Research Paper

Volume : 3 | Issue : 9 | September 2014 • ISSN No 2277 - 8179

Genetic Variation in B-cell lymphoma 2- Associated X (BAX) Gene Confers Susceptibility to Carcinoma of Urinary Bladder

Medical Science

KEYWORDS : Bladder Cancer, BAX, Polymorphism, SNP, Apoptosis

Saranjeet Kaur | Department of Zoology, Panjab University, Sector-14, Chandigarh-160014, India

ABSTRACT

The aim of the study was to investigate genetic polymorphism in apoptotic gene, BAX (B-cell lymphoma 2-associated X), and the risk of bladder cancer through a hospital-based case-control study. This retrospective analysis consisted of 270 cases of bladder cancer and 252 controls.

The AA genotype of BAX 248 G/A polymorphism showed a 2.20-fold risk towards bladder cancer (OR = 2.20, 95% CI = 1.16-4.21) when compared to GG as the referent genotype, hence, proving the additive model. The BAX AA genotype showed a 2.56-fold increased risk (OR = 2.56, 95% CI = 1.23-5.39) of bladder cancer in males and a significantly increased risk towards bladder cancer in inhabitants of urban area (OR = 3.25, 95% CI = 1.19-9.16). Among alcoholics, both BAX AA (OR = 1.66, 95% CI = 1.27-2.17) and BAX GA + AA (OR = 2.01, 95% CI = 1.09-3.73) genotypes showed an increased risk towards bladder cancer, respectively. The BAX AA genotype also showed an increased risk towards bladder cancer among non-vegetarians (OR = 2.68, 95% CI = 0.98-7.60). Among cases having superficial stages, only the BAX AA genotype (OR = 2.19, 95% CI = 1.11-4.32) showed a significantly increased risk towards bladder cancer. The BAX AA genotype also showed a significantly increased risk (OR = 2.69, 95% CI = 1.32-5.51) towards the G2 carcinoma.

INTRODUCTION

Bladder cancer is known to be associated with tobacco smoking and occupational exposure. The aromatic amines present in tobacco smoke may be responsible for the increased risk of bladder cancer which generally includes beta-naphthylamine, 4-aminobiphenyl, and benzidine. Aniline dyes, which are often found in dyes and printing industries; and cyclophosphamide, a chemotherapeutic drug, are also known to be carcinogenic for bladder cancer [Leppert et al., 2006]. Hair dyes have also been associated with an increased risk of bladder cancer [Gago-Dominguez et al., 2003].

DNA repair, genomic instability and apoptosis are intimately linked phenomena, with important implication for the pathophysiology of cancer. While the relationship between populational variability in DNA repair and cancer risk has been extensively studied, the alternative aspect of the DNA damage response, that is, apoptosis, has been unjustly overlooked in the research of tumour-associated gene polymorphisms [Imyanitov et al., 2005]. Research on SNPs in apoptotic genes may uncover new low-penetrance determinants of tumour predisposition [Imyanitov et al., 2005]. Therefore, further analysis of the involvement of apoptosis-related SNPs in cancer susceptibility is highly justified.

Apoptosis is regulated by different pathways involving a number of genes that either promote or inhibit the process. The best-characterized apoptosis regulators include the anti-apoptotic BCL-2 (B-cell lymphoma 2) and the pro-apoptotic BAX (B-cell lymphoma 2-associated X) genes [Chen et al., 2007]. The ratio of expression of BCL-2 to BAX seems to be important in determining both in vitro and in vivo response to chemotherapeutic drugs [Pepper et al., 1998]. The protein products of these two genes physically interact with each other and the relative levels of the two proteins are important determinants of the apoptosis rate [Loro et al., 1999, Cory and Adams 2002, Kim et al., 2004]. Therefore, the relative expression of these two genes plays a key role in cellular homeostasis and cancer development.

The BAX gene has been mapped to chromosome 19q13.3. It encodes a 192-amino acid protein, weighing 21184 Da [Genetics Home Reference Website, 2009]. A single nucleotide polymorphism located within the 5'-untranslated region of the promoter of the BAX gene, BAX 248 G/A (rs4645878), has been reported to be correlated with the reduced expression of its gene (BAX) [Saxena et al., 2002].

MATERIALS AND METHODS

Study design and study subjects

This retrospective case-control study comprised 270 histopathologically proven cases of urinary bladder cancer and 252 cancer-free controls. Peripheral blood samples from patients with urinary bladder cancer, treated at Advanced Urology Centre (AUC) of the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, were collected during routine investigations. The ethical clearance for the present study was obtained from the Institute's Ethics Committee. Cases having HIV, allergies and other cancers, or patients having received chemotherapy were excluded. Informed consents were obtained from all the participants. Data with respect to their age, sex, smoking status, alcohol consumption, occupational status, area inhabited and eating habits were recorded. In patients with bladder tumour, the stage and grade of the tumour were noted.

Genotype analysis

Peripheral blood samples (2-4 ml) were collected from cases and controls in EDTA-coated vials. Genomic DNA was subsequently extracted from peripheral blood lymphocytes by the standard phenol-chloroform method. The BAX 248 G/A polymorphism was then determined by PCR-RFLP assay as per the conditions given in Table I (figure I). A mismatch was introduced near the 3' end of each of the primers, close to the mutation of interest, to create an artificial restriction site in the PCR product (PIRA-PCR – Primer Introduced Restriction Analysis-PCR) [Ke et al., 2001]. Independent repetition of genotyping in randomly selected samples produced the same results and hence, proved concordance.

Statistical Analysis

The power calculations were conducted at 80% with a significance level of 0.05. The sample size used for the present study was adequate. The data showed normal distribution on applying one-sample Kolmogorov-Smirnov Z test when age was taken as the test variable. The data were age-matched, as confirmed by T-test. The odds ratios (ORs) and 95% confidence intervals (CIs) were obtained using $\chi 2$ test and Fisher-Exact test for categorical variables. The odds ratios were calculated without adjustment for potential confounders, i.e., sex, area, job status, smoking, alcohol consumption and diet. The classification of occupations into low-risk and high-risk was done on the basis of previous studies [Manju et al., 2009; Cassidy et al., 2009; Samanic et al., 2008].

To achieve an adequate sample size with power of study at 80%, the various tumour stages were clubbed together and merged into two groups, i.e., superficial (Ta + T1) and muscle-invasive

(T2 + T3 + T4). Both additive and dominant modes of inheritance were considered. The p-values were two-sided. Values less than 0.05 were considered as significant. All statistical analyses were performed using SPSS, version 15.0 and Epi Info, version 3.4.3.

RESULTS

The distribution of the genotype frequencies of BAX 248 G/A polymorphism among cases and controls is summarized in Table II. The allele frequencies were 69.81% for allele G and 30.19% for allele A in the cases. In the control group, the allele frequencies for allele G were 76.39% and for allele A were 23.61%. When calculated on the basis of dominant model (GG vs. GA + AA), no significant differences were observed in the genotype frequencies of BAX polymorphism in relation to bladder cancer. In a dose-dependent manner, the BAX AA genotype had a 2.20-fold risk towards bladder cancer (OR = 2.20, 95% CI = 1.16-4.21) when compared to GG as the referent genotype, hence, proving the additive model.

The interaction of BAX 248 G/A polymorphism with various environmental factors has been summarized in Table III. The BAX AA genotype showed a 2.56-fold increased risk (OR = 2.56, 95% CI = 1.23-5.39) of bladder cancer in males. The AA genotype also showed a significantly increased risk towards bladder cancer in inhabitants of urban area (OR = 3.25, 95% CI = 1.19-9.16). Among alcoholics, both AA (OR = 1.66, 95% CI = 1.27-2.17) and GA + AA (OR = 2.01, 95% CI = 1.09-3.73) genotypes showed an increased risk towards bladder cancer, respectively. The AA genotype also showed an increased risk towards bladder cancer among non-vegetarians (OR = 2.68, 95% CI = 0.98-7.60).

The interaction of BAX 248 G/A polymorphism with each histological subcategory is summarized in Table IV. Among cases having superficial stages, only BAX AA genotype (OR = 2.19, 95% CI = 1.11-4.32) showed a significantly increased risk towards bladder cancer when compared to GG genotype as the referent (assuming an additive mode of inheritance). No other significant observations were seen. Among grades, the BAX AA genotype also showed a significantly increased risk (OR = 2.69, 95% CI = 1.32-5.51) towards the G2 carcinoma. No significant associations were observed with other grades.

DISCUSSION

The overexpression of BAX is prognostic of better survival in transitional cell carcinoma (TCC) of the bladder [Hussain et al., 2003]. In ovarian cancer, high BAX expression was found to be associated with significant improvement of the percentage of complete remissions after first-line chemotherapy with Paclitaxel and a platinum analogue. Survival was also high in other cancer groups with high BAX expression [Tai et al., 1998]. In patients with breast cancer, loss of BAX immunostaining was associated with a decreased response to chemotherapy and shorter survival [Krajewski et al., 1995]. In primary breast tumours and breast cancer cell lines compared with normal breast epithelium and non-malignant epithelial cell lines, BAX expression was reduced. It was possible to induce apoptosis in the cell lines with high BAX expression [Bargou et al., 1995]. Low BAX expression also correlated significantly with poor prognosis for patients with glottic carcinomas who received radiotherapy as primary treatment [Xie et al., 1998].

It is necessary to determine whether BAX expression is modulated by a G to A change [Packham and Stevenson, 2005]. The presence of the BAX promoter polymorphism has been correlated to reduced BAX RNA/protein expression and drug resistance [Saxena et al., 2002; Starczynski et al., 2005]. In the current study, the allele frequency of variant A allele was more in cases as compared to controls. On analyzing in a dose-dependent manner, the AA genotype was shown to have a 2.20-fold risk towards bladder cancer when compared to GG as the referent genotype. There are only a few reports on the association between BAX polymorphism and risk of cancer, mostly chronic lymphocytic leukaemia (CLL) [Saxena et al., 2002; Moshynska et al., 2003; Starczynski et al., 2005; Fegan et al., 2006; Nuckel et al., 2006; Skogsberg et al., 2006] and a few solid tumours [Chen et al., 2007; Yu et al., 2010], but there is no report on urinary bladder cancer.

The BAX 248 G/A polymorphism was reported to correlate with reduced expression of BAX, progression of disease stage and treatment resistance in CLL [Saxena et al., 2002]. These findings were supported by the reports of Starczynski et al. [2005]. Two studies on CLL did not show any significant associations with the genotype frequencies of BAX polymorphism [Starczynski et al., 2005; Skogsberg et al., 2006]. Chen et al. [2007] worked on the association of BAX polymorphism with squamous cell carcinoma of the head and neck (SCCHN). They could not find any statistically significant difference in the frequency distributions of BAX between cases and controls (P = 0.625). Even after the liaisons of variant genotypes, no significant results were seen. This promoter SNP in the BAX gene is particularly interesting because it is located within 100 bases from the TP-53 binding element in the BAX promoter region. There was an increased risk of SCCHN associated with the BAX (-248G>A) polymorphism among the TP53 heterozygotes. This may be due to the weakening of TP53 interaction with the 248AA genotype, leading to a decrease in the expression of pro-apoptotic BAX gene in the cells and a high risk of SCCHN [Chen et al., 2007]. This justification is consistent with the present results, where AA genotype was responsible for an increased risk.

The results of the present study are in contrast to a study carried out on lung cancer by Yu et al. [2010]. They showed that BAX 248A allele carriers yielded a significantly decreased risk of lung cancer, compared with BAX 248G allele carriers. According to Yu et al. [2010], BAX 248A allele exhibited significantly higher transcriptional activity compared with G allele. In another study carried out on multiple myeloma, the A variant of BAX (OR GA + AA = 0.40, 95% CI = 0.21-0.78) was found to be associated with a 60% decreased risk of multiple myeloma (P = 0.018) [Hosgood et al., 2009]. Although of significant interest, further work is required to determine the frequency of the polymorphism in larger cohorts of cancer patients and normal individuals, not only in bladder cancer but also in various other cancers, as the literature pertaining to BAX polymorphism is very less.

Gender

The BAX 248 AA genotype showed an increased risk (OR = 2.56, 95% CI = 1.23-5.39) of bladder cancer in males (Table III). No reports on the effects of gender on BAX gene expression have been published, although testosterone has been shown to induce BAX and decrease BCL-2 protein levels and promote apoptosis in human renal tubular cells [Verzola et al., 2004]. According to this statement, one could say that the BAX AA genotype might overcome the effect of testosterone and reduce BAX induction reducing apoptosis, and hence, leading to cancer.

Area inhabited

The BAX 248 AA genotype showed an increased risk among urban inhabitants (Table III). Various studies reported bladder cancer cases mainly from rural areas [Rafique, 2005; Zarzour et al., 2008]. But no studies on BAX 248 G/A polymorphism in particular were reported.

Alcohol consumption

Acetaldehyde is a major metabolite of ethanol. Both acetaldehyde and ethanol have been shown to accelerate apoptotic cell death in various cells [Baroni et al., 1994; Zimmerman et al., 1995; Oberdoerster and Rabin, 1999]. The BAX GA + AA geno-

type had a 2.01-fold increased risk of bladder cancer in alcoholics (Table III). Any other report regarding the role of BAX polymorphism towards the risk of bladder cancer, especially in alcoholics, has not been seen yet. In a study carried out on squamous cell carcinoma of the head and neck (SCCHN), Chen et al. [2007] did not find any evidence of potential gene-environment interactions among any of the three genotypes of BAX and status of alcohol consumption. Many murine models have shown that BAX expression is critically dependent on ethanol-exposure [Baroni et al., 1994], but such types of studies have not been validated on human cells yet.

A cohort study carried out in Netherlands failed to suggest any important association between alcohol consumption and the risk of bladder cancer. The association, if any, between alcohol consumption and bladder cancer is small [Zeegers et al., 2001]. An explanation for some apparently inconsistent epidemiological findings on alcoholic beverage consumption and cancer of the urinary bladder is that there are different correlates (including tobacco, coffee and diet) of alcoholic beverage drinking in various populations. Alcoholic beverage drinking, in part, may be positively correlated with tobacco smoking, a poorer diet or other recognized risk factors (i.e. social or occupational) for bladder cancer. Thus, residual confounding is possible.

Diet

The BAX AA genotype showed a 2.68-fold increased risk of developing bladder cancer in non-vegetarians (Table III). To date, epidemiologic studies on meat consumption and bladder cancer risk are few and inconsistent, and studies have typically had limited data on different types of meat intake. No case-control study has reported any association for meat intake and bladder cancer till date [La Vecchia and Negri, 1996; Steinmaus et al., 2000]. Certain meat items are found to have nitrosamines that act as bladder carcinogens or their precursors [Scanlan, 1983; Dich et al., 1996]. In addition, processed meats contain varying concentration of nitrites, added for preservation, which can be endogenously converted to nitrosamines [Lijinsky, 1999].

Histopathology

In the current study, the BAX AA genotype showed an increased risk towards bladder cancer in patients with superficial stage (OR = 2.19, 95% CI = 1.11-4.32) as well as in those with G2 grade (OR = 2.69, 95% CI = 1.32-5.51) (Table IV). No study with regard to BAX gene polymorphism on stage and grade of urinary bladder cancer could be found. But studies on BAX gene expression with respect to the same are available in the literature.

Information regarding the prognostic significance of BAX in human tumours is scarce. It has, however, been shown that reduced BAX expression correlates with poor prognosis in patients treated with chemotherapy for metastatic breast adenocarcinomas [Krajewski et al., 1995] and in radiotherapeutic glottis squamous cell carcinomas [Xie et al., 1998]. BAX expression alone had no influences upon survival of stage I patients with radically resected non-small-cell lung cancer [Apolinario et al., 1997], however, a study of the BAX mRNA expression levels in nephroblastoma, was not indicative that its expression could have a role in the prognosis [Re et al., 1999]. This was also confirmed by the outcome of a study showing an absence of any prognostic value of BAX [Ghanem et al., 2001]. BAX protein may be underexpressed in the presence of AA genotype, leading to increased risk of bladder cancer.

CONCLUSION

Although of significant interest, further work is required to determine the frequency of these polymorphisms in larger cohorts of cancer patients and normal individuals, not only in bladder cancer but also in various other cancers, as the literature pertaining to BAX polymorphism is very less.

ACKNOWLEDGEMENTS

This study was supported by Junior Research Fellowship (JRF) grant provided by Council of Scientific and Industrial Research (CSIR), Human Resource Development Group (HRDG), Library Avenue, Pusa, New Delhi – 110012, India. We are indebted to the staff of PGIMER, Chandigarh – 160012, India, for their close cooperation and important contributions.

Table I. Conditions of genotyping assays for the selected polymorphism of BAX gene

Single Nucleotide Polymorphism	Primers	PCR product	Enzyme	Gel band pattern
BAX 248 G/A (Hu et al., 2008)	5'-CATTAGAG CTGCGATTGGACCG-3'	109 bp	Msp1 (4 U, 3 hr, 37°C)	G allele: 89 bp, 20 bp
	5'-GCTCCCTCGG GAGGTTTGGT-3'			A allele: 109 bp (Figure 1)

Table II. Distribution of BAX 248 G/A genotype frequencies among cases and controls

Genotype	Cases (%) (n = 270)	Controls (%) (n = 252)	OR (95% CI)	p-value
GG	145 (53.7)	151 (59.92)	1 (Ref.)	--
GA	87 (32.23)	83 (32.94)	1.09 (0.74-1.62)	0.649
AA	38 (14.07)	18 (7.14)	2.20 (1.16-4.21)	**0.009**
GA + AA	125 (46.30)	101 (40.08)	1.29 (0.90-1.85)	0.152

*** Significant p-values are in bold (p < 0.05).**
OR, odds ratio; CI, confidence interval

Table III. Stratification analysis of the BAX 248 G/A genotype frequencies in cases and controls

Variable	Genotype	Cases (%) (n = 270)	Controls (%) (n = 252)	OR (95% CI)	p-value
Sex					
Males	GG	127 (47.04)	128 (50.79)	1	-
	GA	76 (28.15)	71 (28.17)	1.08 (0.70-1.65)	0.714
	AA	33 (12.22)	13 (5.16)	2.56 (1.23-5.39)	**0.006**
	GA + AA	109 (40.37)	84 (33.33)	1.31 (0.88-1.94)	0.161
Females	GG	18 (6.67)	23 (9.13)	1	-
	GA	11 (4.07)	12 (4.76)	1.17 (0.37-3.69)	0.762
	AA	5 (1.85)	5 (1.98)	1.14 (0.56-2.32)	0.739
	GA + AA	16 (5.93)	17 (6.75)	1.20 (0.43-3.35)	0.694
Inhabitation					
Rural	GG	71 (26.3)	52 (20.63)	1	-

Variable	Genotype	Cases (%) (n = 270)	Controls (%) (n = 252)	OR (95% CI)	p-value
	GA	46 (17.04)	36 (14.29)	0.94 (0.51-1.71)	0.818
	AA	21 (7.78)	11 (4.36)	1.40 (0.58-3.41)	0.418
	GA + AA	67 (24.81)	47 (18.65)	1.04 (0.60-1.81)	0.870
Urban	GG	74 (27.41)	99 (39.29)	1	-
	GA	41 (15.18)	47 (18.65)	1.17 (0.67-2.02)	0.557
	AA	17 (6.30)	7 (2.78)	3.25 (1.19-9.16)	**0.009**
	GA + AA	58 (21.48)	54 (21.43)	1.44 (0.87-2.38)	0.136
Occupation					
High Risk	GG	55 (20.37)	27 (10.71)	1	-
	GA	31 (11.48)	20 (7.94)	0.76 (0.35-1.68)	0.461
	AA	21 (7.78)	6 (2.22)	1.16 (0.90-1.49)	0.294
	GA + AA	52 (19.26)	26 (10.32)	0.98 (0.48-2.00)	0.956
Low risk	GG	90 (33.33)	124 (49.21)	1	-
	GA	56 (20.74)	63 (25.0)	1.22 (0.76-1.97)	0.378
	AA	17 (6.30)	12 (4.44)	1.95 (0.83-4.61)	0.092
	GA + AA	73 (27.04)	75 (29.76)	1.34 (0.86-2.09)	0.172
Smoking					
Smokers	GG	63 (23.33)	27 (10.71)	1	-
	GA	37 (13.70)	18 (7.14)	0.88 (0.40-1.93)	0.730
	AA	26 (9.63)	7 (2.80)	1.59 (0.57-4.60)	0.334
	GA + AA	63 (23.33)	25 (9.92)	1.08 (0.54-2.17)	0.815
Non-smokers	GG	82 (30.37)	124 (49.21)	1	-
	GA	50 (18.52)	65 (25.79)	1.16 (0.71-1.90)	0.521
	AA	12 (4.44)	11 (4.36)	1.65 (0.64-4.24)	0.253
	GA + AA	62 (22.96)	76 (30.16)	1.23 (0.78-1.95)	0.345
Alcohol consumption					
Alcoholic	GG	51 (18.89)	53 (21.03)	1	-
	GA	40 (14.81)	27 (10.71)	1.54 (0.79-3.01)	0.172
	AA	22 (8.15)	5 (1.98)	1.66 (1.27-2.17)	**0.002**
	GA + AA	62 (22.96)	32 (12.70)	2.01 (1.09-3.73)	**0.016**
Non-alcoholic	GG	94 (34.81)	98 (38.9)	1	-
	GA	47 (17.41)	56 (22.22)	0.88 (0.53-1.45)	0.585
	AA	16 (5.93)	13 (5.16)	1.28 (0.55-3.01)	0.533
	GA + AA	63 (23.33)	69 (27.39)	0.95 (0.60-1.52)	0.827
Eating habits					
Vegetarian	GG	76 (28.15)	79 (31.35)	1	-
	GA	45 (16.66)	45 (17.86)	1.04 (0.60-1.81)	0.884
	AA	20 (7.41)	11 (4.36)	1.89 (0.80-4.54)	0.115
	GA + AA	65 (24.07)	56 (22.22)	1.21 (0.73-2.00)	0.440
Non-vegetarian	GG	69 (25.56)	72 (28.57)	1	-
	GA	42 (15.56)	38 (15.08)	1.15 (0.64-2.07)	0.611
	AA	18 (6.66)	7 (2.8)	2.68 (0.98-7.60)	**0.033**
	GA + AA	60 (22.22)	45 (17.86)	1.39 (0.81-2.39)	0.202

*** Significant p-values are in bold (p < 0.05) ; OR, odds ratio; CI, confidence interval**

Table IV. Distribution of the BAX 248 G/A genotypes according to stages and histo-pathological grades

STAGES	Genotype	Cases (%) (n = 270)	OR (95% CI)	p-value
Superficial	GG	115 (42.59)	1	-
	GA	67 (24.81)	1.06 (0.69-1.62)	0.777
	AA	30 (11.11)	2.19 (1.11-4.32)	**0.014**
	GA + AA	97 (35.93)	1.26 (0.86-1.86)	0.218
Muscle-invasive	GG	30 (11.11)	1	-
	GA	20 (7.41)	1.21 (0.62-2.37)	0.545
	AA	8 (2.96)	1.86 (0.96-3.60)	0.102
	GA + AA	28 (10.37)	1.40 (0.76-2.57)	0.253
GRADES				
G1	GG	41 (15.18)	1	-
	GA	26 (9.63)	1.15 (0.63-2.09)	0.616
	AA	7 (2.59)	1.43 (0.50-3.96)	0.451

Volume : 3 | Issue : 9 | September 2014 • ISSN No 2277 - 8179

STAGES	Genotype	Cases (%) (n = 270)	OR (95% CI)	p-value
	GA + AA	33 (12.22)	1.20 (0.69-2.10)	0.488
G2	GG	78 (28.89)	1	-
	GA	48 (17.78)	1.12 (0.70-1.80)	0.621
	AA	25 (9.26)	2.69 (1.32-5.51)	**0.003**
	GA + AA	73 (27.04)	1.40 (0.91-2.15)	0.105
G3	GG	26 (9.63)	1	-
	GA	13 (4.81)	0.91 (0.42-1.96)	0.796
	AA	6 (2.22)	1.70 (0.78-3.71)	0.231
	GA + AA	19 (7.04)	1.09 (0.55-2.17)	0.787

*** Significant p-values are in bold (p < 0.05) ; OR, odds ratio; CI, confidence interval**

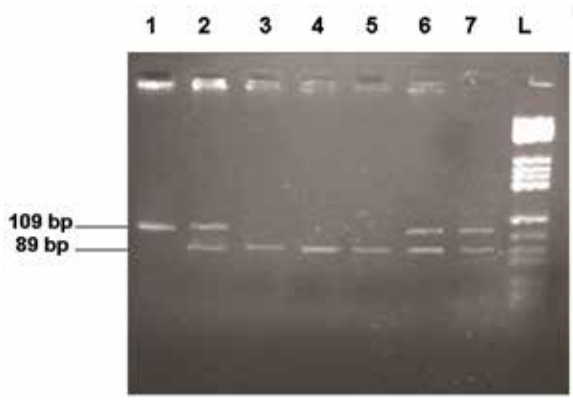

Fig. 1: A representative 4% agarose gel showing RFLP product of BAX after digestion with MspI: lanes 3, 4, 5 = G/G (89 bp); lane 1 = A/A (109 bp); lanes 2, 6, 7 = G/A (109 and 89 bp); and lane L = pBR322 DNA/HaeIII digest. Note that the 20 bp fragment for the BAX assay is not resolved on the gels used.

REFERENCE

Apolinario RM, van der Valk P, de Jong JS, Deville W, van Ark-Otte J, Dingemans AM, van Mourik JC, Postmus PE, Pinedo HM, Giaccone G. 1997. Prognostic value of the expression of p53, BCL-2 and BAX oncoproteins, and neo-vascularisation in patient with radically resected non-small-cell lung cancer. J Clin Oncol 15: 2456-66. | Bargou RC, Daniel PT, Mapara MY, Bommert K, Wagener C, Kallinich B, Royer HD, Dorken B. 1995. Expression of the bcl-2 gene family in normal and malignant breast tissue: low bax-alpha expression in tumour cells correlates with resistance towards apoptosis. Int J Cancer 60: 854-9. | Baroni GS, Marucci L, Benedetti A, Mancini R, Jezequel AM, Orlandi F. 1994. Chronic ethanol feeding increases apoptosis and cell proliferation in rat liver. J Hepatol 20: 508-13. | Cassidy A, Wang W, Wu X, Lin J. 2009. Risk of urinary bladder cancer: a case-control analysis of industry and occupation. BMC Cancer 9: 443. | Chen K, Hu Z, Wang LE, Sturgis EM, El-Naggar AK, Zhang W, Wei Q. 2007. Single-nucleotide polymorphisms at the TP53-binding or responsive promoter regions of BAX and BCL2 genes and risk of squamous cell carcinoma of the head and neck. Carcinogenesis 28: 2008-12. | Cory S, Adams JM. 2002. The BCL-2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer 2: 647-56. | Dich J, Jarvinen R, Knekt P, Penttila PL. 1996. Dietary intakes of nitrate, nitrite and NDMA in the Finnish Mobile Clinic Health Examination Survey. Food Addit Contam 13: 541-52. | Fegan C, Starczynski J, Pratt G, Pepper C. 2006. The role of the bax gene polymorphism G(-248)A in chronic lymphocytic leukaemia. Leukaemia 20: 1460-1. | Gago-Dominguez M, Bell DA, Watson MA, Yuan JM, Castelao JE, Hein DW, Chan KK, Coetzee GA, Ross RK, Yu MC. 2003. Permanent hair dyes and bladder cancer: risk modification by cytochrome P4501A2 and N-acetyltransferases 1 and 2. Carcinogenesis 24: 483-9. | Genetics Home Reference. 2009: http://ghr.nlm.nih.gov/ Accessed: 22nd June, 2009 | Ghanem MA, Van der Kwast TH, Den Hollander JC, Sudaryo MK, Van den Heuvel MM, Noordzij MA, Nijman RJM, Soliman EH, Van Steenbruge GJ. 2001. The prognostic significance of apoptosis-associated proteins BCL-2, BAX and BCL-X in clinical nephroblastoma. Br J Cancer 85: 1557-63. | Hosgood HD 3rd, Baris D, Zhang Y, Berndt SI, Menashe I, Morton LM, Lee KM, Yeager M, Zahm SH, Chanock S, Zheng T, Lan Q. 2009. Genetic variation in cell cycle and apoptosis related genes and multiple myeloma risk. Leuk Res 33: 1609-14. | Hu Z, Li C, Chen K, Wang LE, Sturgis EM, Spitz MR, Wei Q. 2008. Single nucleotide polymorphisms in selected apoptotic genes and BPDE-induced apoptotic capacity in apparently normal primary lymphocytes: A genotype-phenotype correlation analysis. J Cancer Epidemiol 2008: pp 8. | Hussain SA, Ganesan R, Hiller L, Murray PG, El-Magraby MM, Young L, James ND. 2003. Proapoptotic genes BAX and CD40L are predictors of survival in transitional cell carcinoma of the bladder. Br J Cancer 88: 586-92. | Imyanitov E, Hanson K, Zhivotovsky B. 2005. Polymorphic variations in apoptotic genes and cancer predisposition. Cell Death Differ 12: 1004-7. | Ke X, Collins A, Ye S. 2001. PIRA PCR designer for restriction analysis of single nucleotide polymorphisms. Bioinformatics 17: 838-9. | Kim HJ, Jang SI, Kim YJ, Pae HO, Won HY, Hong KH, Oh H, Kwon TO, Chung HT. 2004. 4-Acetyl-12,13-epoxyl-9-trichothecene-3, 15-diol from Isaria japonica mediates apoptosis of rate bladder carcinoma NBT-II cells by decreasing anti-apoptotic Bcl-2 expression and increasing pro-apoptotic Bax expression. Am J Chin Med 32: 377-87. | Krajewski S, Blomqvist C, Franssila K, Krajewska M, Wasenius VM, Niskanen E, Nordling S, Reed JC. 1995. Reduced expression of pro-apoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. Cancer Res 55: 4471-8. | La Vecchia C, Negri E. 1996. Nutrition and bladder cancer. Cancer Causes Control 7: 95-100. | Leppert JT, Shvarts O, Kawaoka K, Lieberman R, Belldegrun AS, Pantuck AJ. 2006. Prevention of bladder cancer: a review. Eur Urol 49: 226-34. | Lijinsky W. 1999. N-nitroso compounds in the diet. Mutat Res 443 (1-2): 129-38. | Loro LL, Vintermyr OK, Liavaag PG, Jonsson R, Johannessen AC. 1999. Oral squamous cell carcinoma is associated with decreased bcl-2/bax expression ratio and increased apoptosis. Hum Pathol 30: 1097-105. | Manju L, George PS, Mathew A. 2009. Urinary bladder cancer risk among motor vehicle drivers: a meta-analysis of the evidence, 1977-2008. Asian Pac J Cancer Prev 10: 287-94. | Moshynska O, Sankaran K, Saxena A. 2003. Molecular detection of the G(-248)A BAX promoter nucleotide change in B cell chronic lymphocytic leukaemia. Mol Pathol 56: 205-9. | Nuckel H, Frey UH, Sellmann L, Bau M, Durig J, Duhrsen U, Siffert W. 2006. Bax gene G(-248)A promoter polymorphism and chronic lymphocytic leukaemia: lack of association with incidence, disease stage and progression-free survival. Leukaemia 20: 724. | Oberdoerster J, Rabin RA. 1999. NGF-differentiated PC12 cells vary in induction of apoptosis by ethanol. Life Sci 64: 267-72. | Packham G, Stevenson FK. 2005. Bodyguards and assassins: BCL-2 family proteins and apoptosis control in chronic lymphocytic leukaemia. Immunology 114: 441-9. | Pepper C, Hoy T, Bentley P. 1998. Elevated Bcl-2/Bax are a consistent feature of apoptosis resistance in B-cell chronic lymphocytic leukaemia and are correlated with in vivo chemoresistance. Leuk Lymphoma 28: 355-61. | Rafique M. 2005. Clinico-pathological features of bladder carcinoma in women in Pakistan and smokeless tobacco as a possible risk factor. World J Surg Oncol 3: 53. | Re GG, Hazen-Martin DJ, El-Bahtimi R, Brownlee NA, Willingham MC, Garvin AJ. 1999. Prognostic significance of BCL-2 in Wilms' tumours and oncogenic potential of BCL-XL in rare tumour cases. Int J Cancer 84: 192-200. | Samanic CM, Kogevinas M, Silverman DT, Tardon A, Serra C, Malats N, Real FX, Carrato A, Garcia-Closas R, Sala M, Lloreta J, Rothman N, Dosemeci M. 2008. Occupation and bladder cancer in a hospital-based case-control study in Spain. Occup Environ Med 65: 347-53. | Saxena A, Moshynska O, Sankaran K, Viswanathan S, Sheridan DP. 2002. Association of a novel single nucleotide polymorphism, G(-248)A, in the 5'-UTR of BAX gene in chronic lymphocytic leukaemia with disease progression and treatment resistance. Cancer Lett 187: 199-205. | Scanlan RA. 1983. Formation and occurrence of nitrosamines in food. Cancer Res 43: 2435S-40S. | Skogsberg A, Tobin G, Krober A, Kienle D, Thunberg U, Aleskog A, Karlsson K, Laurell A, Merup M, Vilpo J, Sundstrom C, Roos G, Jernberg-Wiklund H, Dohner H, Nilsson K, Stilgenbauer S, Rosenquist R. 2006. The G(-248)A polymorphism in the promoter region of the BAX gene does not correlate with prognostic markers or overall survival in chronic lymphocytic leukaemia. Leukaemia 20: 77-81. | Starczynski J, Pepper C, Pratt G, Hooper L, Thomas A, Milligan D, Bentley P, Fegan C. 2005. Common polymorphism G(-248)A in the promoter region of the BAX gene results in significantly shorter survival in patients with chronic lymphocytic leukaemia once treatment is initiated. J Clin Oncol 23: 1514-1521. | Steinmaus CM, Nunez S, Smith AH. 2000. Diet and bladder cancer: a meta-analysis of six dietary variables. Am J Epidemiol 151: 693-702. | Tai YT, Lee S, Niloff E, Weisman C, Strobel T, Cannistra SA. 1998. BAX protein expression and clinical outcome in epithelial ovarian cancer. J Clin Oncol 16: 2583-90. | Verzola D, Gandolfo MT, Salvatore F, Villaggio B, Gianiorio F, Traverso P, Deferrari G, Garibotto G. 2004. Testosterone promotes apoptotic damage in human renal tubular cells. Kidney Int 65: 1252-61. | Xie X, Clausen OP, De Angelis P, Boysen M. 1998. BAX expression has prognostic significance which is enhanced when combined with AgNOR counts in glottic carcinomas. Br J Cancer 78: 100-5. | Yu DK, Guo YL, Tan W, Lin DX. 2010. Functional BAX polymorphism associated with lung cancer susceptibility. Zhonghua Zhong Liu Za Zhi 32: 324-7. | Zarzour AH, Selim M, Abd-Elsayed AA, Hameed DA, Abdelaziz MA. 2008. Muscle invasive bladder cancer in Upper Egypt: the shift in risk factors and tumour characteristics. BMC Cancer 8: 250. | Zeegers MP, Volovics A, Dorant E, Goldbohm RA, van den Brandt PA. 2001. Alcohol consumption and bladder cancer risk: Results from the Netherlands cohort study. Am J Epidemiol 153: 38-41. | Zimmerman BT, Crawford GD, Dahl R, Simon FR, Mapoles JE. 1995. Mechanisms of acetaldehyde-mediated growth inhibition: delayed cell cycle progression and induction of apoptosis. Alcohol Clin Exp Res 19: 434-40. |