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Activation-Induced Cytidine Deaminase Expression in Patients with Chronic Myeloid Leukemia

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ABSTRACT

Objective: The findings on chronic myeloid leukemia (CML) patients suggest that clonal tumoral cells tend to have additional mutations besides the formation of the *BCR-ABL* fusion gene. Previous studies have demonstrated abnormal activation-induced cytidine deaminase (*AID*) expression in various types of cancer, showing *AID* transcript levels to be elevated in the CML blast phase. The study aimed to investigate the *AID* gene expression levels in CML and to investigate the etiopathogenic role of *AID*, which is not yet fully understood.

Materials and Methods: This study analyzed the *AID* transcript levels of 80 CML patients and 50 controls using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Results: The study found the *AID* transcript levels to be significantly elevated ($p < 0.001$) in the CML patients compared to the control group, with no significance in *AID* transcript levels with regard to the patients' age, gender, clinical characteristics, or laboratory findings. No correlation was found between the *AID* and *BCR-ABL* transcript levels, while a positive correlation was present between *AID* transcript levels and presence of polymorphonuclear leukocytes (PMNL; $r = 0.320$, $p = 0.021$). No significant relationship occurred in *AID* transcription levels with the tyrosine-kinase-inhibitor (TKI) resistant mutation profile or cytogenetic response during TKI therapy.

Conclusion: This study found *AID* expression levels to be significantly elevated in CML patients and *AID* to be able to contribute to the etiopathogenesis of CML

Keywords: Chronic myeloid leukemia, gene expression, cytidine deaminase

INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by a reciprocal t(9;22) translocation leading to the formation of the Philadelphia chromosome. The newly formed fusion gene product *BCR-ABL* protein has aberrant tyrosine kinase activity, constituting the pathogenesis of CML (1).

Class-switching recombination (CSR) and somatic hypermutation (SHM) happen after naive B cells meet antigens in secondary lymphoid tissues. The activation-induced cytidine deaminase (*AID*), a member of the apolipoprotein B mRNA editing catalytic polypeptide-like family (APOBEC), takes part in these diversification processes (2). The *AICDA* gene encodes the 24 kDa AID enzyme (3), and SHMs produce mutations in the DNA sequence (4). The AID enzyme converts cytosine residues in the immunoglobulin (Ig) variable region to uracil, and the body attempts to repair this error using base excision or mismatch repair mechanisms (5). *AID* can also alter gene expression through its effect on DNA demethylation, which can induce tumorigenesis due to genomic instability (6). Recent studies have reported ten–eleven translocation (TET) family members, particularly *TET2* and *AID*, to collaborate in DNA demethylation processes (7). *AID* is usually expressed in stimulated B cells, has been shown to be ectopically expressed, and induces CSR and SHM in a variety of non-lymphoid cells (8). Abnormal expression and secretion of Igs has also been reported in non-lymphoid cancer cells (6, 9). The expression of *AID* is strictly controlled

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due to its mutagenic and recombinogenic ability, which can target the non-Ig genes as well as Ig genes that contribute to lymphogenesis (10).

Numerous reports are found stating deregulated AID activity to be able to induce various types of cancer, mainly as a result of its overexpression (11-14). This study aimed to identify the state of *AID* expression in CML patients and to reveal *AID*'s etiopathogenic role, which is not yet fully understood.

MATERIALS AND METHODS

Study Population

This study was comprised of 80 CML cases being followed up in the Hematology Department of Istanbul Medical Faculty between November 1995-January 2014. Laboratory and clinical data, as well as computer records of the CML patients' information were reviewed. The patients' leukocyte, hemoglobin, and platelet counts at the time of diagnosis; leukocyte formula; bone marrow biopsy findings (bone marrow cellularity, fibrosis status); presence/absence of hepatomegaly; splenomegaly level; Sokal Score; disease stage; drug use history; and final status were recorded. Patient diagnoses were based on the criteria the World Health Organization had recommended in 2008 (15). The control group consisted of 50 healthy volunteers with no history of CML. 10 mL peripheral blood samples were drawn in sterile tubes containing EDTA, and complete blood counts were assayed during sampling. The study protocol was handled in accordance with the Declaration of Helsinki (16), with approval being obtained from the local ethics committee. Signed written informed consent was also obtained from all participants.

RNA Extraction and cDNA Synthesis

Total RNA extraction and cDNA synthesis were conducted using the respective High Pure RNA Isolation Kit and RevertAid First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany).

Analysis of AID Gene Expression

Analysis of AID expression was carried out using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) with the Real-Time Ready Universal ProbeLibrary Assay on the LightCycler 480 II Instrument (Roche Diagnostics, Mannheim, Germany) under the following PCR conditions: 95°C for 5 minutes, 95°C for 10 seconds, 58°C for 10 seconds, and 72°C for 15 seconds (40 cycles). The *AID* gene was amplified using forward 5′-TGGACACACCACTATGGAGAGC-3′ and reverse 5′-GCGGACATTTTTGAATTGGT-3′oligonucleotides.The*HPRT1*gene was amplified using forward 5′-GACCAGTCAAACAGGGGACAT-3′ and reverse 5′-GTGTCAATTATATCTTCCACAATCAAG-3′ oligonucleotides. Gene expressions were normalized using the internal control gene hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) and calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical Analyses

Statistical analysis was performed using the statistical software program SPSS (ver. 21.0). The Mann-Whitney U and Student's t tests were used to analyze the distributions of the data. The chi-square test was used to analyze the categorical data. The Spearman correlation test was conducted for the correlation analysis. Continuous variables were determined as mean ± standard deviation (*SD*), with a $p < 0.05$ being accepted as statistically significant.

RESULTS

The patient group consisted of 80 CML cases with a patient mean age of 53.7 ± 15.2 (range = 22–87; 33 females and 47 males). The mean age of the controls was 50.1 ± 16.4 (range = 24–85; 25 females, 25 males). The mean age and female-to-male ratio of the patient and control groups were similar ($p = 0.515$ and $p = 0.329$, respectively). Laboratory findings and clinical features of the patient group are given in Table 1.

Table 1. Laboratory findings and clinical features of the patient group.

Age (mean±SD)	53.7±15.2
Female: male ratio (n)	33: 47
Leukocyte at diagnosis (X10^9/L) (mean±SD)	15.8±10.9
Hemoglobin count at diagnosis (mean±SD) (g/dl)	11.0±1.8
Platelet count at diagnosis (X10^9/L) (mean±SD)	402.7±180.8
Polymorphonuclear leukocytes (PMNL) (%)	48.4 ±15.8
Red cells (%)	17.8±7.7
Eosinophil (%)	1.8±1.8
Basophil (%)	4.4±6.0
Blast (%)	1.6±2.2
Presence of splenomegaly at diagnosis n (%)	24 (30)
Spleen size at diagnosis (cm)	5.9±5.6
Disease phase at diagnosis n (%)	
Accelerated phase	5 (6.25)
Chronic phase	75 (93.75)
Bone marrow cellularity at diagnosis n (%)	
Hypercellular	78 (98.3)
Normocellular	2 (1.7)
Bone marrow reticulin fibrosis degree n (%)	
0	10 (12.5)
1	43 (53.75)
2	26 (32.5)
3	1 (1.25)
Sokal Score (mean ± SD)	1.0±0.4
Sokal classification n (%)	
Low	25 (31.25)
Intermediate	33 (41.25)
High	22 (27.5)
Presence of drug usage before TKI	21 (26.9)
Presence of TKI resistance mutation (n:15)	5 (35)
Presence of complete cytogenetic response	67 (83.3)
Disease duration, years (mean ± SD)	9.6±3.8

TKI: Tyrosine kinase inhibitor

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The mean *AID* expression level was 0.11 ± 0.1205 in the patient group and 0.0073 ± 0.0049 in the control group, with the mean *AID* expression level being significantly higher in the patients than in the controls ($p < 0.001$). The *AID* expression levels for the CML patient and control groups are given in Figure 1. *AID* expression levels do not seem to differ in terms of gender, presence of splenomegaly, disease phase at diagnosis, degree of bone marrow reticulin fibrosis, Sokal score, presence of drug usage before tyrosine-kinase-inhibitor (TKI), or presence of complete cytogenetic response. Table 2 presents the relationships between patients' *AID* expression levels and their clinical features.

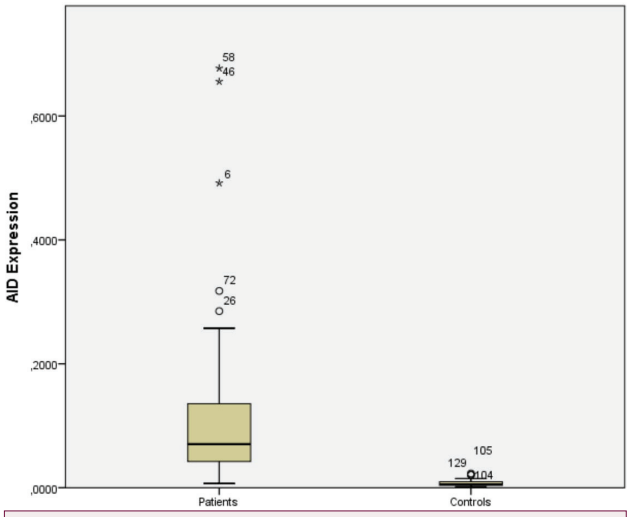

Figure 1. Figure 1. *AID* expression levels in CML patients' and control groups.

A strong correlation exists between patients' *AID* gene expression levels and polymorphonuclear leukocyte (PMNL) counts ($r = 0.320$; $p = 0.021$). The CML patients have a mean *BCR-ABL* fusion gene transcripts level of 0.07 ± 0.31, with *AID* expression not being correlated to *BCR-ABL* expression ($r = -0.101$, $p = 0.374$).

DISCUSSION

Since the late 1990s, tremendous progress has been made in understanding the biology of CML. Studies indicate the *BCR-ABL* fusion gene to be essential for the initiation and progression of CML, while transformation from the chronic phase (CP) to the blast phase (BP) requires additional genetic and epigenetic abnormalities (18). Although the defining event is uniform, a progressive genetic instability is present, especially in patients in the accelerated phase (AP) or BP of CML, with the dependence of the disease on BCR-ABL activity decreasing as it progresses to the AP and BP (19, 20).

Genome sequencing has shed light on the finding regarding non-random mutation signatures in a variety of cancers. Studies

Table 2. Relationship between AID expression levels and clinical features of CML patients.

	AID mRNA expression	p-value
Gender		
Female	0.085±0.061	0.506
Male	0.128±0.146	
Presence of splenomegaly at diagnosis	0.116±0.143	
Disease phase at Diagnosis		
Accelerated phase	0.240±0.266	0.324
Chronic phase	0.103±0.102	
Bone marrow reticulin fibrosis degree (%)		
0	0.100±0.069	0.377
1	0.093±0.100	
2	0.134±0.149	
Sokal classification		
Low	0.087±0.069	0.951
Intermediate	0.126±0.152	
High	0.132±0.169	
Presence of drug usage before TKI	0.094±0.069	0.888
Presence of TKI resistance mutation (n:15)	0.132±0.202	0.462
Presence of complete cytogenetic response	0.113±0.119	0.175

TKI: Tyrosine Kinase Inhibitor

have shown the AID enzyme to recognize these specific motifs in DNA and to create mutations (13). Apart from enhancing the immune response, AID also acts to drive tumorigenesis by causing genome-wide mutations and double-strand breaks (21-23). Subsequently, continued *AID* expression increases the genetic plasticity of tumors (24, 25).

Studies on various types of cancers have provided evidence of aberrant *AID* expression to be able to induce mutations in non-Ig genes, including cancer-related genes, and to possibly participate in the development of solid tumors (e.g., colon, bladder, renal tumors) and hematological malignancies (e.g., B-cell lymphoma, multiple myeloma, CLL, ALL) (11, 12, 26, 27).

Previous studies on myelodysplastic syndrome and myeloproliferative neoplasms have found a strong association between *AID* overexpression and these malignancies (14, 17). Furthermore, Kunimoto et al. (7) demonstrated the important role *AID* has in myeloid and erythroid lineage differentiation by epigenetically regulating genes through active DNA methylation. Liu et al. (28) reported *AID* expression in CML cells to trigger general genetic instability by mutating tumor-suppressor and DNA-repair genes. In addition, they showed overexpression of *AID* to also promote lymphoid blast crisis (BC) in BCR-ABL+ CML and the *AID* and *BCR-ABL* genes to be overexpressed in lymphoid BC compared to CP in CML. Klemm et al. (24) demonstrated a causal role for *AID* activity in the formation of *BCR-ABL* mutations leading to TKI resistance and rapid progression to BC. Feldhahn et al. (11) and Gruber et al. (29) reported the kinase activity of BCR-ABL to upregulate *AID* expression and increase genetic instability, thus contributing to the progression of CML to BC and the leukemogenesis of BCR-ABL+ B-ALL. Assuming *AID* to be an oncogene that initiates tumorigenesis, a therapy targeting *AID* has been suggested for possibly suppressing processes such as cell proliferation and migration (17).

The current study found *AID* expression to be much higher in the CML patient group compared to the control group. However, *AID* overexpression was not detected in patients who were imatinib resistant or in the AP. Considering CML's phases, *ABL1* mutations are most frequently encountered in the AP and BP, with the current study finding most of the patients to be in the CP and no patients in the BC (n_{CP} = 75, n_{AP} = 5). Although patients' *AID* expression levels were higher than the patients in CP, the difference was insignificant, the reason for this finding could possibly be the small number of patients in the AP. However, the highest *AID* gene expression levels detected in this study occurred in a patient in the AP. This finding is consistent with studies reporting increased *AID* gene expression in patients with advanced CML.

The study found a strong correlation between *AID* mRNA levels and the PMNL counts, which involve terminally differentiated myeloid cells that normally travel to the site of infection and destroy foreign bodies (30). Thus, *AID* appears to have a proliferative effect on PMNL cells.

CONCLUSION

The results of this study suggest the aberrant expression of the *AID* gene to be able to play a role in the etiopathogenesis of CML due to its mutation-inducing ability causing genetic instability. Further studies with larger groups are needed to elucidate the role of *AID* in CML.

Ethics Committee Approval: The study was approved by the Istanbul Faculty of Medicine Ethics Committee (Approval number: 2013/1759 date: 13.12.2013).

Informed Consent: Written informed consent was taken from all patients and healthy controls.

Peer-review: Externally peer-reviewed.

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Conflicts of Interest: The authors declare no conflict of interest.

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