The Clinicopathological Impact of Granulocyte-Macrophage Colony-Stimulating Factor Gene Expression and Different Molecular Prognostic Biomarkers in Egyptian Acute Myeloid Leukemia Patients

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Abstract

Background: Acute myeloid leukemia (AML) is characterized by clonal expansion of myeloid precursors with diminished capacity for differentiation. It develops as the consequence of a series of genetic changes in a hematopoietic precursor cell. Purpose This study aimed to investigate the correlation between GM-CSF gene expression and different molecular prognostic markers such as FLT3-ITD, NPM1 mutation A and CEBPA gene expression in 100 Egyptian AML patients. As well as, correlation with the response to induction therapy, DFS and OS in these patients. Methodology: Quantitative assessment of GM-CSF gene expression was performed by qRT-PCR. Additional prognostic molecular markers were determined as FLT3-ITD and NPM1 mutation A together with quantitative assessment of CEBPA gene expression by qRT-PCR. Results: Patients with high GM-CSF expression levels had better OS and DFS with p value 0.004 and 0.02, respectively. However, no statistically significant difference between low and high GM-CSF gene expression was found regarding the response to therapy (p value= 0.08). Most patients with low CEBPA expression had resistant disease together with poor OS and DFS (P value = <0.001 for each). Our results showed that patients with high CEBPA gene expression whether GM-CSF gene expression was high or low had significant higher complete remission rates (p value = 0.1 for each). However, low GM-CSF gene expression and low CEBPA gene expression showed poor response to treatment. Conclusion: Our findings suggest that molecular diagnostic biomarkers for AML are an essential tool that improves prognostication and hence better patients’ management.

Keywords: AML- GM-CSF- CEBPA- FLT3-ITD- NPM1 mutation A

Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematological disorder, characterized by clonal expansion of myeloid precursors with diminished capacity for differentiation resulting in an accumulation of large numbers of abnormal, immature myeloid cells (Hussein et al., 2019). The age-adjusted incidence of AML is 4.3 per 100,000 annually in the United States (US). Incidence increases with age with a median age at diagnosis of 68 years in the US (Shallis et al., 2019). In Egypt, leukemia is the most common presented hematological malignancy (75%), nearly half of leukemic cases were acute myeloid leukemia which develops as the consequence of a series of genetic changes in a hematopoietic precursor cell (Hussein et al., 2019). Many somatic acquired mutations have been identified in AML with normal karyotype such as FLT3-ITD, NPM1, CEBPA...etc. The mutation in FMS-like tyrosine kinase 3 (FLT3) gene is one of the most common genetic abnormalities found in AML patients. The cytogenetic location of FLT3 gene is on chromosome 13q12.2 and is a member of the class III receptor tyrosine kinase (RTK). The FLT3 mutation results in constitutive activation of the receptor with independent dimerization of FLT3 ligand (FL), and auto-phosphorylation which result in uncontrolled proliferation and apoptosis (Kumsaen et al., 2016). Mutations in FLT3 gene have been identified in two functional domains of the receptor, internal tandem duplications (ITDs) in the juxtamembrane domain (JM) and activating point mutations in the second tyrosine kinase domain (TKD). 

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Materials and Methods

Subject and methods

Study population

The present study included 100 AML patients and their ages ranged between 12 and 77 years. They were selected in the period from 2016 to 2018. Twenty age and sex matched healthy volunteers were included in the current study as control group. For patients and controls, 2 ml EDTA blood samples was collected under complete aseptic conditions for molecular studies.
Data analysis

Data was analyzed using IBM® SPSS® Statistics version 22 (IBM® Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test or Fisher’s exact test was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Spearman-rho method was used to test correlation between numerical variables. Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. All tests were two-tailed. A p-value < 0.05 was considered significant.

Results

Patients’ characteristics

Our patients were 48 males and 52 females and their ages ranged between 12 and 77 years with a mean value of 37.3±14.9 years. As regards FAB classification, 1% was M0, 23% were M1, 17% were M2, 27% were M3, 12% were M4, 15% were M5, 3% were M6 and 2% M7. Molecular studies revealed 37 patients had FLT3/ITD mutation and 36 patients had NPM1 mutation A. The cytogenetic patients studies showed 15 patients were t(8;21) positive, 8 patients were inv.16 positive and 27 patients were t (15;17) positive.

CEBP A gene expression levels

AML patients with CEBPA gene expression level below cut off value which was the mean expression level in the control group (1.13) were considered as “CEBPA low expression”, while those with expression level higher than (1.13) were considered as “CEBPA high expression”. The majority of patients (59/100) showed low CEBPA expression levels ranged between 0.0013 and 0.99, a mean value of 0.28 ± 0.35 and median value of 0.08. In forty-one cases, higher expression levels were recorded with a range of 1.15 and 1.99, a mean value of 1.68 ± 0.21 and median value of 1.68. Statistical analysis showed significant difference in expression levels between the two groups with p value < 0.001. Comparison between AML patients with low versus high CEBPA gene expression according to their clinical and laboratory data was described in Table 1.

GM-CSF gene expression levels

Patients with GM-CSF gene expression level below cut off value which was the mean expression level in the control group (1.07) were considered as “GM-CSF low expression”, while those with expression level higher than (1.07) were considered as “GM-CSF high expression”. Many of the patients (55/100) showed low GM-CSF expression levels ranged between 0.001 - 0.99, a mean value of 0.27 ± 0.35 and median value of 0.1. In forty-five cases, higher expression levels were recorded with a range of 1.08 and 2.88, a mean value of 1.87 ± 0.41 and median value of 1.88. Statistical analysis showed significant difference in expression levels between the two groups.

Figure 1. Impact of Studied Molecular Genetic Abnormalities on Overall Survival (OS)
with p value < 0.001. Comparison between AML patients with low versus high GM-CSF expression according to their clinical and laboratory data was described in Table 2.

**Correlation between CEBPA and GM-CSF gene expression levels, FIT3/ITD and NPM1 mut. A and response to therapy**

Complete remission (CR) was defined as recovery of bone marrow morphology with less than 5% blasts, neutrophil count 1/109/L or more, platelet count 100/109/L or more, and no evidence of extra medullary leukemia. Resistant disease (RD) was defined as treatment resistance when evaluation did not meet the criteria of complete remission. Early death was defined as death before completion of the induction therapy cycle. These latter patients were not included in evaluation of resistant disease. Accordingly, only 55 patients were evaluated for response to induction therapy. Twenty-eight patients achieved CR with CEBPA gene expression level ranged between 0.88 and 1.99, with a mean value of 1.69 ± 0.24 and median value of 1.69. Twenty-seven patients had RD with CEBPA gene expression level ranged between 0.003 and 1.98, with a mean value of 0.92 ± 0.73 and median value of 0.88. There was statistically significant difference noticed in CEBPA gene expression between the two patients’ groups with P value <0.001. Regarding high CEBPA expression patients, 27 patients had achieved CR after induction therapy, while 12 patients had RD. However, patients with low CEBPA expression, only one patient achieved CR after induction therapy while, 15 patients had RD. A statistically significant difference was found between high and low CEBPA expression with P value <0.001, where higher number of patients achieved CR had high gene expression levels. In 28 patients who achieved CR, GM-CSF gene expression level ranged between 0.03 and 2.88, with a mean value of 1.32 ± 0.89 and median value of 1.68. In patients with RD, GM-CSF gene expression level ranged between 0.01 and 2.65, with a mean value of 0.86 ± 0.85 and median value of 0.54. There was statistically significant difference noticed in GM-CSF gene expression between the two patients’ groups with P value = 0.04. Regarding high GM-CSF expression patients, 17 patients achieved CR after induction therapy, while 10 patients had RD. However, in low GM-CSF expression patients, 11 patients achieved CR while, 17 patients had RD. There was no statistically significant difference between patients with high and low GM-CSF gene expression as regards the response to therapy with P value = 0.08. Also, we tried to verify the impact of FLT3/ITD and NPM1 mutations on the response to therapy, FLT3/ITD showed no statistically significant difference between cases who achieved CR and those with RD with p-value = 0.11 where, CR rates were higher in patients with wild FLT3/ITD. As regards NPM1 mut. A, no statistically significant difference was found between cases who achieved CR and those with RD with p value = 0.22. Finally, CR rates were higher in patients with high CEBPA gene expression whether GM-CSF gene expression levels were high or low as shown in Table 3.
Impact of GM-CSF Gene Expression and Different Molecular Prognostic Biomarkers in AML Patients

Correlation between CEBPA and GM-CSF gene expression levels, FLT3/ITD and NPM1 mut. A and OS and DFS

Patients were followed up for a median period of 6 months, the Overall Survival rate (OS: defined from the date of diagnosis till the date the patient died, or was last seen) and the Disease Free Survival rate (DFS: defined from the date of CR achievement till the date the patient relapsed) were assessed. AML patients with high CEBPA gene expression had a cumulative OS at 6 months 90.2%, while patients with low CEBPA gene expression had a cumulative OS at 6 months 13.6%, with statistically significant difference between the two patients group with p-value <0.001. Patients with high GM-CSF gene expression had a cumulative OS at 6 months 57.8%, while patients with low GM-CSF gene expression had a cumulative OS at 6 months 34.5%, with statistically significant difference between the two patients group with p-value = 0.004. Regarding FLT3/ITDs, a statistically significant difference between wild and mutant cases was noticed with p-value <0.001 where, FLT3/ITD wild cases had better OS. As regards NPM1 mutation A, a statistically significant difference was found between wild and mutant cases with p-value <0.001 where, NPM1 mutant cases had better OS. Finally, we tried to study the impact of both FLT3/ITD and NPM1 mutations on OS. AML patients were classified into 4 groups where patients with (FLT3/ITD –ve, NPM1 –ve) and (FLT3/ITD3–ve, NPM1 +ve) had higher OS rates than patients with (FLT3/ITD +ve, NPM1 –ve) and (FLT3/ITD +ve, NPM1 +ve) with p-value >0.001 (Figure 1). Regarding DFS, AML patients with high CEBPA gene expression had a cumulative DFS at 6 months 87.8%, while patients with low CEBPA gene expression had a cumulative DFS at 6 months of 8.5%, with statistically significant difference encountered between the two patients group with P <0.001. Patients with high GM-CSF gene expression had a cumulative DFS at 6 months 57.8%, while patients with low GM-CSF gene expression had a cumulative DFS at 6 months of 27.3%, with statistically significant difference between the two patients group with p-value = 0.001. Regarding FLT3/ITDs, a statistically significant difference between wild and mutant cases was noticed with p-value <0.001 where, FLT3/ITD wild cases had better DFS. As regards NPM1 mutation A, a statistically significant difference was found between wild and mutant cases with p-value <0.001 where, NPM1 mutant cases had better DFS. Finally, AML patients were classified into 4 groups where patients with

Table 1. Comparison between AML Patients with Low or High CEBPA Gene Expression According to Their Clinical and Laboratory Data

| Parameter          | CEBPA low expression | CEBPA high expression | P-value |
|--------------------|----------------------|------------------------|---------|
|                    | n=59 (59%)           | n=41 (41%)             |         |
| Hb gm/dL           | Range 5.2-12.4       | Range 5.4-15.3         | 0.03*   |
|                    | Mean±SD 7.9±1.7      | Mean±SD 9.0±2.5        |         |
|                    | Median 7.7           | Median 8.1             |         |
| TLCx10³/cm³        | Range 1.5-272        | Range 2.3-419          | 0.17    |
|                    | Mean±SD 50.6±50.9    | Mean±SD 60.2±67.3      |         |
|                    | Median 36.6          | Median 44.5            |         |
| Plts x10³/cm³      | Range 6-429          | Range 8-337            | 0.01*   |
|                    | Mean±SD 78.2±101.3   | Mean±SD 120.6±103.7    |         |
|                    | Median 45            | Median 77              |         |
| PB blast (%)       | Range 4-94           | Range 5-49             | 0.81    |
|                    | Mean±SD 42.9±27.1    | Mean±SD 45.7±28.4      |         |
|                    | Median 35            | Median 45              |         |
| B.M blast (%)      | Range 20-90          | Range 22-90            | 0.14    |
|                    | Mean±SD 66.0±22.6    | Mean±SD 58.2±25.1      |         |
|                    | Median 70            | Median 60              |         |
| Age                | > 18 years           | > 18 years             |         |
|                    | Range 57 (60.0%)     | Range 38 (40.0%)       | 0.39    |
|                    | Mean±SD 7.9±1.7      | Mean±SD 9.0±2.5        |         |
|                    | Median 7.7           | Median 8.1             |         |
| Gender             | Male                 | Male                   | 0.59    |
|                    | Range 25 (65.6%)     | Range 31 (43.8%)       |         |
|                    | Mean±SD 50.6±50.9    | Mean±SD 60.2±67.3      |         |
|                    | Median 36.6          | Median 44.5            |         |
| Classification     | M3                   | M3                     | 0.01*   |
|                    | Range 10 (37.0%)     | Range 17 (63.0%)       |         |
|                    | Mean±SD 21 (65.6%)   | Mean±SD 11 (34.4%)     |         |
| Mol. studies       | FLT3-ITD wild        | FLT3-ITD mut. A Wild   | 0.00*   |
|                    | Range 23 (36.5%)     | Range 40 (63.5%)       |         |
|                    | Mean±SD 5 (33.3%)    | Mean±SD 36 (42.4%)     |         |
| Cytogenetic studies| t (8;21) positive    | t (8;21) negative      | 0.51    |
|                    | Range 10 (66.7%)     | Range 49 (57.6%)       |         |
|                    | Mean±SD 5 (33.3%)    | Mean±SD 36 (42.4%)     |         |
|                    | Median 36 (42.4%)    | Median 49 (57.6%)      |         |
|                    | inv.16 positive      | inv.16 negative        | 0.56    |
|                    | Rank 16 (64.0%)      | Rank 9 (36.0%)         |         |
|                    | Median 32 (42.7%)    | Median 16 (59.3%)      |         |
|                    | t (15;17) positive   | t (15;17) negative     | 0.02*   |
|                    | Range 11 (40.7%)     | Range 48 (65.8%)       |         |
|                    | Mean±SD 16 (59.3%)   | Mean±SD 25 (34.2%)     |         |
|                    | Median 16 (59.3%)    | Median 25 (34.2%)      |         |

*, Significant at P ≤ 0.05

Table 1. Comparison between AML Patients with Low or High CEBPA Gene Expression According to Their Clinical and Laboratory Data
Acute myeloid leukemia is a group of hematological malignancies whose leukemogenesis and clinical behavior were deeply influenced by the underlying cytogenetic and molecular abnormalities (Zhu et al., 2017). Here, we aim to investigate GM-CSF gene expression using quantitative RT-PCR as GM-CSF is a known autocrine/paracrine cytokine that stimulates growth, differentiation, and function of normal and leukemic myeloid progenitors together with different molecular prognostic markers such as FLT3/ITD, NPM1 mutation A and CEBPA gene expression in Egyptian AML patients. In addition to response to therapy, DFS and OS in these patients were assessed which help in understanding their impact on the pathogenesis of the disease and hence predict prognosis. Our results showed statistically significant difference between low and high GM-CSF gene expression levels in AML patients with p value < 0.001. No significant difference was found between low and high GM-CSF gene expression as regards their age, gender, clinical data, total leukocytic count, and initial peripheral blood blasts percentage. This is in agreement with previously reported by (Kassem et al., 2018) who found no significant correlations between GM-CSF gene expression and different demographic, clinical and laboratory data. As regards cytogenetic analysis, we found no statistically significant difference between low and high GM-CSF gene expression as regards different cytogenetic markers. This is in disagreement with (Weng et al., 2017) who found GM-CSF gene significantly downregulated in t(8;21) positive leukemic patients. Our results revealed no statistically significant difference between low and high GM-CSF gene expression regarding the response to therapy which in agreement with (Kassem et al., 2018) who found no significant correlations between GM-CSF gene expression and response to treatment. Our AML patients with high GM-CSF expression levels had better OS and DFS with statistically significant difference between high and low GM-CSF gene expression regarding the response to therapy which in agreement with (Kassem et al., 2018) who found no significant correlations between GM-CSF gene expression and OS. The CCATT enhancer binding protein alpha (CEBPα) transcription factor is a critical regulator of proliferation.

### Table 2. Comparison between AML Patients with Low or High GM-CSF Gene Expression According to Their Clinical and Laboratory Data

| Parameter                  | GM-CSF low expression | GM-CSF high expression | P-value |
|----------------------------|------------------------|-------------------------|---------|
|                            | n=55 (55%)             | n=45 (45%)              |         |
| Hb gm/dL                   | Range                  | Mean±SD                 | Median  |
|                            | 5.2-14.1               | 8.2±2.2                 | 7.8     |
| TLCx10⁶/cm³                | 1.5-419                | 60.2±71.5               | 44.7    |
| Plts x10⁹/cm³             | 6-429                  | 89.3±103.5              | 54      |
| P:B blast (%)              | 4-90                   | 44.3±29.4               | 37      |
| B:M blast (%)              | 20-90                  | 64.0±23.9               | 66      |
| Age                        | > 18 years             | 52 (54.7%)              | 43 (45.3%) |
|                            | < 18 years             | 3 (60.0%)               | 2 (40.0%)  |
| Gender                     | Male                   | 26 (54.2%)              | 22 (45.8%) |
|                            | Female                 | 29 (55.8%)              | 23 (44.2%) |
| FAB                        | M0,M1,M2               | 22 (53.7%)              | 19 (46.3%) |
|                            | M3                     | 13 (48.1%)              | 14 (51.9%) |
| Mol. studies               | FLT3-ITD wild          | 29 (46.0%)              | 34 (54.0%) |
|                            | FLT3-ITD mutant        | 26 (70.3%)              | 11 (29.7%) |
|                            | NPM1 mut. A Wild       | 41 (64.1%)              | 23 (35.9%) |
|                            | NPM1 mut. A mutant     | 14 (38.9%)              | 22 (61.1%) |
| Cytogenetic studies        | t (8;21) positive      | 7 (46.7%)               | 8 (53.3%) |
|                            | t (8;21) negative      | 48 (56.5%)              | 37 (43.5%) |
|                            | inv.16 positive        | 15 (60.0%)              | 10 (40.0%) |
|                            | inv.16 negative        | 40 (53.3%)              | 35 (46.7%) |
|                            | t (15;17) positive     | 14 (51.9%)              | 13 (48.1%) |
|                            | t (15;17) negative     | 41 (56.2%)              | 32 (43.8%) |

* Significant at P ≤ 0.05

(FLT3/ITD –ve, NPM1 –ve) and (FLT3/ITD –ve, NPM1 +ve) had higher DFS rates than patients with (FLT3/ITD +ve, NPM1 -ve) and (FLT3/ITD +ve, NPM1 +ve) with p-value < 0.001 (Figure 2).

### Discussion

Acute myeloid leukemia is a group of hematological malignancies whose leukemogenesis and clinical behavior were deeply influenced by the underlying cytogenetic and molecular abnormalities (Zhu et al., 2017). Here, we aim to investigate GM-CSF gene expression using quantitative RT-PCR as GM-CSF is a known autocrine/paracrine cytokine that stimulates growth, differentiation, and function of normal and leukemic myeloid progenitors together with different molecular prognostic markers such as FLT3/ITD, NPM1 mutation A and CEBPA gene expression in Egyptian AML patients. In addition to response to therapy, DFS and OS in these patients were assessed which help in understanding their impact on the pathogenesis of the disease and hence predict prognosis. Our results showed statistically significant difference between low and high GM-CSF gene expression levels in AML patients with p value < 0.001. No significant difference was found between low and high GM-CSF gene expression as regards their age, gender, clinical data, total leukocytic count, and initial peripheral blood blasts percentage. This is in agreement with previously reported by (Kassem et al., 2018) who found no significant correlations between GM-CSF gene expression and different demographic, clinical and laboratory data. As regards cytogenetic analysis, we found no statistically significant difference between low and high GM-CSF gene expression as regards different cytogenetic markers. This is in disagreement with (Weng et al., 2017) who found GM-CSF gene significantly downregulated in t(8;21) positive leukemic patients. Our results revealed no statistically significant difference between low and high GM-CSF gene expression regarding the response to therapy which in agreement with (Kassem et al., 2018) who found no significant correlations between GM-CSF gene expression and response to treatment. Our AML patients with high GM-CSF expression levels had better OS and DFS with statistically significant difference between high and low GM-CSF gene expression group, p value 0.004 and 0.02, respectively. This is discordance with (Kassem et al., 2018) who found no significant correlations between GM-CSF gene expression and OS. The CCATT enhancer binding protein alpha (CEBPα) transcription factor is a critical regulator of proliferation.

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Table 3. Impact of Studied Molecular Genetic Abnormalities on Response to Induction Therapy

|                           | Complete remission (CR) | Resistant disease (RD) | P-value   |
|---------------------------|-------------------------|------------------------|-----------|
|                           | n=28                    | n=27                   |           |
| High CEBPA expression     | 27 (96.4%)              | 12 (44.4%)             | <0.001*   |
| Low CEBPA expression      | 1 (3.6%)                | 15 (55.6%)             |           |
| High GM-CSF expression    | 17 (60.7%)              | 10 (37%)               | 0.08      |
| Low GM-CSF expression     | 11 (39.3%)              | 17 (63%)               |           |
| FLT3/ITD +ve              | 0 (0%)                  | 3 (11.1%)              | 0.11      |
| FLT3/ITD -ve              | 28 (100%)               | 24 (88.9%)             |           |
| NPM1 +ve                  | 16 (57.1%)              | 11 (40.7%)             | 0.22      |
| NPM1 -ve                  | 12 (42.9%)              | 16 (59.3%)             |           |
| FLT3/ITD -ve, NPM1 +ve    | 16 (57.1%)              | 10 (37%)               | 0.21      |
| FLT3/ITD -ve, NPM1 -ve    | 12 (42.9%)              | 14 (51.9%)             |           |
| GM-CSF high expression, CEBPA high expression | 17 (100%) | 6 (60%) | 0.01* |
| GM-CSF low expression, CEBPA low expression | 0 (0%) | 4 (40%) |      |
| GM-CSF low expression, CEBPA high expression | 10 (90.9%) | 6 (35.3%) | 0.01* |

*, Significant at P ≤ 0.05

and differentiation in myeloid cells (Zhang et al., 2004).

Quantitative assessment of CEBPA gene expression was done by real time PCR and our study showed that there was a statistically significant difference between low and high CEBPA gene expression where the majority of patients had low CEBPA expression levels. This is in accordance with the results previously reported by (Barjesteh et al., 2003; D’Al’o et al., 2008) who reported that the majority of their patients showed low CEBPA expression level. Also, we found no statistically significant difference between low and high CEBPA gene expression as regards their gender. However, Gholami et al., (2019) found that a significant up-regulation of CEBPA gene was detected in male AML patients. Also, we found no statistically significant difference between low and high CEBPA gene expression as regards their clinical and laboratory data except for hemoglobin (Hb) and platelet count where patients with high CEBPA expression levels had significant higher Hb and platelet counts. However, Gholami et al., (2019) found patients with a lower level of CEBPA gene expression had leukopenia. Our results revealed that M3 patients had significant higher CEBPA gene expression levels than non M3 patients with p-value 0.01. This is in accordance with (Kassem et al., 2013; Gholami et al., 2019) who found significant CEBPA gene over-expression was in M3. Regarding cytogenetic analysis, our results showed no statistically significant difference between low and high CEBPA gene expression but most of low CEBPA expression patients harboring t(8;21). This is in accordance with (Grossmann et al., 2012) who found cases harboring t(8;21) presented a lower CEBPA expression than patients without, where no significant difference was detected between CEBPA expression levels and RUNX1 mutations. Our results showed statistically significant difference between patients with high and low CEBPA gene expression levels as regards the response to therapy with most of patients with low CEBPA expression having resistant disease together with poor OS and DFS. This is in accordance with (Kassem et al., 2013) who found patients with higher CEBPA gene expression had higher OS and DFS and (Barjesteh et al., 2003) who found that particularly patients with low CEBPA gene expression seemed to have a relatively poor OS and DFS but didn’t reach significant difference. Our results showed that AML patients with high CEBPA gene expression whether GM-CSF gene expression was high or low had significant higher complete remission rates. However, low GM-CSF gene expression and low CEBPA gene expression showed poor response to treatment. We also assessed NPM1 mutation A and FLT3/ITD by conventional RT-PCR because of their known prognostic value besides being potential targets for therapy. NPM1 mutation A was detected in 36% of AML patients. This frequency was in agreement with many previous studies in Egypt and worldwide as reported by (Falini et al., 2008; Farawela et al., 2014; Kassem et al., 2019) where the frequency of NPM1 mutation ranged between 30–52.9% among their AML patients. FLT3/ITDs was detected in 37% of our AML patients, this frequency was close to that previously reported by (Gorin et al., 2013; Farawela et al., 2014; Kassem et al., 2019) where the frequency of FLT3/ITDs ranged between 15.4–36% among their AML patients. Our results revealed no statistically significant difference between NPM1 mut. A and FLT3/ITD wild and mutant patients regarding response to therapy. This is in accordance to (Akla et al., 2012) where no significant difference was detected between FLT3/ITD positive and negative cases after induction chemotherapy. Although, he recorded a significant difference between NPM1-positive and -negative patients with a P-value 0.004 where 62.5% of NPM1-positive patients achieved CR. We classified our patients regarding NPM1 mut. A and FLT3/ITD mutational status into 2 subgroups (NPM1 +ve, FLT3/ITD +ve and NPM1 –ve, FLT3/ITD -ve). Fifty seven percent of our patients who achieved CR were in (FLT3/ITD –ve, NPM1 +ve) group. This in accordance to (Akla et al., 2012; Testa and Pelosi 2013) who found that 60% who achieved CR were NPM1+/FLT3/ITD – and had favorable
outcome. Regarding DFS and OS, there was a statistically significant
difference between FLT3/ITD mutant and wild
patients with p-value < 0.001 for each, where FLT3/ITD
wild cases had better DSF and OS. This is in accordance with
(Medinger et al., 2016; Garcia and Baer, 2017) as they
reported that FLT3/ITD-positive AML patients had
higher relapse incidence and lower DFS as well as OS. A
statistically significant difference was detected between
NPM1 mutant and wild cases as regards DFS and OS with
p-value 0.001 and< 0.001, respectively. Most of patients
with positive NPM1 mutation A had better DFS and OS.
This is in accordance with (Port et al., 2014) who found a
better outcome for DFS and OS for patients harboring
NPM1 mutations. In the current study, we classified our
patients into 4 groups as regards FLT3-ITD and NPM1
mutations where higher DFS and OS were detected in (NPM1
mutant, FLT3/ITD wild) group .This is in accordance to
(Medinger et al., 2016; Velloso et al., 2011)
who found that absence of FLT3 ITD mutations, positive
NPM1 mutations are associated with improved outcome
for patients and NPM1+/FLT3-, currently recognized as
of good prognosis.

In conclusion, this study identified GM-CSF gene
expression in AML patients providing additional evidence
for the possible role of that as a prognostic marker
and indicator for treatment outcome together with already
known prognostic molecular biomarkers such as CEBPA,
NPM1 mut. A and FLT3-ITD. Additional researches in
this field with larger sample size involving the majority of
oncology centers throughout Egypt’s governorates are
required for understanding the molecular mechanisms
underlying AML pathogenesis and risk stratification in
Egypt. In conclusion, our findings suggest that molecular
diagnostic biomarkers for AML are an essential tool
that improves prognostication and hence better patients’
management.

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Ethics approval and consent to participate

This study was approved by Kasr Al-Ainy Center of
Clinical Oncology and Nuclear Medicine Institutional
Review Board (IRB)-2-2017. All procedures performed in
the study involving human participants were in accordance
with the ethical standards of the institutional research
committee and with the 1964 Helsinki declaration and
its later amendments (GCP guidelines) or comparable
ethical standards. Consent to participate was obtained
from all individual participants included in the study in
a written form.

Conflicts of interest

Authors declared no conflicts of interest.
Informed consent was obtained from all individual
participants included in the study.

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