DETECTION OF BCR-ABL1 FUSION GENE IN SALIVA OF CHRONIC MYELOID LEUKAEMIC PATIENTS

A DISSERTATION PRESENTED TO THE UNIVERSITY OF NIGERIA, FOR THE DEGREE OF MASTER OF SCIENCE

BY

UZOMA IJEOMA CHINWE
PG/M.Sc/10/54991

DEPARTMENT OF MEDICAL LABORATORY SCIENCES.
FACULTY OF HEALTH SCIENCES AND TECHNOLOGY
COLLEGE OF MEDICINE
UNIVERSITY OF NIGERIA, ENUGU CAMPUS.

2013
ABSTRACT

The fusion between the “breakpoint cluster region” gene (BCR) and Abelson gene (ABL1) is the molecular hallmark of most cases of chronic myeloid leukaemia and some cases of acute lymphoblastic leukaemia. The objective of this study was to detect BCR-ABL1 in saliva of known CML patients collected in Oragene-RNA kit (DNA Genotek, Canada) and to see if mRNA could be preserved for a longer period in the saliva samples than in blood samples. A comparison of the BCR-ABL1 ratios in blood and saliva samples was done. Forty two paired blood and saliva samples from 13 female and 29 male patients referred to Safety Molecular Pathology Laboratory as part of their care were collected in k3EDTA anticoagulant tubes and Genotek Oragene RNA kits respectively. Total RNA was extracted using RNeasy kit and reverse transcribed by random hexamer priming using murine molony reverse transcriptase (MuMLV). BCR-ABL1 transcript types were first detected by multiplex PCR and then quantified by a duplex Real Time PCR- TaqMan chemistry with minor groove binders (MGB) probes and primers of HPLC grade and black hole quencher (BHQ). BCR-ABL1 transcripts, e14a2 and e13a2 were detected in 27 (64%) and 10 (24%) samples respectively. The median BCR-ABL1 ratio values were 14.50% (range: 0.00-75.03) and 12.01% (0.00-76.33) in saliva and blood respectively. The median ABL1 values were 3.11x10^3 (range: 1.28x10^1-1.02x10^4) and 4.22x10^3 (range: 1.3x10^1-2.11x10^5) in saliva and blood respectively. The median BCR-ABL1 transcript level were 9.38x10^2 (range: 7.76x10^1-15.98x10^2) and 10.29x10^4 (range: 8.3x10^1-23.21x10^5) in saliva and blood respectively. The BCR-ABL1 ratios in saliva and blood did not differ significantly (p > 0.05). However, there was a statistically significant difference in the ABL1 level in blood and saliva (p<0.05) and in the BCR-ABL1 transcript level (p<0.05). From the result of this study, it can be concluded that the detection of BCR-ABL1 fusion gene in saliva of CML patients provides an alternative method of sampling that is non-invasive and prevents repeated sampling of patients as a result of the fast rate of decay of mRNA in blood after 72 hours of sample collection.