Novel e8a2 BCR/ABL Fusion Transcript in Case of a Myeloproliferative Neoplasm

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Abstract Main objective of this work was to confirm the occurrence of rare BCR-ABL fusion variant involving the a2 region of the ABL gene and e8 of BCR gene in a patient of Myeloproliferative neoplasm positive for t(9;22) translocation but negative for common major and minor breakpoint cluster regions. A patient with elevated white blood cell count was subjected to classical cytogenetics, FISH as well as RT-PCR testing using commercial kits as well as published primers and in house testing protocol. The translocation event in chromosome 9 and 22 could be successfully detected. BCR/ABL dual color, dual fusion probe generated a classical balanced translocation scenario within the nucleus. In RT-PCR we found an unexpectedly large amplification band around 1700 bp, which is consistent with e8a2 transcript. Nine metaphases showed 46,XY,t(9;22)(q34;q11.2)[9] by cytogenetic. A rare e8a2 break point in the BCR-ABL gene in myeloproliferative neoplasm disease detected in India. It also emphasizes the utility of cytogenetic and FISH for primary diagnosis of any neoplasm in blood. Our Patient detected rare BCR-ABL fusion variant e8a2 was on imatinib 400 mg since last 3 months. After 3 months fluorescent in situ analysis and reverse transcriptase pcr analysis showed negative results.

Keywords MPN · FISH · BCR-ABL

A 59 years old female presented (in October 2015) with pain in abdomen, fever and weight loss for one week. Her initial blood count were: hemoglobin 9.4 g/dl, white blood cell 19,800/cumm, platelets 2,295,000/cumm with a differential count of 58 % polymorphs, 26 % lymphocytes, 3 % monocytes, 5 % eosinophils, 4 % basophils and 4 % myelocytes. Bone marrow biopsy features were suggestive of myeloproliferative neoplasm with differentials CML with thrombocytosis and cellular phase of myelofibrosis were suggested.

Cytogenetics—24 h unstimulated culture of bone marrow using RPMI 1640 with appropriate serum and antibiotics. Banding resolution was 450–550.

FISH—Fluorescent in situ hybridization was done on interphase nuclei using vysis LSI BCR-ABL dual colour dual fusion probe (Abbott molecular Inc., USA).

RT-PCR—RNA isolation was done by QiAMP Mini Spin Kit (Qiagen, German) (cat no 52304), 1 µg RNA was used for cDNA synthesis using Omniscript cDNA synthesis Kit (Qiagen, German) (cat no. 205113). Reverse transcriptase pcr was done using primers as per JJM van Dongen et al. [1].

In RT-PCR we found an unexpectedly large amplification band around 1700 bp, which is consistent with e8a2 transcript. Gel picture and FISH showed positive in 15 % interphase nuclei fusion shown in Fig. 1. Nine metaphases showed 46,XY,t(9;22)(q34;q11.2)[9] by cytogenetics.

This report deals with novel BCR-ABL mRNA fusions transcript by RT-PCR in a patient of diagnosed with Myeloproliferative neoplasm in which cytogenetic analysis also revealed the presence of t(9;22)(q34;q11.2).

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Fig. 1 In *left side* 7th lane of gel picture shows e8a2 transcript (~1700 bp) and in *right side* BCR-ABL fusion signal by FISH indicated by arrow

BCR-ABL positive CML contributes more than 90 % of CML cases and found in the form of major break points (M-bcr), which result in b2a2 (e13a2) or b3a2 (e14a2) fusion mRNAs. And associated with protein p210. Approximately 50 % of cases of Ph positive ALL are associated with the p190 [2].

Compared to the typical p210, the protein derived from an e8a2 fusion (p200) lacks the pleckstrin homology (PH) domain but, unlike p190, retains the DBL-like and CDC24 homology domains, PH domains are present in multiple proteins and bind phosphoinositides with varying degrees of affinity [3]. As these findings suggest BCR-ABL e8a2 break point is not an aggressive phenotype. Our Patient was on imatinib 400 mg since last 6 months. After 6 months fluorescent in situ analysis and reverse transcriptase PCR analysis showed negative results.

Compliances with Ethical Standard

Conflict of interest All the authors mentioned above declares that there is no conflicts of interest with any individual or organization.

Informed Consent Informed consent obtained from the patient (Form No- F/MISC/09-11-04).

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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