myelomonocytic leukemia (CML) and juvenile myelomonocytic leukemia (JMML) [1].

AMLis characterized by a maturation block and accumulation of myeloid progenitor cells. Clinically, cytogenetically, and molecularly it has been recognized as a heterogeneous disorder [4]. Although in AML N-RAS mutations were first reported 25 year ago, the prognostic impact of N-RAS mutations is still under discussion and seems to vary from disease to disease. Several studies indicate an association with poor outcome; others found a negative prognostic impact of N-RAS mutations only in AML with favorable karyotypes; other found N-RAS mutations associated with a favorable prognosis and at last some studies could not defined a prognostic impact of N-RAS mutations [5]. The current study aimed to determine the frequency of N-RAS mutation, its influence on response to induction therapy in AML patients in Iraq.

Method

Fifty eight newly diagnosed untreated AML patients and thirty individuals with reactive bone marrow (including 19 individuals presented with pyrexia of unknown origin and 11 presented with idiopathic thrombocytopenic purpura) served as control group were enrolled in thisstudy at Department of Hematology / Baghdad Hospital at Baghdad Medical City for the period April 2011 to July 2012.

The study was approved by the Ethics Committee of College of Medicine, Al-Nahrain University and informed consent in accordance with the Declaration of Helsinki was obtained from patients, control individuals or their legal guardians prior to the collection of samples and data.

DNA extraction from peripheral blood, amplification and enzyme restriction was done at the Department of Pathology/Baghdad Collage of Medicine. Genomic DNA was extracted from peripheral blood specimens of patients at time of presentation, N-RAS gene amplificationperformed; briefly 1 µL of the extracted DNA was added to a 20µL PCR reaction mixturecontaining 5 µL of AccuPower TLA PCR Premix, 10 pmol of each forward and reverse primer (Table 1) and 13µL of nuclease free water. The first round of PCR consisted of 30 cycles (denaturation at 94^{o}C for 30 seconds, annealing at 55^{o}C for 30 seconds, and extension at 72^{o}C for 30 seconds). 1 µL of the amplified product of the first round was then added to a second 20-µL PCR reaction mixture using 2nd set of primers for a further 30 cycles under identical conditions to the first round. Each round was preceded by heating at 95^{o}C for 10 minutes. Negative control (no DNA template) tube was included with each batch of samples analyzed. Beta globulin gene also amplified as control for amplification [6].

Table 1. Sequences of DNA primers

First Round N-RAS Gene Primers: [6]
• RS 12 (Forward) 5’ GCTCGCAATTAACCCTGATTAC • RS7 (Reverse) 5’ ATTCCTTTAATACAGAATATGG
Second Round N-RAS Gene Primers: [6]
• RS6 (Forward) 5’ACTGAGTACAAACTGGTGGTGGTTGGACCA • RS5 (Reverse) 5’ GGTCAGCGGGCTACCCCTGGACCA

Mutation sensitive digestion analysis (MSDA) was used for detection of mutations at codon 12 and codon 13. The second round PCR primers (RS6 and RS5) are both mismatched at a single base from their target sequence. This creates a 5’ *Bst*NI restriction site at codon 12 and 3’ restriction site within sequence at the downstream end of amplified DNA. If the amplified DNA has normal sequence at the first two bases of codon 12, it is cleaved at both the 5’ and 3’ sites by *Bst*NI to produce an 87 bp fragment, whereas mutant DNA with a