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Evaluation of Oncogene Protein p190/bcr-abl in some Iraqi chronic myelogenous leukemia patients

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Abstract: Philadelphia chromosome is the most common specific cytogenetic chromosomal abnormality in human leukemias that is associated with chronic myelogenous leukemia (CML). However, the presence of the Philadelphia (Ph) chromosome is not sufficiently specific to diagnose CML, it is also found in acute lymphoblastic leukemia (ALL) and occasionally in acute myelogenous leukemia (AML). The purpose of this study was to use an enzyme-linked immunosorbent assay (ELISA) to detect Philadelphia chromosome in small samples of cells for ALL patients. Sera from 50 ALL Iraqi patients were used for evaluation of Oncogene Protein p190/bcr-abl. Also, Sera from a total of 38 healthy humans were used as negative controls to determine the referral intervals value for ELISA. While the range of oncogene protein p190 / bcr-abl concentration value for healthy control group was 0.111-0.161 ng/ml, the upper limit considered as a referral interval value of the 99% confidence interval (CI). However, ELISA values with concentrations above the assay referral interval of 0.161 ng/ml revealed Ph+ALL. The sera of 9 out of 50 ALL Iraqi patients showed a significantly higher levels than the referral interval ($p>0.01$).

Key words: Oncogene Protein, p190 / bcr-abl , Acute Lymphoblastic Leukemia, ELISA.

تقييم بروتين الجين الورمي p190/bcr-abl لدى بعض مرضى سرطان الدم اللمفاوي الحاد العراقيين

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الخلاصة: كروموسوم فيلادلفيا شذوذ خلوي محدد في الكروموسومات والأكثر شيوعا في لوكيميا الإنسان والمقترن بابيضاض الدم النقوي المزمن (CML). ومع ذلك، فإن وجود كروموسوم فيلادلفيا (Ph) لم يعد كافياً CML ، حيث انه وجد في سرطان الدم الليمفاوي الحاد (ALL)وأحيانا في ابيضاض الدم النقوي الحاد (AML). الغرض من هذه الدراسة تقييم بروتين الجين الورمي p190/bcr-abl باستخدام اختبار الإنزيم المرتبط المناعي (ELISA) وهي طريقة للكشف عن كروموسوم فيلادلفيا في عينات صغيرة من خلايا مرضى سرطان الدم الليمفاوي الحاد (ALL) . استخدمت 50 عينة أمصال لمرضى سرطان الدم الليمفاوي الحاد (ALL) العراقيين لتقييم بروتين الجين الورمي p190/bcr-abl . كما أستخدمت 38 عينة من أمصال الأشخاص الأصحاء كضوابط سلبية لتحديد قيمة فترات الإحالة لأختبار ELISA . أظهرت النتائج أن قيمة تركيز بروتين الجين الورمي p190/bcr-abl لمجموعة السيطرة الصحية كانت 0.111 - 0.161نانوغرام / مليلتر، والحد الأعلى يعتبر قيمة فترة الإحالة بفاصل الثقة 99 ٪ (CI). على كل حال قيم ELISA ذات التراكيز الاعلى من قيمة فترة الإحالة لهذا الاختبار والتي هي 0.161 نانوغرام / مليلتر تعتبرعينة موجبة لكروموسوم الفيلادلفيا Ph+ALL . مصل 9 من أصل 50 لمرضى سرطان الدم الليمفاوي الحاد (ALL) العراقيين أظهروا مستويات أعلى بكثير من من قيمة فترة الإحالة ($p < 0.01$).

Introduction

Philadelphia chromosome (or translocation) is the most common specific cytogenetic chromosomal abnormality in human leukemias that is 95% associated with chronic myelogenous leukemia (CML) and 20–30% of adult and 2-4% of children of acute lymphoblastic leukemia(ALL) and occasionally in acute myelogenous leukemia (AML) (1,2,3). The Ph chromosome is a translocation between the abl proto-oncogene on the long arm of chromosome 9 and a breakpoint cluster region (bcr) on the long arm of chromosome 22, resulting in a fusion oncogen , bcr-abl on a shortened chromosome 22 designated as t(9;22) (q34;q11) (1). Based on the specific breakpoints of the rearrangement, three isoforms of the bcr-abl distinct fusion proteins of 190 or 210 kd and, rarely, 230 kd can be generated. CML typically carries p210 bcr-abl , while ALL is most often associated with p190 (4) . Rowley (2000) (5) reported that among the specific chromosome translocations identified and causally linked to leukemogenesis, bcr-abl gene rearrangements represent one of the best characterized .

Over expression of the bcr-abl fusion oncogenes activates a number of downstream signaling pathways , involves cell cycle-controlling proteins and enzymes , speeding up cell division , causing genomic instability and contributing further to the pathogenesis of the disease (6,7) .

Over time, different methodologies have been applied to determine the presence of fusion genes for diagnosis or for monitoring disease . These included cytogenetics, FISH, Southern blot, and RT-PCR (8,9). These procedures are time-consuming , or are not appropriate for quantification of the transcript level. Real-time RT-PCR using TaqMan or hybridization probes strategies have also been described for detection of bcr-abl (10,11,12). These strategies have the inherent advantages of allowing quantification, specificity, and high sensitivity. However, all of these real-time RT-PCR assays require using expensive fluorescently labeled oligonucleotides. A single assay that allows simultaneous determination of the breakpoint cluster region and quantification of the transcript is not available. Although several independent reactions with sequence-specific probes can be used, this increases the complexity, workload, and cost. Alternatively, a real-time quantitative RT-PCR followed by capillary electrophoresis has been recently proposed but it requires additional equipment and manipulation of PCR products (13). We have here and for the first time used ELIZA technique to identify bcr-abl transcript e1-a2 and quantify its level without any further procedures (14). Also this assay had applied to serum from healthy individuals and confirmed that the potential background bcr-abl positivity in normal cells does not interfere with the quantification of leukemic cells.

Materials and Methods

Requirements of research had been done for the samples under study in the molecular genetics laboratory at the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies/Baghdad University and in the laboratories of the Iraqi Centre for

Cancer and Medical Genetics Researches. One hundred blood samples were collected from subjects. The subjects were divided into two enrolled groups : Fifty Iraqi patients with Acute Lymphoblastic Leukemia (ALL) (34 males and 16 females),age 2-70 years. The clinically diagnosis of the patients had been made at a consultant medical staff in the following medical centers : The National Center of Hematology / Al Mustansyria University ; Central Pediatric Hospital and Baghdad Teaching Hospital (those patients included 15 under-treated ,15 relapsed and 20 pre-treated) ; and fifty apparently healthy individuals who had been randomly selected to be matched with the patients regarding to age, gender and ethnicity (Iraqi, Arabs) , they were non-smokers and without indication of any exposure to therapeutic or diagnostic irradiation or other potential genotoxic substances for 1 year prior to blood collection . No member of either groups had consumed alcohol (wine and/or beer). Before beginning the study, all participants were informed about the objectives and experimental details of this research and to complete a standardized questionnaire to obtain necessary data on life styles and personal factors (age, occupation, health, etc).

Finally , blood samples from all cases and control were collected .Serum for Evaluation of Oncogene Protein p190/bcr-abl were separated from blood samples and stored at -20 ºC until tested. ELISA assay that was used in Oncogene Protein p190/bcr-abl screening done by using Human Oncogene Protein p190/bcr-abl ELISA Kit (CUSABIO BIOTECH CO., China ,Catalog No.CSB-E08968h "96 T") and ELISA multi well plate reader (Organon Teknika, Australia). The test was performed performed as per the manufactures' instructionmanufactures' instruction.

Results and Discussion

Sera from a total of 38 healthy humans were used as negative controls to determine the referral intervals value for ELISA .While the range of oncogene protein p190/bcr-abl concentration value for healthy control group were 0.11112-0.161395 ng/ml ,the upper limit considered as a referral interval value of the 99% confidence interval (CI).However, ELISA values with concentrations above the assay referral interval of 0.161395197 ng/ml revealed Ph+ALL.

The sera of 9 patients showed a significantly higher levels of than the referral interval ($p>0.01$) .Thus, they had evidence of Ph+ALL . One (2%) was at 1-10 age group , 6 (were at 11-20 age group and 2(4%) at 21-30 age group. However, this result suggests that the levels of oncogene protein p190/bcr-abl were significantly higher in adults than others. This result isn't far from what reported by Maurer et al. (1991) (15) and who suggested that the Philadelphia chromosome (Ph) is found in 2% to 10% of children and 20% to 55% of adults with acute lymphoblastic leukemia (ALL).Also, Mullighan (2012) (16) mentioned that with increasing age, the frequency of genetic alterations associated with favourable outcome declines and alterations associated with poor outcome such as BCR-ABL are more common . Anyhow, BCR-ABL, a common molecular defect in adult ALL, is a valuable tumor marker whose detection influences prognosis and clinical management decisions (9).

ALL patients who expressed the oncogene Protein p190/bcr-abl were most at relapse phase (one was undertreated and nine were relapsed), suggesting a strongest association between bcr-abl expression and relapse. However, despite all of the improvements in first-line therapies, at least one-third of standard-risk (SR) patients and up to two-thirds of high-risk (HR) patients eventually experience relapse, which is still a major therapeutic challenge (17).

As philadelphia positive acute lymphoblastic leukemia (Ph+ ALL) is a high-risk, aggressive form of acute leukemia (18) , the serum level of oncogene protein p190/bcr-abl above the referral-interval value revealed positive to the correlation with bad prognosis. Thus, despite the introduction of tyrosine kinase inhibitor therapy, the prognosis for p190-bcr-abl (+) acute lymphoblastic leukemia remains poor (19).

The levels of some plasma protein may reflect processes specific to a particular cancer and correlate with disease activity, so they could behave as prognostic factors and changes in their plasma level may associate with prognosis and disease outcome. There are many prognostic factors but unfortunately, some of these parameters are difficult or inconvenient to evaluate serially in individual patients or routinely in medical centers and therefore, cannot be widely applied and it will be more convenient to find a limited useful prognostic factors for such evaluation. This what evoked us to adopt such assay in our study to evaluate serum levels of oncogene protein p190/bcr-abl as one of the possible prognostic factors that may help in follow up the disease.

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