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Original Article

Cox-2 gene polymorphisms in Turkish patients with myelodysplastic syndrome

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Abstract: Cyclooxygenase-2 gene polymorphisms have been studied and known its role one on cancerogenesis even though there has not yet been any studies myelodysplastic syndrome. We aimed to provide the first data on COX-2 gene polymorphisms in myelodysplastic syndrome. A total of 39 patients with MDS and 50 healthy controls were recruited from undertaken hematology departmentand compared in terms of COX-2-765 G→C and COX-2-1195 A→G genes. Statistically significant difference was observed between patients with MDS and controls in terms of COX-2-765 G→C genotype and distribution of alleles and COX-2-765 GG genotype was more frequently found in the MDS group (P<0.001). Moreover, COX-2-765 C+ (CC+CG) genotype was found to provide 5.6 times more protection against MDS. In conclusion, our results indicate that polymorphisms of the C allele of the COX-2 gene may provide protection against MDS; however, its predictive value and potential as a marker in oncology remain to be investigated in further trials.

Keywords: Cyclooxygenase, genes, polymorphism, myelodysplastic syndrome

Introduction

Myelodysplastic syndrome (MDS) is a group of acquired clonal illnesses that proceed to acute leukemia [1]. It is often seen in the elderly and is one of the hematologic malignancies with high mortality and mobidity rates. Its incidence is 4/100,000 per year [2]. Its etiology is not clear but some environmental factors and genetic mutations have been described. There is no exact therapy but as genetic studies continue there is hope for progress in therapy.

Inflammation is a critical component of cancer development. Numerous studies have reported the association between genetic polymorphisms in cytokine genes and the susceptibility to different hematologic cancers. However, the effects of such SNPs on modulating HD risk have not yet been investigated [3].

The relationship between the COX-2 gene and childhood leukemia risk is ambiguous. In this study, the association of genotypic polymorphisms in cyclooxygenase 2 (COX-2) with childhood leukemia were investigated. COX-2–specific inhibitors have demonstrated preventive and therapeutic effects as anticancer drugs for breast, bladder, lung, and pancreas cancers in several animal and clinical studies. However, the association of COX-2 genotypes with childhood acute lymphoblastic leukemia (ALL) has never been investigated. Although COX-2 overexpression and COX-2 inhibitor drugs have been studied extensively in cancer, very few studies have reported on the effects of COX-2 inhibition in hematologic malignancies, not least childhood ALL. In 2002, it was reported that COX-2 overexpression was frequent in patients with chronic myelocytic leukemia (CML) and was also found to be associated with shorter sur-

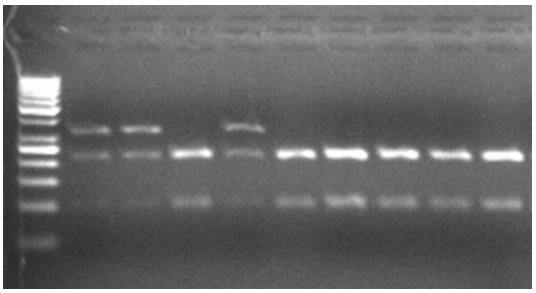

Figure 1. COX-2-765 G→C genotypes; CC: 309 bp; GG: 209 bp, 100 bp and GC: 309 bp, 209 bp and 100 bp bands.

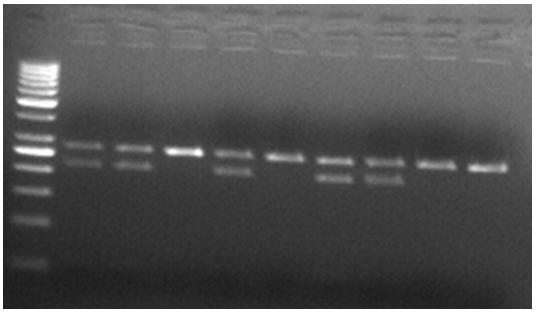

Figure 2. COX-2 1195 A→G genotypes yielded AA: 273 bp; GG: 220 bp, 53 bp and AG: 273 bp, 53 bp bands.

vival. This work was motivated by the biologic possibility that a genetic variation in COX-2 could alter enzyme expression levels or biochemical function and may consequently impact on modifying individual risk of childhood ALL [4]. We were motivated by these studies to investigate the role of COX-2 gene polymorphism in MDS.

COX is an enzyme that catalizes the transformation of arachidonic acid to prostaglandins. COX-1 is normally seen in tissues; there is limited expression of COX-2 if there is no stimulus [5]. Both enzymes are important in the inflammatory response but COX-2 can be induced by cytokines, growth factor, and oncogenes. Some studies showed that polymorphisms in the COX-2 gene causes cellular overexpression and malfunction of these enzyme, which leads to an increase in predisposition to cancer [6].

It is not clearly understood at the molecular level how COX-2 expression contributes to the transformation to malignancy but it is considered to cause effects that diminish apoptosis and intercellular adhesion, but augments angiogenesis and proliferation [6, 7].

The aim of our study was to investigate the frequency and clinical implications of COX-2 gene polymorphisms in MDS.

Materials and methods

Study design

This study was approved by the local Institutional Review Board and written informed consent was obtained from all subjects. Thirty-nine patients with MDS and 50 healthy controls who were admitted to the hematology department of our tertiary center, between January 2011, and May 2012, were enrolled in this study. The study was approved by the Ethics Committee of Haseki Training and Research Hospital and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients for being included in the study.

DNA isolation

DNA isolation from blood samples was performed using an Invitrogen Purelink Genomic DNA kit (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. Purified DNA samples were maintained at -20°C. DNA samples were diluted at 1/100 with Tris-EDTA solution. After the spectrophotometer was calibrated, absorptions of DNA and RNA were measured at 260 nm and 280 nm, respectively. Purity of DNA was evaluated with respect to ratio of absorption at 260 nm to the absorption of 280 nm. DNA samples with OD_{260}/OD_{280} ratio at 1.7-1.8 were accepted as valid.

Absorbance of double-stranded DNA at 260 nm is 50 μg/mL. The DNA concentration (ng/μL) was calculated as a dilution coefficient (100) × A_{260} × 50.

Alleles of COX-2-765 G→C and COX-2-1195 G→C loci in genomic DNA samples were produced using polymerase chain reaction (PCR). A total 25 μL PCR mixture was prepared for amplification of DNA samples. Amplification reactions were performed in a thermal cycler. Polymorphisms were analysed using restriction fragment length polymorphism (RFLP).

PCR samples were loaded on 2% agarose gel and subsequent to an electrophoresis procedure, products were observed under ultraviolet light. The PCR products were viewed and

Table 1. Distribution of COX-2-765 G→C genotype

COX-2-765 G→C genotype		Control (n=50)	MDS patients (n=39)	*P* value
		n (%)	*n (%)*	
CC		3 (6%)	1 (2.6%)	<0.001*
GG		17 (34%)	29 (74.4%)	
CG		30 (69%)	9 (23.1%)	
Alleles	C	36 (26.40%)	11 (14.10%)	0.001*
	G	64 (73.60%)	67 (85.60%)	

recorded under transillumination. At the end of the PCR reactions, 309 bp products were formed for COX-2-765 G→C and 173 bp products represented COX-2-1195 A→G transcription.

For analysis of COX-2-765 G→C polymorphism with RFLP, PCR products were transected with restriction enzyme and exposed to 2% agarose gel electrophoresis. Subsequent to staining of DNA fragments with ethidium bromide, DNA fragments were viewed and genotyping was performed under UV light. Genotypes yielded CC: 309 bp; GG: 209 bp, 100 bp and GC: 309 bp, 209 bp and 100 bp bands (**Figure 1**).

To detect COX-2 1195 A→G polymorphism, PCR products were transected with restriction enzyme PvuII. Following the staining of DNA fragments with ethidium bromide, DNA fragments were viewed and genotyping was made under UV light. Genotypes yielded AA: 273 bp; GG: 220 bp, 53 bp and AG: 273 bp, 53 bp bands (**Figure 2**).

Statistical analysis

Analysis of data was performed using Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS Inc., Chicago, IL, USA). Level of significance was set at P<0.05. Frequency of genotypes and alleles were compared using Chi-square and Fisher tests. Demographic variables were anayzed using Student's t-test and ANOVA. Fisher's exact and Chi-square tests were used to find relationships between disease parameters of MDS and gene polymorphisms.

Results

The average age of the control and MDS groupswas 66.2±7.0 and 68.5±9.7 years, respectively. Female to male ratios in the control and study groups were 1.38 (29/21) and 1.29 (22/17). There was no difference between patients with MDS and controls with respect to descriptive parameters such as sex (P>0.05) and age (P>0.05).

A significant difference was found between patients with MDS and controls in terms of COX-2-765 G→C genotype and allele distributions (P=0.0007, and P=0.001, respectively) (**Table 1**).

Interestingly, patients with MDS had a higher frequency of COX-2-765 GG and this genotype was found to bring about a 5 times increased risk for MDS (P<0.001; ^{2}=14.29; OR=5.62; 95% CI=2.22-14.22).

COX-2-765 CG was detected more frequently in the control group (P=0.000, ^{2}=12.13; OR=0.20; 95% CI=0.07-0.51).

Distribution of COX-2-765 CC genotype seemed not to display a remarkable difference between controls and patients (P>0.05). A combined analysis of genotypes revealed that COX-2-765 C+ (CC+CG) genotypes were more frequent in the control group and patients with this genotype have 5.6 times greater protection against MDS (P<0.001; ^{2}=14.29; OR=0.178; 95% CI=0.07-0.44) (**Figure 3**).

COX-2-765 G+ genotype was encountered 2.4 times more commonly in patients with MDS, but this difference was not statistically significant (P=0.438; ^{2}=0.603; OR=2.42; 95% CI= 0.24-24.2) (**Figure 4**). There was no statistically significant difference between patients with MDS and controls in terms of distribution of COX-2-1195 A→G genotype and allele distributions (P=0.122, and P=0.267, respectively) (**Table 2**).

COX-2-1195 AA genotype was observed two times more frequently in patients MDS; however, this difference was statistically insignificant (P=0.133; ^{2}=2.26; OR=2.04; 95% CI=0.79-5.22). COX-2-1195 GG genotype was not detected in the control group and its frequency in patients with MDS was 2.6%. No remarkable difference was noted between the patient and control groups (P>0.05). The COX-2-1195 AG genotype was observed 2.3 times more frequently in the control group; however, this difference was no statistically significant (P= 0.107; ^{2}=3.17; OR=0.42; 95% CI=0.16-1.10).

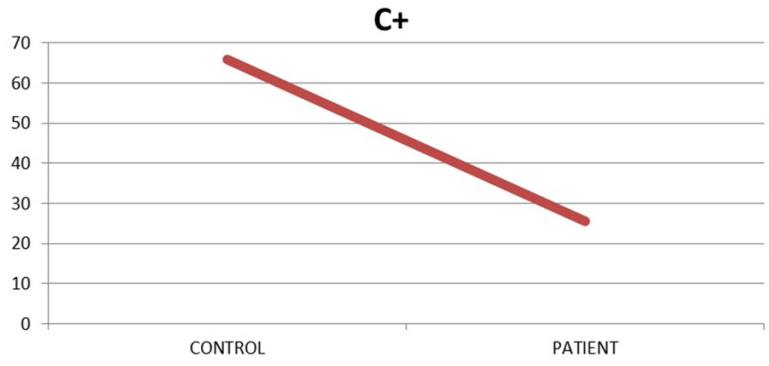

Figure 3. COX-2-765 C+ (CC+CG) genotypes frequencies.

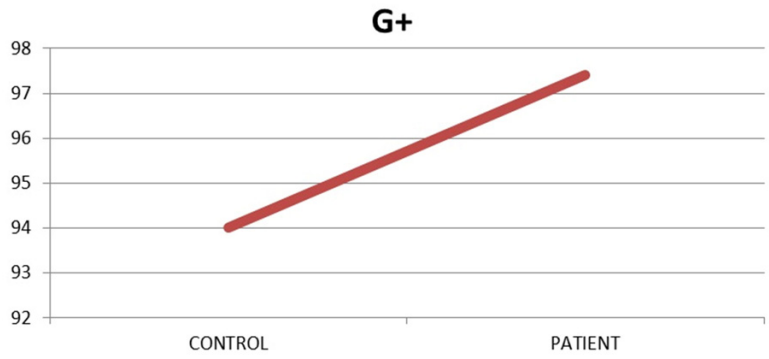

Figure 4. COX-2-765 G+ genotypes frequencies.

Table 2. Distribution of COX-2-1195 A→G genotype

COX-2-1195 A→G genotype		Control group (n=50)	MDS patients (n=39)	*P* value
		n (%)	*n (%)*	
AA		31 (62%)	30 (76.9%)	0.122
GG		0 (0%)	1 (2.6%)	
AG		19 (38%)	8 (20.5%)	
Alleles	C	81 (81%)	68 (87.2%)	0.267
	G	19 (19%)	67 (12.8%)	

COX-2-1195 G+ (AG+GG) genotype was observed two times more frequently in the control group, but no statistically significant difference was found (P=0.133; ^{2}=2.26; OR=0.48; 95% CI=0.19-1.25) (**Figure 5**).

Discussion

In the present study, we attempted to demonstrate whether there was a relationship between COX-2 gene polymorphisms and MDS. Our results indicated that there was a remarkable difference between patients with MDS and controls in terms of COX-2-765 G→C genotype and allele distribution.

Several potentially functional, single-nucleotide polymorphisms (SNP), -765G>C (reference SNP ID, rs20417), -1195G>A (rs689466), and 8473T>C (rs5275) have been identified in the COX-2 gene and have been given more attention in relation to the risk of human cancers than other SNPs. These three SNPs could affect gene transcription and/or mRNA stability, modulate the inflammatory response, and consequently contribute to individual variations in susceptibility to cancers [8].

Many publications have confirmed the association of many diseases with increased COX-2 expression of the COX-2 gene [9]. In vivo and in vitro studies have shown that COX-2-1195 G allele decreased transcription level and mRNA amount compared with COX-2-1195 allele [10]. Our results yielded a remarkable difference between patients with MDS and controls in terms of COX-2-765-GC genotype and allele distribution.

Patients with MDS had a higher frequency of COX-2-765 GG and this genotype was found to bring about a 5 times increased risk for MDS (P<0.001). Moreover, the control group revealed an obvious increased polymorphism in COX-2-765 C+ genotype. This difference was statistically significant and reminded a less likelihood for vulnerability for MDS. The COX-2-765 G+ genotype was detected more frequently in patients with MDS, but this difference was not statistically significant.

Limitations of the present study include its relatively small series size and the lack of definite criteria for selection of the control group. Due to these restrictions, associations should be interpreted with caution. However, we hope

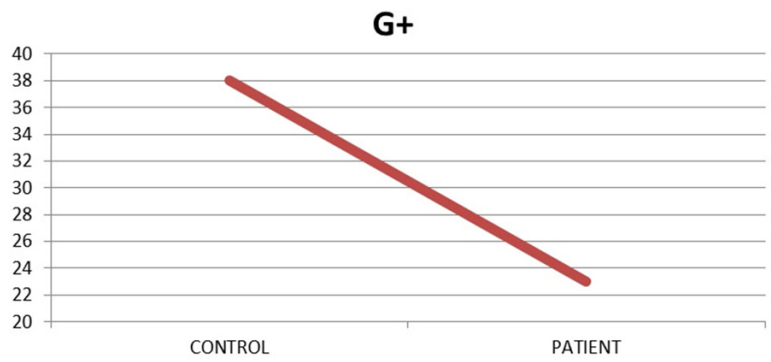

Figure 5. COX-2-1195 G+ genotypes frequencies.

that this study will pioneer further genetic studies on this topic.

To the best of our knowledge, this study was the first to be conducted on the relationship between MDS and COX-2 gene polymorphisms. Further trials are warranted to unveil the actual potential of COX-2 gene polymorphisms as a molecular marker in MDS.

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Disclosure of conflict of interest

None.

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