Detection of The Optimum Conditions For The Amplification of Some Regions In The Multidrug Resistance Gene In Acute Myeloid Leukemia Patients In Iraq

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Abstract: A molecular study on MDR1 gene by a specific PCR is carried out for the patients of acute myelogenous leukemia before and after chemotherapy to study the possible alteration in MDR1 gene and its correlation with development of multidrug resistance for acute myelogenous leukemia patients from Iraq. Samples were collected from 57 patients who were admitted to the Hematology Department of Baghdad Teaching Hospital and were diagnosed as acute myelogenous leukemia patients from 2017 to 2018. The studied patients are classified in to two groups; the first includes 19 patients in the stage of early diagnosis and the second group involved 38 patients in the stage of after taking the treatment in addition to a control group that include 15 healthy people. Using specific primers for the promoter region, MDR1 gene has been detected in 21.1% of 19 untreated (AML) samples, in 3 of 23 patients (13.0%) treated with chemotherapy, and in only 6 of 15 (40.0%) normal blood controls. And the region of MDR represent exon 21 Using DR Primer, MDR1gene has been detected in 11 of 19 (57.9%) untreated (AML) samples, in 11 of 23 patients 47.8% treated with chemotherapy, and in only 9 of 15(60.0%) normal blood controls. Using Mdr primer, MDR1 gene has been detected in 3 of 4 (75%) untreated (AML) samples, in 5 of 8 patients (62.5%) treated with chemotherapy, and in 6 of 6 (100%) normal blood controls.

Keywords: MDR-1, AML, PCR

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I. Introduction

Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion, and sometimes the metastasis. Cancer cells contain many alterations which accumulate as tumors develop. Over the last 25 years, considerable information has been gathered on the regulation of cell growth and proliferation leading to the identification of the proto-oncogenes and the tumor suppressor genes. The proto-oncogenes encode proteins which are important in the control of cell proliferation, differentiation, cell cycle control and apoptosis. Mutations in these genes act dominantly and lead to a gain in function. In contrast, the tumor suppressor genes inhibit cell proliferation by arresting progression through the cell cycle and block differentiation [1].

Leukemia is a cancer of the white blood cells and bone marrow. The bone marrow is the spongy inner part of bones where blood cells are made. White blood cells arise from the marrow and circulate in the blood. Leukemia is characterized by uncontrolled growth of blood cells. In acute leukemia, immature, functionless cells accumulate in the marrow and blood. The marrow often can no longer produce enough normal red and white blood cells and platelets [2]. Leukemia represented about 9.59% out of the total cases of cancer in Iraq, 5.48% males and 4.11% females (Iraqi cancer registry, 2004). While in 2005, it was 6.35% out of all cases of cancers in Iraq, 3.45 persons affected with Leukemia per 100,000 of the population [3].

Acute myeloid leukemia (AML) has not been a common disease in childhood and represents about 15% of childhood leukemia, with a poor outcome recently improved with the use of intensive chemotherapy and bone marrow transplantation to about 50% survival at 5 years [4]. One of the limitations in the treatment of cancer patients with chemotherapy is the development of multidrug resistance. Many evidences have indicated that the drug resistance can be acquired as a result of chemotherapy [16]. The current study objective is to the study of MDR1 gene and its relationship with the development of the multidrug resistance of AML patients in Iraq.
II. Patients And Methods

Patients, this prospective study was carried out (55) patients of acute myelogenous leukemia (AML), only 42 samples of blood were successfully DNA extracted from them, through the period from October 2008 to April 2009 from the hematology clinic of Baghdad Teaching Hospital, the selected patients were classified in to two groups: the first group included 19 patients in the stage of the early diagnosis; the second group included 23 patients in the stage after taking the treatment. Blood was placed in tubes containing anti-coagulant solution acid citrate dextrose (ACD) solution buffer and stored at -20°C. Blood samples were used to extract the DNA for PCR amplification of certain regions within MDR1 gene. Extraction, Genomic DNA Extraction from Frozen Blood using (DNA Wizard Promega USA). Measurement of the DNA concentration, DNA concentration and purity determined to be 4.6 – 9.3 µg/µl concentration and the purity range was 1.06-1.24 [5].

Agarose Gel Electrophoresis, agarose gel prepared in two different concentrations that were 0.8% for the detection of extracted DNA and 2.5% for detection of PCR products. Gel was run horizontally in 0.5 x TBE buffer. Samples of DNA were loaded into the wells of the gel Generally the gel buffer added up to the level of horizontal gel surface and gel were run for 1.40-2 hours at 3-5 v/cm.

PCR primers: The primer sequences for amplification of promoter region were: 5'GGGTGTTGTTGATATAGTGT-3' for forward primer; 5'-CCAACCTTTACATACCCCTACCTCACA-3' for reverse primer with this primer the DNA was denatured at 95 °C for 5 minutes, followed by 45 cycles at 95 for 30 second, 56 °C for 1 minute, and 72 °C for 1 minute with a final extension at 72 for 7 minutes. For dr primer: forward 5', - GTTAGGAGTTTGGATT3; and reverse 5', - AAAACTATCCCATATAAAC-3, and for Mdr primer: forward 5', - CACGGTGTTGGAAGCTAACC-3, and reverse 5', GAAGGCCAGAGCATAAGGTC-3, the DNA was denatured at 94 °C for 4 minutes, followed by 45 cycles at 94 for 40 second, 51 for dr primer and 60 for mdr primer for 45second and 1 minute, and 72 °C for 1 minute with a final extension at 72 for 7 minutes. Stain and detection, Agarose gels were stained with ethedium bromide by immersing the gel in the distilled water containing the dye final concentration of 0.5 mg/ml for 30-45 minutes. DNA bands were visualized by U. V. Transilluminator at 365nm wave length described by Maniatis and his co-worker [5].

III. Results and Discussion

In this study found that the mean age was 35.41 years and the median age was 30 years. The highest age incidence was between21-30 years (9 cases) which represented 26.4% of the whole AML of this study. (Figure 1.1). Eight patients (23.5%) were below the age of 20, and twenty-Six patients (76.5%) were above the age of 20 (adults). A mirghofran and his co-worker [6] found that the mean age in AML patients was 32.7years while Legrand et al [7] found 57 years mean age in AML patients, the difference presented in this study could be due to the certain criteria used for the selection of the cases, in addition to racial and geographical factors.

![Age distribution in AML patients](image)

Using UNM primer for the promoter region of MDR1 designed to include six CPG dinucleotides that have been linked to regulation of MDR1 expression. MDR1 gene was detected in16.6% of 42acute myelogenous leukemia samples. MDR1 gene has been detected in (4) 21.1% of 19 untreated (AML) samples, in (3) 13.0% of 23 patients treated with chemotherapy, and in only 6 of 15(40.0%) normal blood controls. Figure 1.2.
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Figure 1.2: PCR amplification of the promoter region, lanes 1-4 represent DNA from AML patients before treatment, 5-7 DNA from AML patients after treatment, all give the expected PCR product of 330 bp. C: negative control of PCR reaction. M: (1) Kb DNA ladder. Agarose 2.5% used in 0.5X TBE using 5V/cm for 1 h. Using DR Primer for the Exon 21, the MDR1 gene was detected in 2% of 42 acute myelogenous leukemia samples. MDR1 gene has been detected in (11) 57% of 19 untreated (AML) samples, in (11) 47% of 23 patients treated with chemotherapy, and in only 9 of 15 (60.0%) normal blood control Figure 1.3.

Figure 1.3: PCR amplification of Exon 1, lanes 1-4 represent DNA from AML patients before treatment, 5-12 DNA from AML patients after treatment, all give the expected PCR product of 200 bp. M: (1) Kb DNA ladder. Agarose 2.5% used in 0.5X TBE using 5V/cm for 1 h.

When this quantitative system was applied to clinical samples, MDR1 gene detection was found to correlate with response to chemotherapy. The detection MDR1 gene at after treatment was obviously lower than those at before treatment. Furthermore, all cases who were MDR1-positive at after treatment did not respond to chemotherapy or relapsed after short remission periods, due to resistance to chemotherapy. The amplified DNA with MDR1 gene was 200bp is lengths of DR and Mdr primers while in UNM primer was 330 of human blood samples in this study. These results were in disagreement with those obtained by Shin-ichi et al [8]. Who showed that the product size was 120bp for MDR1 gene and by Yousuf et al [9] who showed that the product size of MDR1 gene was 258bp.

The presence of single band but not with the same size of the expected product in some samples of the patients and the absence of the expected band as I Figure 1.3 could be attributed either to the non-specific product or to the presence of major change in the genetic material of the patient that resulted from the accumulation of mutations or insertion or deletion of a DNA fragment in the amplified area.

In Figure 1.3 it has found that the presence of a double band with a very little difference in the size could be attributed to the heterozygosity of this locus. Although the primer binding sites were the same there might be an insertion or deletion inside the amplification area that led to this size differences.

In this study when compared genotype frequency in AML patients with healthy controls, no statistical difference between AML and healthy controls were observed. Similar results were reported in other studies on different cancers have found no statistical difference in the frequency of this genotype between patients and
controls [10, 11]. In contrast, in a study performed in Turkey, a difference in the frequency of C3435T polymorphism between breast cancer patients and healthy controls was observed [12].

Several mechanisms were thought to be involved in the development of resistance to chemotherapy, including overexpression of the membrane-associated ATP-dependent efflux pump, p-glycoprotein, which was encoded by MDR1 [13,14]. Because many kinds of antileukemic agents could be substrates for this efflux pump, overexpression of MDR1 led to insufficient concentrations of agents in leukemic cells even at maximum doses. [15].

IV. Conclusions

According the results of this study the following can be concluded: The multidrug resistance phenomena could be developed as a result from an induction of mutation in MDR-1 gene as a result of chemotherapy. The targeted regions of amplification used in this study could be informative enough to detect the presence of mutation in MDR-1 gene. The measurement of MDR1 in AML might contribute the establishment of therapeutic plan and the estimation of the prognosis.

References


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