Molecular effect MDR1(C3435T) gene polymorphism on leukemia patients in Babylon province, Iraq

Asmaa Mohammed Mekkey¹, Alyaa Saad Abed²*, Zainab Mohammed Jasim¹, Nawres Najah Jawad¹

1. College of Medicine, University of Babylon, Hilla, Iraq.
2. Genetic engineering department/Biotechnologies college, Al-Qasim green University, Babylon province, Iraq.

* Corresponding author:
Alyaa Saad Abed
Genetic engineering Department,
Biotechnologies College, Al-Qasim Green University, Babylon province, Iraq.
E-mail: saadaliaa85@yahoo.com

Abstract

Background: Acute leukemia is the causing death between many people. Many previous discussions have showed an correlation between MDR1 polymorphisms and leukemia risk. Objective: This study is used to show a strong significance between the genetic MDR1 polymorphism and leukemia in gene site (C3435T). Methods: A case control study involving 70 leukemia patient and 40 healthy was conducted. Specimens of all patients and controls that carry MDR1.C3435T gene polymorphism were identified by conventional PCR followed by RFLP methods. MDR1 (C3435T) polymorphism, was detected by PCR amplification by using the following primers: forward, primer 5’ TTG ATG GCA AAG AAA TAA AGC 3’ and reverse primer 5’ TTT ACA TTA GGC AGT GAC TCG 3’.

Results: Our results were significantly related with development of acute leukemia patients. C3435T allele frequency widely varies between variants people.

Conclusion: These polymorphisms are correlated with the advanced risk of acute leukemia.

Keywords: MDR1, Gene polymorphism, Leukemia, Patients.


Introduction

Leukemia is a ailment of the bone marrow, characterized through pathogenic boom of immature white blood cells [1]. Two peaks of acute leukemia have an impact in the early formative years, at the same time as the influence of second top was take vicinity at 50 years [2]. Cancer happens whilst genetic mutations or rearrangements disrupt the ordinary machinery of the mobile, causing doubtlessly limitless cell proliferation and expansion. Many cancers start with one or a few mutations with increase promoting properties; the fast cell department rate, frequently in aggregate with impaired DNA restore mechanisms, outcomes in new mutations, and one of a kind subclones emerge. Alternatively, several mutations can appear simultaneously in a few catastrophic genomic events [3]. Malignant sickness has historically been categorized in keeping with tissue type in place of mutational profile; however, new genome-extensive DNA and RNA sequencing technologies have dramatically changed the knowledge of the cancer genome [4]. Tumors across unique tissue sorts can display hanging molecular similarities, while tumors of the equal tissue type can display absolutely different mutational spectra, suggesting that the current tissue-precise type is probably insufficient [5]. The kinds of genetic occasions underlying cancer improvement have historically been categorized into.
mutations, copy range changes (CNAs) and rearrangements/translocations. In current years, the time period mutation, which refers to single nucleotide versions (SNVs) or small Insertions/deletions (indels), has been widely replaced with the term sequence version. In the context of cancer, collection variations motive stimulation of oncogenes or inactivation of tumor suppressor genes. Similarly, CNAs result in amplifications of oncogenes or, greater regularly, deletions of tumor suppressors [6]. Many studies revealed that MDR1 (C3435T) gene might give the influence of cancer risk [7]. MDR1 (C3435T) gene is a, member of the (ATP-binding cassette) family in which code for a membrane-bound protein (P-gp). This gene existing on chromosome number 7 and contain 28 exons, and the coding region which make a change in the sequence of the protein accounts for less than 5% of the total [8]. This gene act crucial position as efflux pump and guide cellular protecting towards special substances together with organic, cations, amino acids, polysaccharides, proteins, and some antibiotics [9]. Accordingly, a few crucial organs in human frame consist of excessive quantity of P-gp [8].

In this study, we investigate the correlation between genetic polymorphisms MDR1 at one sites (C3435T), and the results may help to discover new markers for acute leukemia.

Materials and Methods

Study design and Patients

A total of 70 acute leukemia cases and 40 control samples were used in this study. Blood specimen was extracted from diagnostic patients that carry acute leukemia, which obtained from Merjans teaching hospital, Babylon province, Iraq. The age and sex control samples were included (19-61 years), male and female selected from different areas of hospital. Genomic DNA was isolated by extraction by special purification kit (Faverogen) due to the manufactures protocol.

Ethical approval

A study protocol was reviewed and granted permission by the Ethical committee at Babylon University, College of Medicine. After explaining the objectives of the study to the patients, verbal consent was obtained from them prior to interviewing. Names of participant were replaced by identification codes to keep data confidential. To carry out the study, official agreement was obtained from Babylon Health Directorate.

Genotyping Methods

Specimens of all patients and controls that carry MDR1.C3435T gene polymorphism were identified by conventional PCR followed by RFLP methods [10]. MDR1 (C3435T) polymorphism was detected by PCR amplification by using the following primers: forward primer 5’ TTG ATG GCA AAG AAA TAA AGC 3’ and reverse primer 5’ CTT ACA TTA GGC AGT GAC TCG 3’. PCR product was carried out in 50μL reaction mixture containing PCR buffer (10mM Tris- HCl, pH 9.0 50mM KCl, 1.5mM MgCl2) 200 μM of each dNTP, 1 unit of Taq DNA polymerase (due to, Biotech, USA), 20μM of each primer and 100ng of genomic DNA. PCR conditions was primary denaturation at 94C for 60s, annealing at 56C for 60s, extension at 72C for 60s, and final extension 72C for 5min. PCR product of (206) bp digested with restriction enzymes MboI, incubated at 37C for 24hr (homzygote(TT) 130 and 76 bp fragments, polymorphic homozygote (tt) with 206 bp fragments and Tt heterozygote with 206, 130, and 76 bp fragments) and then separated by agarose gel electrophoresis 3.5%, and staining by Ethidium bromide that used for visualized.

Statistical Analysis:
Genetic analysis was performed using Chi-square ($\chi^2$) test. These tests were significant correlated when $p$ value was less than 0.05.

**Results and Discussion**

A total number of (100) subjects were enrolled in this study. The subjects included (70) leukemic patients and (40) control groups. Leukemic patients and control groups were the same age (19-61 years). PCR product of (206) bp figure (1) digested with restriction enzymes *Mbo*I, incubated at 37°C for 24 hr (homozygote (TT) 130 and 76 bp fragments, polymorphic homozygote (tt) with 206 bp fragments and Tt heterozygote with 206, 130, and 76 bp fragments) figure (2). Results in this study were showed that C3435T CC is significantly correlated with increased association with acute leukemia, allele frequency 0.3 in control groups compared with patient that showed 0.39 in all patients of acute leukemia, these results were reported in Table(1). Other study showed MDR1 gene polymorphism may modulate risk of leukemia [11].

**Figure (1):** PCR product (206) bp

**Figure (2):** Digested fragments of MDR1 C3435T; (TT) 130 and 76 bp fragments, (tt) with 206 bp fragments and (Tt) 206, 130, and 76 bp
MDR1 is still an important factor through the leukemia therapy. The P.gp function is attributable to the occurrence of variant MDR1 genotype among all patients. Ren et al.,[12] demonstrated the importance of SNPs were either intronic or noncoding SNPs, and as such do not change P.gp amino acid composition. Some variants have been discovered in the MDR1 gene to date. Komar,[13] reported that the first MDR1 SNP to be correlated with modification of gp transport function was the silent mutation 3435C-T exon 26. Other study from cell lines and patients sample were suggested that this polymorphisms leads to several alteration, proteins expression and protein folding.[14] Our results were significantly related with development of acute leukemia patients. C3435T allele frequency widely vary between variants people. Hamidovic et al.,[15] reported that these variations of allele frequency in Caucasians, the 3435 frequency as 22% CC, 50% CT, and 28% TT. Other study among Spanish breast cancer patients MDR1 C3435T allele frequency was C 0.52 and T 0.48 which were not different from controls, while genotypes were distributed as 14 (28%) CC, 24 (48%) CT and 12 (24%) TT.[16] In contrast, in Korean community, TT, CT, and CC genotypes were reported in 38.9% 10.2%, and 50.9%, patients, respectively. A study among Jordanian and Sudanese people by Salem, et al.,[17] showed that T allele was more dominant among Jordanians than C allele CC= 20.7%, CT= 51.7%, TT= 27.6%; C: 47%, T=53%). The human adenosine triphosphate-binding cassette, subfamily B, member 1 (ABCB1) gene, additionally named multidrugresistance 1 (MDR1) gene, is located at 7q21.1, with 28 exons encoding a one hundred seventy kDa membrane transporter called P-glycoprotein (P-gp). The presence of a extraordinarily conserved adenosine triphosphate (ATP)-binding web site in two homologous halves as well as the linker vicinity makes this protein a member of the adenosine triphosphate-binding cassette (ABC) superfamily. P-gp acts as an efflux pump in an ATP dependent style, transporting exogenous and endogenous substrates from the inner of cells to the outside. P-gp become first diagnosed in human cancer cells as a protein liable for resistance towards many anticancer drugs. Thereafter, the efflux transporter was determined in diverse regular human tissues, including inside the intestinal epithelium, adrenal gland, placenta, kidney, liver, capillary endothelial cells of the brain, and testes. Expression of P-gp physiologically in excretory tissues provides a cellular vindication mechanism against potentially harmful compounds. The C3435T SNP in ABCB1 has been associated with the development of various cancers, including breast cancer, hepatocellular...
carcinoma, and non-Hodgkin lymphoma. Many case–control studies have been conducted to investigate whether the ABCB1 C3435T polymorphism is associated with leukemia risk but these have yielded controversial results [18].

Conflicts of interest: None of the authors have any conflicts of interest relevant to this research subject.

References: