High Frequency of Nucleophosmin Mutations in Thirty Two Iraqi Adult Patients with Acute Myeloid Leukemia

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Abstract

Background: Genetic characterization of all AML patients at presentation is nowadays regarded as mandatory to determine treatment choices. In Iraq no previous study on genetic level that determine the frequency of NPM1 mutations in adult AML patients was previously done and for this reason the current study was designed.

Methods: Thirty two adult AML patients in addition to 15 healthy were enrolled in this study. The AML cases were classified according to the FAB classification. Molecular analysis for nucleophosmin mutations was done using Single Strand Confirmatory Polymorphism- Reverse transcriptase- Polymerase Chain Reaction with specific primers.

Results: The NPM1 mutations were found in 46.88% of adult AML patients, whereas the control group showed absence of these mutations. The NPM1 mutations were detected non significantly in older age and female gender AML patients, (P>0.05). Furthermore; patients with the NPM1 mutations showed lower blast cell % and higher platelet count as compared to non mutated cases, and were mainly FAB M1 and M3, (P>0.05). Moreover, higher failure rate of induction therapy was found in adult AML patients without NPM1 mutations 55.55% as compared to NPM1 mutated patients 44.44%, but this finding was not significant, (P= 0.453).

Conclusions: Molecular detection of NPM1 mutations in Iraqi adult AML patients was reported for the first time where the frequency of NPM1 mutations in adult AML patients was higher than that reported in different studies worldwide. NPM1 mutations were associated with good prognostic factors in AML as female gender, lower blast cell percent and higher rate of response to induction therapy thus, this mutation is can be considered in risk stratification of AML patients.

Keywords: Acute myeloid leukemia, Nucleophosmin mutations, prognosis, RT-PCR, Iraqi patients.

Background

In recent years several recurrent molecular markers were identified that allowed further sub classification and prognostic predictions in the vast majority of AML patients especially those with normal karyotype, (Marcucci et al.,2005).

Mutations interfering with tumor suppressor pathways such as Nucleophosmin mutations (NPM1) are the most frequent known genetic alterations in AML occur in about 30% of all cases, (Renneville et al., 2008).

Nucleophosmin, also known as B23,1 NO38,and numatrin, is an abundant, highly conserved, ubiquitously expressed nucleolar phosphoprotein which belongs to the nucleolar/Nucleophosmin family of nuclear chaperones (Pier and Di , 2010).

The NPM1 gene contains 12 exons, and in humans maps to chromosome 5q35 and the protein exists in two alternatively spliced isoforms: B23.1, the prevalent isoform in all tissues, contains 294 amino acids whereas B23.2, a truncated protein, lacks the last 35 C-terminal amino acids of B23.1 and is expressed at very low levels (Falini et al., 2007a).
It is well known that in several hematologic malignancies, the NPM1 locus is lost or translocated leading to the formation of oncogenic fusion proteins (Hayami et al., 2005), whereas in early 2005, it was reported that NPM1 mutations closely associate with AML carrying a normal karyotype (AML-NK) (Falini et al., 2005). Although mutational studies of NPM1 in tumors other than AML are scarce, immunohistochemical analysis of NPM in thousands of hematopoietic and extra hematopoietic neoplasm has consistently revealed nucleus-restricted NPM expression, which indicates no NPM1 mutations are present (Falini et al., 2007b).

All NPM1 mutations are consistently heterozygous and a wild-type allele is retained. (Falini et al., 2007a). Independently of their type, all mutation variants generate common alterations at the C-terminus of the NPM leukemic mutants which are responsible for their dislocation into cytoplasm, (Mariano et al., 2006).

Longitudinal studies had demonstrated that NPM1 mutations are stable throughout the course of the disease, (Giovanna et al., 2009) and detection of a mutant NPM1 allele may also be of clinical benefit in following the treatment course of patients with AML. Thus, an NPM1 mutation, if present, represents a marker that can be used to monitor for minimal residual disease. Moreover, investigators have found that cytoplasmic/mutated NPM predicts a good response to induction therapy (Schlenk et al, 2009).

**Methods**

Thirty two adult patients with acute myeloid leukemia (AML) attending Baghdad Teaching Hospital were recruited in this study.

The diagnosis of AML cases was based on the finding of peripheral blood and bone marrow aspirate smears which were examined by two hematology specialists for diagnosis and sub-classification of patients according to FAB classification in the teaching laboratories of Baghdad teaching hospital.

Each patient was assessed at the admission and after the first course of induction therapy. The initial response to chemotherapy was assessed whether there was complete hematological remission (CR), treatment failure or death. Ten healthy adult individuals served as negative control group for the mutations were collected randomly in relation to age and gender. Extracted RNA from OCI/AML3 cell line was used as positive control for molecular analysis of NPM1 mutations.

This research was approved by the ethical committee at the college of Medicine, Al-Nahrain University, Baghdad-Iraq, and informed consents were obtained from all participants.

From patients 1.5 ml of peripheral blood samples or bone marrow aspirate samples according to the availability were collected in EDTA tube whereas from the control group 1.5 ml of peripheral blood samples were collected in EDTA tube divided as 1 ml for analysis of hematological parameters and 0.5 ml was equally divided into 2 eppendorff tubes each contain (1 ml) trizol reagent mixed well and kept in deep freeze(-70°C) until the day of analysis.

White blood cell count (WBC), platelet count and hematocrit percent were assessed for each patients and control group using automated hematology analyzer (Sysmix) in the Teaching Laboratories of Baghdad Teaching Hospital. Blood film and bone marrow aspirate slides were prepared and stained with leishman stain and special stains Sudan Black B & Periodic Acid Schiff using the standard procedures for staining (Lewis and Barbara,2006).

Total RNA was extracted from bone marrow cells or peripheral blood cells using bioZOL™-G RNA Isolation Kit (BioWORLD-US) following the instruction manual.

**Mutation detection**

In order to assess the expression of different types of NPM1 mutations in exon 12, Single Strand Confirmatory Polymorphism-Reverse transcriptase- Polymerase Chain Reaction (SSCP-RT-PCR) was used. A forward and backward primers “NPM-F, 5_ATCATCAACACCAAGATCA 3_ and NPM-R, 5-catgtctga ccaccgc TACT 3_” were designed to specifically amplify NPM exon 12 only if the NPM1 mutations that are specific for AML were expressed.(Brown et al., 2007).
Single step Accu power® RocketScript RT/PCR Premix Kit (BiONEER-Korea) was used for cDNA synthesis and amplification using Rocket Script™ reverse transcriptase and Taq DNA polymerase in one tube following the manufacturer manual.

To the 0.2 ml ready to use tube, approximately 1 µg of RNA and 5 picomoles of each primer (BiONEER,Korea) "NPM-F , 5'-ATCATCAACACCAAGATCA-3' and NPM-R , 5'-CATGTCTGACCACCGCTACT‘-3" were added. The volume was completed to 50µl using nuclease free water.

For the negative control tube, nuclease free water was added instead of the template RNA, whereas to the positive control tube 1 µg of RNA extracted from OCI-AML3 cell line was added. The reaction was performed under the conditions in table 1 (Brown et al., 2007). PCR product was visualized by electrophoresis on 3% agarose gel. 

Statistical Methods

Data were analyzed using SPSS program (Statistical Package for Social Sciences) version 16 and Microsoft Office Excel 2007. Numeric data were expressed as mean± SE, frequency was used to express discrete data. Student t-test was used to analyze numeric data while Chi-square was used to analyze discrete data. Values were considered statistically significant when P < 0.05.

Results

Interpretation of the results

In the current study, the mutated cases had showed hetero duplex formed from mutant allele and the wild type allele presented as 2 bands, the first band approximately 550 bp, whereas the second band approximately 320 bp.

Cases negative for NPM1 mutation did not showed hetero duplex formation. Control persons showed the same results of wild type (no hetero duplex formation). OCI/AML3 cell line was used as positive control showed hetero duplex formation on agarose gel electrophoresis, figure 1.

NPM1 mutations were found in 15/32 adult AML patients (46.88%). The mean age of patients with NPM1 mutations was higher than non mutated patients [40.53±6.17; 35.70±4.00 year,( mean ±SE)], respectively, (P= 0.507), table 2.

Out of 32 adult AML patients 16 were male and the other 16 were female. Nine out of 16 females (60%) were mutated with mutant female: male ratio 1.5:1, (P= 0.411), table 2.

Regarding the relation of NPM1 mutations to hematological parameters of the patients, the mean WBC count in adult mutated patients was lower than non mutated patients [40.09±8.52; 40.52±8.67x10^9/L,( mean ±SE )], respectively, (P= 0.972) whereas platelet count was higher in adult patients with mutation than in patients without mutation [73.94±12.06; 53.60±9.00x10^9/L, (mean ±SE )], respectively, (P= 0.196), table 3.

The mean hematocrit % was nearly the same in mutated patients and non mutated patients [24.33±1.10; 26.67±1.69 %,(mean ±SE )], respectively, (P= 0.270), table 3.

The mean peripheral blood blast cells percent was lower in NPM1 mutated cases than wild cases[60.40±6.69; 67.82±6.43%, (mean ±SE )], respectively, (P= 0.431) similarly the mean bone marrow aspirate(BMA) blast cells percent in NPM1 mutated cases was lower in patients with mutations than that in patients without mutation[67.53±5.51; 71.29±6.66%, (mean ±SE )], respectively, (P= 0.672), table 3.

Regarding the distribution of NPM1 mutations within the FAB subtypes; the mutations were higher in adult patients with M1 and M3(33.33% for each), followed by M4 and M2(13.33% for each), and the least was found in M5(6.66%), (P= 0.191), table 4.

Furthermore, no significant correlation was found between the presence of NPM1 mutations and clinical presentations of adult AML patients, lymphadenopathy, splenomegaly, hepatomegaly, fever, pallor, bleeding and weight loss (P > 0.05), table 5.

NPM1 mutations were detected more in de novo adult AML patients than in secondary cases 9/15(60%) and newly diagnosed cases than relapsed cases 12/15(80%), (P >0.05), table 6.
Furthermore, 12/32 (37.5%) of adult AML patients had achieved complete hematological remission 5 out of them (41%) were mutated representing (33.3%) of all mutated cases whereas, higher failure rate 10/18(55.55%) was found in wild type patients as compared to mutated patients 8/18(44.44%), (P= 0.453) table 6.

Discussion

In this study NPM1 mutations were found in 15 /32 adult AML patients (46.88%), this result was higher than previously reported findings by Pazhakh et al., study in Iran, in 2011 in which the mutation was detected in 17.5% of AML patients who had used the same technique but with different primers set and larger sample size (Pazhakh et al.,2011). However the incidence of this mutation in the Iranian study was the lowest among most of the recent studies on NPM1 mutation and he had attributed this low frequency of the mutation to the higher frequency of cytogenetic abnormalities among Iranian AML patients in general. Furthermore; the frequency of NPM1 mutations in this study was higher than the incidence of these mutations in different studies including over 4300 AML patients worldwide, where the overall frequency of NPM1 mutations was 31.4% (range 25.4–41%), (Falini et al., 2005; Suzuki et al., 2005; Verhaak et al., 2005; Chou et al., 2006; Thiede, et al., 2006; Gale et al., 2007; Wu et al., 2007; Boonthimat et al., 2008).

In this study the frequency of NPM1 mutation was nearly the same as that in cytogenetically normal karyotype AML reported by Egyptian studies by Kassem et al. and Nafea et al (Kassem et al., 2011;Nafea et al., 2011) in which the mutation was detected in 45.8 % and 47.9%, respectively. Moreover, it was similar to other researchers worldwide in which the range of the detected mutation incidence was between 40-60% in cytogenetically normal karyotype AML,(Falini, et al., 2005; Thieda et al., 2006;Angela et al., 2008). NPM1 mutations in AML usually detected in cases who had normal karyotype and lack recurrent cytogenetic abnormalities,( Rau and Brown , 2009).

The high frequency of NPM1 mutation in the present study in relation to other studies may be attributed to difference in method of detection as all the above studies used different PCR circumstances and different primers. Furthermore, it may be attributed to different sample size and different environmental and geographical factors. Regarding the relation of the NPM1 mutations to age, the mean age of adult patients with NPM1 mutations (40.53) year) was higher than that of non mutated patients (35.70) years), (P=0.507).This result was in accordance with the finding in other studies (Schnittger et al., 2005 ; Thiede et al., 2007 ; Nafea et al., 2011).

It had been found that high frequency of NPM1 mutations occur in middle-aged adults or more elderly individuals, (Suzuki et al., 2005) with rare incidence of NPM1 mutations in individuals younger than 35 years (Schnittger et al., 2005).

This may be explained by epidemiological data which suggested that the risk of acquiring a mutation such as NPM1 mutations in a myeloid stem/progenitor cell is cumulative, and the latency between the acquisition of NPM1 mutation and the acquisition of cooperating mutations required for the development of AML may be on the order of years, so these mutations may be secondary events. (Rau and Brown, 2009).

Regarding the relation of gender to NPM1 mutations most of the mutation was detected in female (P= 0.411).This finding was similarly reported by different clinical studies ( Alcalay et al., 2005; Döhner et al.,2005; Schnittger et al., 2005; Thiede et al., 2006; Falini et al.,2010;Kassem et al., 2011).

Regarding the relation of NPM1 mutations to hematological parameters, adult patients with NPM1 mutations presented with the same mean WBC count of non mutated patients,(P=0.972) Kassem et al. , had reported no significant difference in the mean WBC count between NPM1 mutated patients and wild type patients, (Kassem et al., 2011). Others had reported a higher WBC count in NPM1 mutated patients as compared to non mutated patients ,(Dohner et al., 2005; Verhaak et al., 2005; Chen et al., 2006 ;Wang et al., 2010;Nafea et al., 2011).

A non significant higher platelet count was found in patients with NPM1 mutations than patients without these mutations,(P=0.196),this finding was similarly reported by other researchers, (Dohner et al., 2005;Chou et al., 2006; Thiede et al., 2006) .This might imply that blasts with NPM1 mutations retain a certain capacity for thrombocytic differentiation (Hsu and yung , 2003).

In this study no significant difference in hematocrit between mutated and non mutated patients which was also reported by others (Cazzaniga et al., 2005; Chou et al. 2006).
The mean blast cells percent in peripheral blood and bone marrow aspirate were lower in NPM1 mutated cases than wild type cases,(P= 0.431,0.672), respectively. Cazzaniga et al. and Kassem et al had reported no significant difference in blast cell percent between NPM1 mutated patients and patients without this mutation (Cazzaniga et al., 2005; Kassem et al, 2011), whereas other researchers had reported higher blast cell count in mutated patients as compared to non mutated patients (Suzuki et al., 2005; Chou et al., 2006; Thiede et al., 2006).

In the current study higher frequency of NPM1 mutation was found in adult patients with both M1 (33.33%) and (M3 and M3V) (33.33%) followed by M4 and M2(13.33%) for each and the least was found in M5 subtype (6.66%), (P= 0.191). In agreement to these results Verhaak et al., had reported high frequency of these mutations in FAB M1 34%, (Verhaak et al., 2005); whereas Pazhakh et al., had reported the occurrence of NPM1 mutation mostly in M4, M5 and M3 and M3V subtypes (Pazhakh et al., 2011). Other studies had reported high frequency of NPM1 mutations in M4 and M5 subtypes and the least in M2 with no mutations were detected in M3 subtype (Thiede et al., 2006; Falini et al., 2011; Kassem et al., 2011; Nafea et al., 2011). Furthermore; similar to the current study Thiede et al. had reported the absence of NPM1 mutations in M6 patients (Thieme et al., 2006). The high frequency of NPM1 mutations in the M3 and M1 subtypes may be attributed to the high number of these subtypes among our patients or it may be due to other environmental and geographical factors.

No significant correlation was found between the presence of NPM1 mutations and the clinical presentations of the adult patients including fever, pallor, hepatomegaly, splenomegaly, lymphadenopathy and weight loss, (P > 0.05) which was similarly reported by other studies (Boonthimat et al., 2008; Kassem et al., 2011). Other studies had reported that NPM1 mutations correlate with extramedullary involvement, mainly gingival hyperplasia and lymphadenopathy, possibly because the mutation was mostly detected in M4 and M5 FAB subtypes in those studies which usually presented with extramedullary involvement (Dohner et al., 2005; Falini et al., 2007a). NPM1 mutations in adult AML patients were detected more in de novo cases than in secondary cases 9/15(60%) (P=0.485) and in newly diagnosed cases than relapsed cases 12/15(80%), (P =0.264). These results were consistent with the results reported by other researchers (Dohner et al., 2005; Falini et al., 2005; Schnittger et al., 2005; Suzuki et al., 2005; Verhaak et al., 2005).

Regarding the relation of the NPM1 mutations with response to induction therapy, 12 adult AML patients out of 32(37.5%) had achieved complete hematological remission, 5 out of those 12 (41%) were mutated and they represent (33.33%) of all mutated adult patients, whereas, higher failure rate 10/18(55.55%) was found in wild type patients as compared to mutated patients 8/18(44.44%), (P= 0.453).

Thus we may propose that the effect of NPM1 mutations in adult AML patients was a favorable prognosis which was similarly found in most studies (Dohner et al., 2005; Scholl et al., 2005; Schnittger et al., 2005; Thiede et al., 2005; Gale et al., 2007; Schlenk et al., 2009). However Boonthimat et al. and Wang et al., found no relation between the mutation and the overall survival of AML patients (Boonthimat et al., 2008; Wang et al., 2010).

Conclusions

In this novel study for molecular detection of NPM1 mutations in Iraqi adult AML patients the frequency of NPM1 mutations in adult AML patients was higher than that reported in different studies worldwide and the NPM1 mutations were associated with good prognostic factors as female gender, lower blast cell percent and higher rate of response to induction therapy thus, we may propose that this mutation is one of the good prognostic parameters although larger molecular study with follow up is beneficial in Iraqi AML patients as those patients may benefit from molecular target therapy.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

EK, contributed to collection of data, performing larger part of the molecular studies, hematological assays, data analysis and drafting of the manuscript, contributed the concept and design; MA, contributed to part of the molecular work and interpretation of the molecular results, and revision of the manuscript. All authors revised and approved the final submitted version of the manuscript.
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References


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Table 1: PCR reaction for NPM1 mutation analysis

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
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<tbody>
<tr>
<td>c DNA synthesis</td>
<td>42 °C</td>
<td>60 min</td>
<td>1</td>
</tr>
<tr>
<td>Inactivation</td>
<td>95 °C</td>
<td>5 min</td>
<td>1</td>
</tr>
<tr>
<td>Pre-denaturation</td>
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<td>3 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94 °C</td>
<td>45 sec</td>
<td>35</td>
</tr>
<tr>
<td>Annealing</td>
<td>57 °C</td>
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<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72 °C</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72 °C</td>
<td>10 min</td>
<td>1</td>
</tr>
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Table2: Relation of NPM 1 mutations with age and gender of AML patients

<table>
<thead>
<tr>
<th>Patients characteristics</th>
<th>NPM 1 Wild</th>
<th>NPM1Mutated</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Age /years</td>
<td></td>
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<tr>
<td>Adult Mean ±SE</td>
<td>35.70±4.00</td>
<td>40.53±6.17</td>
<td>0.507</td>
</tr>
<tr>
<td>Gender</td>
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<td></td>
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</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>6</td>
<td>0.411</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>9</td>
<td></td>
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Table 3: Hematological parameters of AML patients in relation to NPM1 mutations.

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>NPM1 Wild</th>
<th>NPM1 Mutated</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCx10⁹/L</td>
<td>Mean±SE</td>
<td></td>
<td>40.52±8.67</td>
</tr>
<tr>
<td>PlateletX10⁹/L</td>
<td>Mean±SE</td>
<td></td>
<td>53.60±9.00</td>
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<tr>
<td>Hematocrit%</td>
<td>Mean±SE</td>
<td></td>
<td>26.67±1.69</td>
</tr>
<tr>
<td>Peripheral Blast %</td>
<td>Mean ±SE</td>
<td></td>
<td>67.82±6.43</td>
</tr>
<tr>
<td>BMA Blast%</td>
<td>Mean±SE</td>
<td></td>
<td>71.29±6.66</td>
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Table 4: Relation of NPM1 mutations with FAB subtypes of AML

<table>
<thead>
<tr>
<th>FAB subtype</th>
<th>Wild type NPM1</th>
<th>Mutated NPM1</th>
<th>% From mutated</th>
<th>P-value</th>
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<tbody>
<tr>
<td>M1</td>
<td>5</td>
<td>5</td>
<td>33.33</td>
<td>0.191</td>
</tr>
<tr>
<td>M2</td>
<td>6</td>
<td>2</td>
<td>13.33</td>
<td></td>
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<tr>
<td>Classical M3</td>
<td>1</td>
<td>4</td>
<td>26.66</td>
<td></td>
</tr>
<tr>
<td>M3w</td>
<td>1</td>
<td>1</td>
<td>6.66</td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>3</td>
<td>2</td>
<td>13.33</td>
<td></td>
</tr>
<tr>
<td>M5</td>
<td>0</td>
<td>1</td>
<td>6.66</td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>M7</td>
<td>0</td>
<td>0</td>
<td>0</td>
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Table 5: Relation of clinical presentation of AML patients with NPM1 mutations

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Wild type NPM1</th>
<th>Mutated NPM1</th>
<th>% From mutated</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphadenopathy</td>
<td>7</td>
<td>5</td>
<td>33.33</td>
<td>0.952</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>9</td>
<td>11</td>
<td>73.33</td>
<td>0.170</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>6</td>
<td>9</td>
<td>60</td>
<td>0.153</td>
</tr>
<tr>
<td>Pallor</td>
<td>18</td>
<td>10</td>
<td>66.66</td>
<td>0.141</td>
</tr>
<tr>
<td>Fever</td>
<td>10</td>
<td>11</td>
<td>73.33</td>
<td>0.300</td>
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<tr>
<td>Bleeding</td>
<td>6</td>
<td>8</td>
<td>53.33</td>
<td>0.733</td>
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<tr>
<td>Weight loss</td>
<td>3</td>
<td>4</td>
<td>26.66</td>
<td>0.451</td>
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Table 6: Relation between type of AML and response to induction therapy with NPM1 mutations in AML patients

<table>
<thead>
<tr>
<th>Characteristic of AML</th>
<th>Wild type NPM1</th>
<th>Mutated NPM1</th>
<th>% From mutated</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>De novo AML</strong></td>
<td>20</td>
<td>9</td>
<td>60</td>
<td>0.485</td>
</tr>
<tr>
<td><strong>Secondary AML</strong></td>
<td>1</td>
<td>6</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td><strong>Newly diagnosed AML</strong></td>
<td>9</td>
<td>12</td>
<td>80</td>
<td>0.264</td>
</tr>
<tr>
<td><strong>Relapsed AML</strong></td>
<td>8</td>
<td>3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><strong>Response to induction therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remission</td>
<td>7</td>
<td>5</td>
<td>33.33</td>
<td></td>
</tr>
<tr>
<td>Failure</td>
<td>10</td>
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<tr>
<td>Death</td>
<td>0</td>
<td>2</td>
<td>13.33</td>
<td></td>
</tr>
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</table>

Figure 1: Detection of NPM1 mutations using Single Strand Confirmatory Polymorphism- RT-PCR in AML patients.

**Lanes 1:** positive control OCI/AML3 cell line show hetero duplex formed from mutant and wild type allele appear as 2 bands app.(550 & 320 bp arrow).

**Lanes 2,3,4,5&6:** amplified products from mutated AML patients show hetero duplex formation from mutant & wild-type allele of NPM1 gene, arrows.

**Lanes 7,8:** amplified product from healthy control showed absence of hetero duplex.

**Lanes:9,10,11,12&13:** amplified product from wild type patients show no hetero duplex formed.

**Lane 14:** negative control (no template).

**M:** Molecular weight marker(DNA ladder). Electrophoresis was carried in 3% agarose gel at (4V/cm) for 120 min.