of the Arg allele results in products of 374 bp and 221 bp. The G to A transition in exon 10 of XRCC1 abolishes the recognition site for *MspI* enzyme, and remains undigested. Thus the GA (Arg/Gln) genotype shows three fragments with 615, 374 and 221 bp and AA (Gln) genotype with a single fragment of 615 bp (Fig 1). In case of XPD polymorphism all PCR products contain an internal *PstI* site, resulting in 234 and 110 bp products in the 751 Lys/Lys (AA) allele. In addition, an extra *PstI* site is present in the Gln allele, resulting in 234/171/110/63 bp products for Lys/Gln (AC) and 63/171/ 110 bp for Gln/Gln (CC) genotypes (Fig. 2).

**Statistical Analysis**

Unconditional logistic regression was used to estimate odds ratio and 95% confidence interval (CI) to assess the effect of each gene on AML risk. The genotypes were combined to assess a potential synergism of polymorphisms. The effect of genetic polymorphism on the survival was estimated using Kaplan-Meier survival and assessed using log rank test. Quantitative variables were summarized using median (range) and analyzed using non-parametric test. Qualitative data were summarized using frequencies and percentages. Pearson Chi-square test was used to calculate the significance of association between polymorphism and other discrete variables. Probability value <0.05 were considered statistically significant. All the statistical analysis was carried out using the SPSS (version 17) software programme for windows.

**Results**

The XRCC1 and XPD gene polymorphism status was investigated in all the samples from study participants which include 211 AML cases and 211 controls. Cases and controls ranged in age from 18-70 years. Cancer group included 107 (51%) males and 104 females (49%). The cancer free population control group comprised of 110 males (52%) and 101 (48%) females. The mean age among AML cases was 40.09 years and among controls 40.50 years. The median age of the study subjects was 40 and 42 years for case and control group respectively. Regarding the distribution of genotypes in the study population, in cases 26% were GG, 66% were GA, and 8% were AA for the XRCC1 Arg399Gln polymorphism compared to 49%, 37% and 14% in controls. For XPD Lys751Gln polymorphism, 41% were AA, 42% AC and 17% CC genotypes in AML patients whereas the respective genotype frequencies were 65%, 30% and 5% in controls. Significantly higher odds ratios were encountered for XRCC1 399Gln genotype (OR=3.3; 95% CI: 2.16 – 5.09), P<0.001) as well as XPD 751AC genotype with an OR of 2.35 (95% CI: 1.54-3.59) with P<0.001, compared to their normal counterparts. The distribution of XRCC and XPD genotypes in patients and controls and their
association with AML risk is shown in Table 1 & 2. A very high increase in the odds ratio was observed for the CC genotype of XPD compared with others, showing a 5 fold increase in the risk for that particular genotype to induce cancer. The presence of a variant genotype (either heterozygous or homozygous) of XPD and XRCC was associated with an increased risk for AML with OR 2.8 (95% CI: 1.89-4.19) and 2.7 (95% CI: 1.79-4.07) \( [P < 0.001] \) respectively. We also looked for the combined effect of these variant genotypes in AML susceptibility. Compared to the XRCC1 399 GG/ XPD 751 AA genotype which being the reference genotype, the other combined variants namely, XRCC1 399 GG (wild) / XPD 751 AC+CC (variant) (OR=2.4, 95% CI: 1.226-4.7, \( P = 0.011 \)), XRCC1 399 GA+AA (variant) / XPD 751 AA (wild) (OR=2.33, 95% CI: 1.32-4.14, \( P = 0.004 \)), XRCC1 399 GA+AA (variant) / XPD 751 AC+CC (variant) (OR=6.8, 95% CI: 3.8-12.3, \( P<0.001 \)), had significantly higher odds ratios (Table 3), indicating a higher risk for AML susceptibility. The associations involving variant genotype combinations of XRCC1399GA + AA/XPD751AC + CC (both variants) emerged as a highly significant risk factor with odds ratios greater than 6.

There was no significant differences in the distribution of XRCC and XPD polymorphisms with respect to age, gender, clinical parameters like WBC, platelet, hemoglobin and bone marrow blast %, compared to their wild genotype. Cytogenetics was evaluated in all the cases, of whom 55% had normal, 34% had abnormal and in 11% cytogenetic analysis failed. A statistical significance was not observed for both XRCC and XPD polymorphism with the cytogenetics patterns and FAB subtypes. The details regarding the clinical parameters and association with genotypes were shown in Table 4.

Prognostic significance of DNA repair gene polymorphism was evaluated in 136 AML cases. An overall survival up to 30 months was analyzed. Kaplan-Meier survival analysis was performed for the single polymorphism and also for their combination genotypes. Analysis of overall survival in unstratified AML patients with respect to different polymorphisms compared to respective wild type genotype was not statistically significant. The XRCC 399 polymorphism and XPD 751 polymorphism didn’t showed any effect on the survival pattern of AML patients with normal and aberrant karyotype when compared to respective wild genotypes.

Discussion
Polymorphism in genes involved in carcinogen metabolism and DNA repair are reported as a source of inter-individual variability in human response to carcinogens. Studies have noted associations between risk of de novo AML and DNA repair gene polymorphisms\(^\text{18,45}\). In our study we assessed two

<table>
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<th>Case / Control</th>
<th>OR (95% CI)</th>
<th>P Value</th>
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<tr>
<td>XRCC1399 GG</td>
<td>55 / 103</td>
<td>1 (ref)</td>
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<td>XRCC1399 GA</td>
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<td>XRCC1399 GA/AA (Variant)</td>
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<td>XPD751 AA</td>
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<td>XPD751AC</td>
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<tr>
<td>XRCC1399GG/ XPD751 AA</td>
<td>25 / 69</td>
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</tr>
<tr>
<td>XRCC1399GG/ XPD751AC+CC</td>
<td>30 / 34</td>
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<tr>
<td>XRCC1399 GA+AA / XPD751 AA</td>
<td>61 / 70</td>
<td>2.337</td>
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<tr>
<td>XRCC1399GA+AA/XPD751AC+CC</td>
<td>95 / 38</td>
<td>6.8</td>
<td>&lt;0.001</td>
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(Fig. 3 & 4). Thus in our study, no difference in the OS was encountered between different groups studied. Combination genotypes were also analyzed for the survival and found no significant difference between the groups analyzed (data not shown), showing that the XRCC and XPD genotypes were not associated with the survival of de novo AML cases, even though the risk for onset of the disease is high for certain genotypes.

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(Table 1 — XRCC1 Arg399Gln polymorphism genotypes and risk for developing AML)

(Table 2 — XPD Lys751Gln polymorphism genotypes and risk of developing AML)

(Table 3 — Association between combination genotypes of XRCC1 Arg399Gln and XPD Lys751Gln with AML susceptibility)

(Table 4 — Distribution of XRCC and XPD polymorphisms with respect to age, gender, clinical parameters and cytogenetics patterns and FAB subtypes).
common polymorphisms of the XRCC1 and XPD genes that may influence DNA repair capacity and their association with AML risk and overall survival. The polymorphisms chosen for the study have been shown to have functional significance leading to decreased repair capacity and modulate cancer susceptibility via gene environment interactions.

In the present study higher odds ratios were noted for XRCC1 399 GA genotype (P= 0.0001) compared to their normal counterparts. There are reports suggesting that the presence of at least one XRCC1 399Gln allele indicated an increased risk of AML. Similar reports were observed with a significantly high risk of AML for the heterozygous and homozygous variant of XRCC 399 (OR=1.90 for
Various studies have reported the XRCC1 399 AA variant genotype and risk for several types of cancers due to reduced DNA repair capacity. In our study we didn’t observe an association of AA genotype and AML risk. The protective effect of XRCC Arg399Gln in the development of AML, particularly therapy related AML has been explained in the study by Seedhouse et al. The association of XRCC1 Arg399Gln and XPD Lys751Gln polymorphism with an increased risk for therapy related AML was reported in some studies. The present study showed an increased risk for the XPD 751 AC and CC genotypes (P<0.001) with a significantly higher odds ratio for the CC genotype showing a 5 fold risk for developing AML. Excessive risk for CC genotype was observed for the de novo and secondary AML in a study carried out in British AML patients. Another study showed a 1.6 fold increased risk for AC genotype to develop AML. Reports showing lack of association of XPD 751 polymorphic genotype for t-AML was reported. In a pediatric study, the author found no influence of genotype on susceptibility to de novo AML in children. A significant association with age, gender, clinical parameters like WBC and platelet count, haemoglobin and bone marrow blast percentage, cytogenetics risk group and FAB subtypes were not observed for the XRCC1399 and XPD751 polymorphic genotypes compared to wild genotype in our study population. Similar reports were observed in other studies also.

Polymorphisms in single genes are unlikely to alter the expression or function of specific protein to the extent of producing a pathological phenotype, a combined effect of different single nucleotide
polymorphism (SNPs) may produce a change in expression or protein function thus by increasing the AML pathogenesis. So we looked for the effect of combined genotypes with regard to AML and found that compared to the XRCC1 399 GG/ XPD 751 AA genotype, the other combined variants such as, XRCC1 399 / XPD 751 AC+CC (P=0.011), XRCC1 399 GA+AA/ XPD 751 AA (P=0.004), XRCC1 399 GA+AA/XPD 751 AC+CC (P<0.001), had significantly higher odds ratios indicating a increased risk for AML susceptibility. The presence of both XRCC1 & XPD variant genotypes was found to have more risk compared to others. Overall survival was analyzed for the single /combination genotypes of XRCC1 or XPD and found no significant association for the polymorphic genotype and survival compared to wild genotype. Similar results were reported in another study.54 The XPD codon 751 polymorphism is an independent prognostic marker for disease-free survival and overall survival in elderly AML patients treated with chemotherapy, having a modestly increased hazard ratio (HR) of 1.30 and 1.19 respectively showing that the glutamine variant was associated with a poorer prognosis relative to the lysine variant.53 But in a study of pediatric AML patients conducted by the Children’s Oncology Group, lack of association for survival and treatment-related mortality with XPD codon 751 genotypes was reported.56 Regarding the survival, diploid patients with the XPD AC/CC genotype survived shorter than those having the wild-type genotype, but disease free survival was not affected by the polymorphism.57, 58 Association of the different studied polymorphism with respect to overall survival was not observed in our study. Stratified analysis in different cytogenetic groups were also performed for checking the survival difference with respect to polymorphism, but found no association. Normal and abnormal karyotypes also does not differ in their survival with respect to polymorphisms of XRCC or XPD.

In brief, to our knowledge, this is the first report of XRCC1 and XPD polymorphisms in AML patients from India. Our results suggests that XRCC1 399 AG, XPD 751AC and CC genotypes might be risk genotypes for de novo AML, indicating that NER and BER pathways were important in AML pathogenesis. Due to the shorter survival period (30months) taken for the OS analysis, the actual prognostic significane was not detected. Extensive analysis with more number of samples could reveal the interaction of the genes in detail.

Acknowledgments
We wish to thank all the study participants for their contribution. We also thank the support provided by the staff members of various clinical departments of RCC, Trivandrum, for providing samples and patient clinical data.

References


