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RESEARCH ARTICLE

B-RAF V600E AND B-RAF CODON 464-469 MUTATIONS IN HAIRY CELL LEUKEMIA PATIENTS AND THEIR RELATION WITH CLINICAL PARAMETERS

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ABSTRACT

Purpose: Hairy cell leukemia (HCL) is a chronic lymphoproliferative disorder which counts %2-3 percent of the leukemias. B-RAF is a member of mitogen activated protein kinase pathway, associated with cell proliferation. The most common B-RAF mutation V600E has been shown in hairy cell leukemia recently. The aim of our study is to investigate B-RAF V600E and B-RAF codon 464-469 mutations in HCL patients and compare the results with clinical parameters.

Methods: Thirteen individuals who were diagnosed and followed up with hairy cell leukemia in Dokuz Eylul University Faculty of Medicine Hematology outpatient clinic are included in our study. Demographic and clinical data are collected and B-RAF mutations are analyzed with pyrosequencing based molecular methods.

Results: B-RAFV600E mutation was positive in 10 (%76,9) patients. B-RAF G464E was mutated in one patient, B-RAF G466E was positive in another and B-RAF G469E mutation has been found in a patient. Two patients had both codon 600 and codon 464-469 mutations, showing no invincible difference clinically. All the patients having lymphadenopathy had B-RAFV600E mutations (p=1.000). Response rates were similar in the groups having B-RAFV600E mutation and/or B-RAF codon 464-469 mutations.

Conclusions: B-RAF is a commonly mutated gene in hairy cell leukemia with different types of muatations. Especially B-RAFV600E mutation can be used as a supportive diagnostic test, in cases with contraversial diagnosis or differential diagnosis of other peripheral B cell neoplasms. Also it can be used as a marker to select the candidate patients for target therapies, who did not respond to the conventional therapies.

Keywords: Hairy cell leukemia, B-RAF V600E, B-RAF G464E, B-RAF G469E

INTRODUCTION

Hairy cell leukemia (HCL) is a B cell lymphoproliferative disorder, presenting with spleen, liver and bone marrow infiltration; which was first described as Leukemic Reticuloendothelosis in 1958 (1). Later on, Schrek and Donnely named the disease "Hairy cell leukemia", because of the mononuclear cells with irregular cytoplasmic projections observed in the blood and marrow (2). HCL accounts for %4,5 of non-Hodgkin lymphomas, more commonly seen in man (3). Most of the patients present with fatigue, infectious complications, abdominal pain due to cytopenias and splenomegaly. Diagnosis is based on the examination of peripheral blood smear, flow cytometry and the bone marrow aspiration-biopsy (4,5). HCL is highly responsive to purine analogs if diagnosed correctly (6,7).

Recently, Tiacci et al. demonstrated B-RAFV600E mutation in %100 of their HCL cases (8). B-RAF (rapidly accelerated fibrosarcoma) is a protein-serine/threonine kinase; which is a member of MAPK (mitogen activated protein kinase) pathway that regulates cell growth, differentiation and proliferation (9). The most common B-RAF mutation; B-RAFV600E occurs as a result of substitution of the valin by glutamate in codon 600. The mutation causes constitutive activation of MAPK pathway (10). Later on; Tschernitz et al. reported two rare B-RAF mutations in exon 11(F468C and D449E) of two B-RAFV600E negative HCL patients (11).

Aim of our study is to investigate the frequency of B-RAFV600E mutation and other rare mutations of B-RAF in exon 11 (B-RAFG464E, B-RAFG466E, B-RAFG469V) and their relation with clinical data and treatment responses.

MATERIAL AND METHODS

Charts of 13 patients diagnosed with HCL were retrospectively analyzed. Patients' age, gender, sypmtoms at the time of diagnosis, secondary malignancies, hematologic paremeters were evaluated. For all patients the diagnosis was confirmed morphologically and immunhistochemically. HCL variant type patients were excluded. All patients were hospitalized and treated with one cycle of cladribine; 0,1 mg/kg/day intravenous infusion for 7 days. One patient received subcutanous IFNα at a dose of 4,5 mIU/day for eleven days prior to cladribine therapy. Complete response criteria are defined as the disapperance of HCL cells in peripheral blood and bone marrow, normalization of blood count (absolute neutrophil count =ANC>1500/10^9l, hemoglobin=Hb >12gr/dl and platelets=Plt>150000/10^9l) and resolving of organomegaly and lympadenopathy by physical and radiolographic examination. Partial response is defined as normalization of cytopenias along with a minimum %50 improvement in organ involvment and bone marrow and the absence of hairy cells in peripheral blood. Responses are evaluated according to posttreatment results at 4 or 6 months (12).

Spleen and bone marrow tissue samples were obtained from paraffin-embedded blocks archived at the Department of Pathology, Dokuz Eylul University Faculty of Medicine. Tissue samples were prepared 10 micron slides. Molecular analyzes were performed in the laboratory of Molecular Oncology. DNA was isolated from paraffin-embedded tumor tissue with a spin column-based commercial DNA extraction kit (QIAamp DNA FFPE Tissue Kit; Qiagen, Germany) according to the manufacturer's instructions. Quantification of the amount of extracted DNA by spectrophotometry. BRAF mutations were determined with pyrosequencing method (Qiagen PyroMark Q24 system, Therascreen BRAF Pyrokit 24, V1 (1/2) kit) Mutation and clinical data analysis was conducted using the SPSS 15.0 software. (IBM, NY, USA) Minimum, median and maximum values of numerical variables are summarized and analyzed with Mann Whitney U test. Percent distributions of categorical variables are summarized and analyzed with chi-squared test. $p<0,05$ was accepted as statistically significant. The study was approved by the local ethics board of Dokuz Eylul University.

RESULTS

Patient demographics

Nine of the patients were male, four patients were female. Median age at diagnosis was 48 (37-59). Median follow-up was 59 (3-96) months.

Clinical Findings

At the time of diagnosis, %46,2(n=6) of patients were asymptomatic. Fatigue, left upper quadrant pain, weight loss, dyspnea were the symptoms at diagnosis and two patients were hospitalized for hemorrhagic diarrhea and neutropenic fever. All of the patients had splenomegaly (n=13), five patients had hepatomegaly and two had intraabdominal lymhadenopathy. Approximately half of the patients (%46,2) diagnosed with splenectomy; none of the

other patients had splenectomy for treatment or palliation. Only one patient was pancytopenic at diagnosis. Four patients were anemic (Hb<10 gr/dL), six were thrombocytopenic (Plt<150000/10^9l). Leucopenia was %84,6 (n=11), four of these patients were also neutropenic (ANC<1500/10^9l). Monocytopenia commonly seen in HCL was detected %61,5(n=8) among our patients. One of the patients was diagnosed with Mantle cell lymphoma (MCL) and treated a year ago and in remission for both MCL and HCL; one was diagnosed Kaposi carcoma just before the diagnosis of HCL and lost in follow-up. None of the patients had autoimmune disease.

Treatment and Responses

Eleven patients were treated with cladribine 0,1mg/kg/day continously infusion; for 7 days. One patient was treated with interferon-α at a dose of 4,5mIU/day, for 11 days due to neutropenic fever and pneumoniae; then continued the same cladribine regimen as the others. One patient was asymptomatic with mild cytopenias (Hb>10gr/dL, Plt>100000/10^9l, ANC>1000/10^9l) and followed without treament for a year. At the time of study this patient was on cladribine treatment for detoriation of hematologic parameters. Eleven patients were eligible for the evaluation of response with CR at all. 2 patients were lost in follow-up; one with CR at 62th month, and one at third month without evaluation of treatment response. None of other patients relapsed or died in follow-up. We couldn't perform survival analysis because all the patients were CR at the time of study with no relapse or death.

B-RAF Mutation Analysis Results

B-RAFV600E - common mutation of BRAF in HCL - was detected in 10(%76,9) of our patients. Rare mutations of B-RAF codon 464-469 in exon 11 were detected in 3 patients (one patient with B-RAFG464E, one patient with B-RAFG466E, one patient with B-RAFG469E mutation) Two patients were positive for both mutations and showed no significant clinical differences compared to other patients. B-RAF mutation rates (common and rare) were similar in both men and women. All patients with lymphadenopathy were positive for B-RAFV600E mutation but there was no statistical significance. (p=1.000) As a remarkable finding, B-RAFV600E was mutated in all patients with normal thrombocyte count; whereas the mutation was positive in %50 of the thrombocytopenic patients. (p=0,70) Nine out of ten patients with B-RAFV600E mutation were leucopenic. The patients which have B-RAF 464-469 codon mutations were all leucopenic and lymphopenic. There was no statistical significance in relationships between the hematologic parameters and mutations. Treatment responses were similar in

Table 1. Mutation analysis results of our cases

PATIENT	TISSUE	CODON 600	MUTATION TYPE	CODON 464-469	MUTATION TYPE
Patient 1	Spleen	Mutant	1799 T>A	Mutant	1397 G>A
Patient 2	Spleen	Mutant	1799 T>A	Wild	Wild
Patient 3	Spleen	Mutant	1799 T>A	Wild	Wild
Patient 4	Spleen	Mutant	1799 T>A	Wild	Wild
Patient 5	Spleen	Mutant	1799 T>A	Wild	Wild
Patient 6	Spleen	Mutant	1799 T>A	Wild	Wild
Patient 7	Bone marrow	Mutant	1799 T>A	Wild	Wild
Patient 8	Bone marrow	Mutant	1799 T>A	Wild	Wild
Patient 9	Bone marrow	Mutant	1799 T>A	Wild	Wild
Patient 10	Bone marrow	Wild	Wild	Mutant	1406 G>T
Patient 11	Bone marrow	Mutant	1799 T>A	Mutant	1391 G>A
Patient 12	Bone marrow	Wild	Wild	Wild	Wild
Patient 13	Bone marrow	Wild	Wild	Wild	Wild

groups with B-RAFV600E and/or B-RAF 464-469 codon mutations.

DISCUSSION

HCL is most commonly seen among men and at middle age. In our study demographic data was consistent with the literature (1). Approximately half of the HCL patients are symptomatic at diagnosis with the symptoms due to cytopenias or splenomegaly. Infections, splenomegaly, hepatomegaly, lymphadenopathy findings were observed at similar rates reported in other studies (4,5,13). Pancytopenia was rarer, and the difference may arise from the early diagnosis of patients investigated due to splenomegaly. Dearden et al. reported that, the disease free survival of HCL patients recieving pentostatin or cladribine therapy is better if Hb>10gr/dL and/or platelet>100000/dL. (14). In our cohort, no relaps or death occured in follow-up so the relationship of these parameteres couldn't be analyzed. HCL patients may develop non-Hodgkin lymphomas, chronic myeloproliferative diseases, leukemias, solid organ cancers (lung, prostate, colon), melanom or other skin cancers before the diagnosis or after the treatment (15,16). One patient was treated for Mantle cell lymphoma before HCL diagnosis and one patient had concurrent Kaposi carcoma disease with HCL diagnosis, none of the other patients developed secondary malignancies in follow-up. Many researchers investigated the long term effects of first line therapy on secondary malignancies with purine analogues. Goodman et al. investigated the long term effects of cladribine treatment of 209 HCL patients and reported an approximately 2 fold increase in secondary malignancies; the risk was related with previous cancer history and age (15). Else et al. assessed the long term effects of pantostatin and cladribine therapies and the secondary malignancy incidence was similar with normal population at same age and gender (7). Secondary malignancy incidence was reported similar with the normal population after pentostatin or IFNα treatments in other studies (17,18). As a result, we can suggest that there is no clear evidence for development of secondary malignancies related to HCL treatment.

Cladribine was preferred for all patients who received treatment, depending on the experience of the center and the availability of the drug. There is no randomised controlled trial comparing cladribine and pentostain. The data of treatment responses and side effects are obtained from large case series (14,17,19,20). Else et al. showed no significant difference between the responses of pentostatin and cladribine therapies in a group of 233 HCL patients (7). Similar results of pentostatin and cladribine were reported by Dearden et al. and randomised controlled studies comparing these agents were suggested to obtain real data (14). In our patients, whom were eligible for response evaluation, complete response rate with cladribine was %100; similar with the literature (21,22). Treatment response was reported as the most significant factor affecting disease free survival (7). None of our patients were relapsed and this result may be associated with treatment outcome as well as the short median follow-up time.

B-RAFV600E mutation was reported up to %100 of HCL patients using different molecular methods such as Sanger sequencing, allele-specific PCR, high-resolution melting analysis (8,23–25). Ten of our patients (%76,9) were positive for B-RAFV600E mutation. Similar rates were reported by Shao et al. (%76) and Xi et al. (%79) using the same pyrosequencing based molecular method as our study (26,27). But there are also other studies reporting %100 B-RAFV600E mutation positivity with pyrosequencing method. Thus, the lower rate in our patient group was thought to be independent of molecular method, but may be associated about the genetic and geographic differences or the small number of study group (28,29). Therefore a multi-center study with a larger patient group may reveal the real data for Turkey.

As mentioned before, %15,4 of our patients had lymphadenopathy as a rare clinical finding, and all these three patients were positive for mutation. Such comparison about lymphadenopathy and mutation wasn't done in studies where all patients were positive for B-RAFV600E mutation (8,23–25,28–30). Also this clinical finding wasn't evaluated in studies which our mutation rates were similar (26,27). We think that there may be a clinical association, but this prediction should be verified in larger patient group.

As a result of our literature and Catalogue of Somatic Mutations in Cancers (COSMIC) Database research, we found out that B-RAF codon 464-469 in exon 11 mutations were not identified in HCL patients before (31). Three of our patients (%27,1) had these mutations and this may be related to ethnic factors, but also may be mutations that have not been reported in HCL patients before.

Study Limitations

Hairy cell leukemia is an uncommon lyphomproliferative disorder. Small number of our patient group and the high responsive nature of the disease resulted in a lack of statistical assesment.

CONCLUSION

B-RAF is a frequently and variably mutated gene in hairy cell leukemia. In particular, B-RAFV600E mutation can be used as a supportive genetic test in cases with contraversial diagnosis or differential diagnosis of other peripheral B cell neoplasms. It may also be an important biomarker in selection of the cases where the targeted therapies will be applied.

Author contribution: The project was constructed by A.O., Y.B., and M.A.O. Y.B., A.O. M.A, T.U.K., S.O. and O.B. designed and performed experiments. A.O., Y.B., and H.E. analysed the data. A.O., I.A. and S.S. were responsible for data collection and processing. A.O., Y.B., S.S., I.A, S.O. and H.E. wrote the manuscript, and M.A.O edited the manuscript.
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