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# The Role of β-Catenin and c-Myc Dysregulation in Acute Myeloid Leukemia: A Study of Egyptian Patients

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**Abstract**

**Acute Myeloid Leukemia (AML) is a treatable but aggressive hematologic malignancy attributed to both genetic and epigenetic mutations in hematopoietic stem cells (HSCs). The disease is highly genetically heterogeneous with mutations in important genes like FLT3, NPM1 and TP53. Wnt/β-catenin dysregulated signaling pathways3839 are implicated in the majority of AML pathogenesis as they promote tumor progression and cancer stem cell-like properties also inhibit normal haemopoiesis. One of the crucial mediators of Wnt pathway, β-catenin plays a pivotal role in regulating HSC self-renewal, differentiation, and expression of oncogenes such as *c-Myc*. Dysregulation of *c-Myc*, often observed in AML, is associated with poor prognosis and chemoresistance.**

**The present study aimed to investigate the expression of β-catenin and c-Myc mRNA in Egyptian patients with acute myeloid leukemia (AML), and its relationship to clinical parameters as well as AML subtypes. Clinical characteristics of AML patients were analyzed in our cohort relative to healthy controls, we demonstrated that kidney and liver dysfunction were frequent manifestations among AML patients which showed significantly higher serum creatinine, urea, ALT and AST levels compared with healthy individuals.**

**Plasma β-catenin levels were significantly lower in AML patients, particularly in the M4/M5 subtypes characterized by monocytic differentiation. In contrast, no significant difference in β-catenin levels was observed between M0-M2 subtypes and controls. Age and FAB subtypes influenced β-catenin levels, with lower levels common in middle-aged patients (30–45 years) and the M5 subtype, while higher levels were associated with younger patients (18–30 years) and the M1 subtype.**

**Expression of *c-Myc* mRNA did not differ significantly between overall AML patients and controls. However, subgroup analysis revealed substantially lower *c-Myc* expression in M4/M5 subtypes compared to M0-M2 subtypes and controls.** **Keywords:** AML; Liver**; *β-catenin* ; *c-Myc*.**

## 1. Introduction

Acute myeloid leukaemia (AML) is an aggressive disease in which hemopoietic stem cells (HSCs) or the early progenitors undergo malignant transformation [1,2] Genetic damage in HSCs lead to increased proliferation, decreased apoptosis and a block in cellular differentiation [3,4]. These result in the accumulation of early hematopoietic cells known as blast cells in the bone marrow (BM). The dominant clinical feature of acute leukaemia is usually BM failure which results from the accumulation of blast cells at the expense of the normal production of the red blood cells (RBCs), platelets and white blood cells (WBCs), also organ infiltration occurs [5].

According to Globocan 2020, Leukaemia is ranked among the top 10 cancers affecting the Egyptian population [6] with a slightly higher incidence rate in male population (6.3/100,000) compared to the

female population (5.1/100,000). Leukaemia in Egypt generally has an incidence rate of 5.7 per 100,000 cases and a mortality rate of 4.5 per 100,000 cases [7].

Acute myeloid leukaemia, the most predominant type of acute leukaemia occurring in adults, represents about 25% of all leukaemia types with most cases appearing as a de novo malignancy in previously healthy individuals. Around 20% of cases arise as a consequence of treatment with topoisomerases II inhibitors, alkylating agents or radiation and in this case it can be identified as therapy-related AML (t-AML) [8,9].

The direct and specific causes of AML remain mostly unknown, despite recurrent acquired genetic abnormalities found in AML blasts. However, there are some proposed risk factors and causes that are related to the incidence of AML, such as aging of HSCs, environmental exposures, and genetic and familial predisposition [10].

Acute myeloid leukemia develops as the result of a series of genetic and epigenetic mutations in HSCs or their precursors [11]. These genetic and epigenetic changes affect normal cellular growth and differentiation, resulting in the collection of abnormal, immature myeloid cells in the BM and peripheral blood known as blast cells at the expense of normal hematopoiesis which becomes suppressed. These AML blasts proliferate without differentiation [12].

Multiple mutations involved in AML pathogenesis are frequently found in the preleukemic clone. These alterations include mutations that may affect epigenetic regulators or chromosomalabnormalities that may affect various transcription factors and transcriptional activators [13].

Many factors contribute to gene transcription alteration which results in the inhibition or activation of various signalling pathways that control normal hemopoiesis such as RAF/MEK/ERK, JAK/STAT or phosphatidylinositol 3-hydroxy kinase/protein kinase B (PI3K/AKT) pathways [14]. In addition, evolutionary conserved signalling pathways such as Notch, Wnt or Hedgehog in LSCs were shown to be reactivated [15,16].

Acute myeloid leukemia is considered a heterogeneous group of diseases as there is no single prevalent mutation that is found in all or even in most patients. The top genes mutated in AML with incidence more than five percent each are are fms-related tyrosine kinase 3 (FLT3), nucleophosmin (NPM1), DNA methyltransferase 3 alpha (DNMT3A), isocitrate dehydrogenase 1 and 2 (IDH1/2), tet methylcytosine dioxygenase 2 (TET2), AML1, tumor protein 53 (TP53), neuroblastoma RAS viral oncogene homolog (NRAS), CCAAT enhancer binding protein alpha (CEBPA) and Wilms tumor 1 (WT1) [13]. This genetic heterogeneity is reflected on blast cells morphologically resulting in maturation defects corresponding to the specific stages of hemopoietic differentiation [17].

Beta-catenin is a signal transducer in the Wnt pathway which controls the self-renewal of HSCs and transcription of genes [18]. In HSC, β-catenin suppresses differentiation while regulating survival and growth factor responsiveness both in vitro and in vivo. In many haematological cancers of lymphoid or myeloid origin, the significance of β-catenin was reflected in the preservation of the HSC pool [19,20].

Wnt/β-catenin signaling pathway is known to be active in AML and increase oncogene expression [20-22]. An activating mutation in the β-catenin gene can lead to AML, where β-catenin is the major Wnt signal transducer in the canonical pathway [23]. The Wnt/β-catenin signaling pathway is a well-known promoter of cancer that drives tumor progression by modulating the tumor immune cycle [24,25].

Wnt is a glycoprotein that can attach to frizzled (Fz) receptors (atypical G protein coupled receptor) and low-density lipoprotein receptor-related protein (LRP) on the cell surface [26].

In the canonical pathway, degradation of cytoplasmic β-catenin occurs through phosphorylation by a destruction complex is formed of APC, axin, CK1 and GSK3, when Wnt ligand is absent [26].

When Wnt ligand is present, the destruction complex formation is prevented, increasing the intracellular concentration of β-catenin, leading to translocation of β-catenin into the nucleus where it frees TCF from its repressor [carboxy terminal binding protein (CtBP) and Groucho (Gro)] and binds to it leading to activation of Wnt target genes transcription, one of these target genes is c-Myc [27,28]. Hematopoiesis must be intensely regulated by large group of cytokines and coordinated activity of transcription factors. One of these significant transcription factors regulating hematopoiesis and a target gene of an activated Wntpathway is c-Myc.

c-Myc is part of the Myc family which involves three nuclear transcription factors (c-Myc, n-Myc, and i-Myc). c-Myc, encoded by MYC, is a master regulator of normal and cancer-associated processes [29]. c-Myc is also involved in normal hematopoiesis and its expression is highest in HSCs and as myeloid differentiation occurs, c-Myc level decreases [30].

The c-Myc is a proto-oncogene that is present on

chromosome 8 q24.21. It is transcribed into nuclear phosphoproteins that have a pivotal role in cellcycle progression, and apoptosis. c-Myc also has a role DNA repair, through regulating genes responsible for DNA mismatch repair, as well as binding to the promoters of genes required for double-strand break repair, and metabolism, especially regulating of glycolysis andglutaminolysis [29].

The c-Myc proto-oncogene is a central promoter in numerous malignancies as breast cancer, HCC, colorectal cancer and prostatic cancer. c-Myc was also associated with chemoresistance in different cancers [29,31].

c-Myc proto-oncogene was found to be dysregulated in more than 70% of malignancies, where its dysregulation was implicated with poor prognosis and decreased survival. Excess c-Myc expression can be due to chromosomal translocation, amplification due to degradation malfunction or pathways that increase the stability of c-Myc and its oncogenic actions [31,32].

The current study aimed to analyze the correlation between acute myeloid leukemia and total β-catenin expression as well as c-Myc mRNA expression in Egyptian Patients

## 2. Materials and Methods

### *2.1 Study Population and Sampling Strategy"*

The study was conducted on 90 subjects divided into two groups: Group I; This group included 60 newly diagnosed adult patients with acute myeloid leukemia aged from 18 years to below 60 years who were recruited from the Department of haematology, Alexandria University Hospital before initiation of treatment from January 2021 to February 2022. Group II; This group included 30 healthy age and sex-matched subjects as controls. Patients The following criteria were excluded from the study; pregnant female subjects, patients with concomitant chronic disease, patients with acute promyelocytic anaemia – M3, patients with associated other malignancies. Following FAB criteria [3], 21 (35%) AML patients were M5, 20 patients (33.3%) were M1, 13 patients (21.7%) were M4, 4 patients (6.7%) with M2, and 2 (3.3%) patients were M0.

The study was conducted following the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Local Ethics Committee of the Faculty of Medicine, Review Board of the Alexandria University, Faculty of Medicine obtained under the number of 0201437. Informed consent was obtained from all subjects included in the study.

Genetic testing was done to detect mutations for all patients using real-time quantitative polymerase chain reaction (RT-qPCR).University of Alexandria.

AML patients were diagnosed based on hypercellular bone marrow with more than 20% blasts, morphologic findings, cytogenetics and immunophenotyping. Six ml of venous blood was withdrawn from every patient and control subject. Each blood sample was then divided into three aliquots: a plain tube and two tubes of tri-potassium and ethylenediamine tetraacetic acid ($K_3$EDTA) tubes. In one of the latter, blood was centrifuged at 1200 xg for 10 minutes to separate the plasma sample, which was kept frozen at -80$^{o}$C until use. The following laboratory investigations were performed.

### *2.2 Standard Laboratory Investigations for AML Patients*

Complete blood count (CBC) and was implemented to evaluate anaemia, thrombocytopenia, leucocytosis, leukopenia, count and type of blasts. Kidney function tests were conducted using colorimetric methods to measure serum creatinine and urea concentrations. Liver function tests included the measurement of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, also determined by colorimetric methods. Additionally, bone marrow aspiration was performed for morphological studies, cytogenetics, and immunophenotyping to gain further insights into haematological conditions. The plasma beta-catenin level was determined using an enzyme-linked immunosorbent assay (ELISA) kit provided by NOVA, located at No. 18, Keyuan Road, DaXing Industry Zone, Beijing, China. This comprehensive panel of tests ensured a detailed assessment of physiological and biochemical parameters.

### *2.3 Biochemical characterization of beta-catenin Determination in plasma using the Sandwich-ELISA method.*

The micro-ELISA strip plate provided in the kit was pre-coated with an antibody specific to beta-catenin. Plasma samples and standards were prepared and added to the wells of the micro-ELISA strip plate, where beta-catenin molecules were bound to the specific pre-coated antibodies. Following this, a horseradish peroxidase (HRP)-conjugated antibody specific for beta-catenin was added to the wells, and the plate was incubated to allow binding interactions.

To ensure accuracy, all plasma samples were brought to room temperature before the assay. Standards were prepared in duplicate using a serial dilution protocol to achieve a range of concentrations (270 pg/mL to 15 pg/mL). The

detailed preparation included mixing defined volumes of the standard solution with the dilution buffer. Each dilution was conducted sequentially, with excess solution discarded as needed to maintain precise concentrations. One well was left blank as a control.

After sample addition, the plate was sealed with a closure membrane and incubated at 37°C for 30 minutes to promote binding. Subsequently, the concentrated washing buffer provided in the kit was diluted 30 times with distilled water. The closure membrane was carefully removed, and each well was washed five times by aspirating and refilling with the wash solution. During each washing step, the plate was allowed to rest for 30 seconds before the solution was discarded.HRP-conjugate reagent (50 µL) was added to each well except the blank control, and the plate was incubated again at 37°C for 30 minutes. Following incubation, the washing process was repeated to ensure the removal of unbound reagents. Chromogen Solution A (50 µL) and Chromogen Solution B (50 µL) were then added to the wells, and the plate was gently shaken to mix the contents. The plate was incubated at 37°C for 15 minutes in the dark to prevent interference with the color development.

To terminate the enzymatic reaction, 50 µL of stop solution was added to each well, which changed the color from blue to yellow. The absorbance of each well was measured at 450 nm using an ELISA reader, with the absorbance of the blank control well set to zero. The concentration of beta-catenin in each sample was calculated using a standard curve generated from the serially diluted standards. Sample concentrations were adjusted for the dilution factor used during the assay.

### *2.4 Measurement of Liver function*

#### *2.4.1 Assay of Aspartate Aminotransferase (AST)*

AST was measured according to Reitman and Frankel (1957) [33]. The incubation reaction contained 0.5 ml of AST substrate (0.1 M phosphate buffer pH 7.5, 0.002 M α-oxoglutarate and 0.2 M L-aspartic acid). The protein samples 0.1 ml were then added and the reactions were proceeded at 37°C for 60 minutes. Color forming reagent, 2,4-dinitrophenylhydrazine, was then added and the reactions tubes were left for 20 minutes at room temperature after which the alkaline reagent, 0.4 N NaOH was then added and left for 5 minutes. The absorbance was read at 505 nm against a blank solution containing distilled water.

#### *2.4.2 Assay of Alanine Aminotransferase (ALT)*

ALT was measured according to Reitman and Frankel (1957) [33]. The reaction mixture contained 0.5 ml of ALT substrate (0.1 M phosphate buffer pH 7.5, 0.002 M α-oxoglutarate and 0.2 M dL-alanine). The mixture was incubated at 37 °C for 5 minutes followed by the addition of 0.1 ml of sample after which the reaction was continued for 60 minutes at 37 °C. Color forming reagent (2,4-dinitrophenylhydrazine) was then added and left for 20 minutes at room temperature after which alkaline reagent 0.4 N NaOH was added and left for additional 5 minutes. The absorbance was read at 505 nm against blank solution containing distilled water.

### *2.5 RNA Extraction and Quantitative polymerase chain reaction*

Total RNA, including mRNA and lncRNA isolation from fresh whole blood samples (collected on K3EDTA) was carried out with the miRNeasy Mini Kit (QIAGEN, Maryland, USA) according to the manufacturer's instructions. (QIAGEN, Germany, cat. no.217004).

Cells were homogenized in QIAzol Lysis Reagent. After the addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase, while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase. The upper, aqueous phase is extracted, and ethanol is added to provide appropriate binding conditions for all RNA molecules from 18 nucleotides (nt) upwards. The sample is then applied to the RNeasy Mini spin column, where the total RNA binds to the membrane and phenol and other contaminants are efficiently washed away. High quality RNA is then eluted in RNase-free water.

Quantitative real-time PCR was performed using Thermo Scientific Maxima SYBR Green qPCR Master Mix (2X) provided by Thermo Fisher Scientific Inc. (catalog No. K0251) and Two primer sets were utilized to amplify the cDNA for mRNA c-Myc. In addition to that, GAPDH gene primers were used as an internal control. (Provided by Thermo Fisher Scientific Inc). Primer Sequence of c-Myc Forward primer; 5'-AGAGTTTCATCTGCGACCCG-3' Reverse primer 5'-GAAGCCGCTCCACATACAGT-3', GAPDH gene Forward primer; 5'-GAAGGTGAAGGTCGGAGTCAAC-3' Reverse primer 5'- CAGAGTTAAAAGCAGCCCTGGT-3'. RT-qPCR was programmed for an initial activation cycle of 50 °C for 2 min then 95 °C for 10 min; followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for c-Myc and GAPDH for 30 s and extension at 72 °C for 30 s

The expression of targeted genes was normalized to the reference gene GAPDH expression levels within the same sample to determine ΔCT. This step

serves to correct for non-treatment-related variation among wells such as potential differences in cell number. ΔCT was then normalized to the expression of the targeted gene in treated animals from a separate untreated sample to find ΔΔCT. % Expression was calculated by the equation (%Expression = $2^{-\Delta\Delta CT}$).

### *2.6 Statistical analysis*

Data were analysed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) [34]. Qualitative data were described using numbers and percentages. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. The significance of results was judged at the 5% level. Chi-square test, t-test, Mann Whitney test, Kruskal Wallis test

## 3. Results

### *3.1 Evaluating Kidney Function in AML Patients vs. Healthy Controls*

#### *3.1.1 Creatinine and Urea Analysis in AML Patients and Healthy Control*

This study evaluated kidney function in acute myeloid leukemia (AML) patients by analyzing serum creatinine and urea levels, comparing them to those of healthy controls. The results indicate significant increases in these markers among AML patients, suggesting potential renal dysfunction or metabolic disturbances associated with the disease (Table 1).

For creatinine levels, the median value in AML patients (n = 60) was 0.90 mg/dL, with an interquartile range (IQR) of 0.7–1 mg/dL. In contrast, the healthy control group (n = 30) exhibited a lower median creatinine level of 0.60 mg/dL, with an IQR of 0.6–0.8 mg/dL. This difference was statistically significant, with a p-value of <0.001. The elevated creatinine levels in AML patients point to potential kidney impairment.

Similarly, urea levels were significantly higher in AML patients compared to the control group. The median urea level in AML patients was 31.4 mg/dL, while the median level in the control group was 23.77 mg/dL. This difference was also highly significant, with a p-value of <0.001. Elevated urea levels may reflect altered renal function, increased protein breakdown, or a combination of factorssuch as the disease itself and treatment-induced metabolic stress. Elevated urea, in conjunction with increased creatinine, highlights the need to distinguish between pre-renal causes, such as dehydration or catabolic stress, and intrinsic kidney dysfunction.

Table 1: Creatinine and Urea in AML and control

| | AML patients (n = 60) | Control (n = 30) | P |
|---|---|---|---|
| **Creatinine (mg/dl)** | | | |
| **Min. – Max.** | 0.50 – 1.20 | 0.55 – 1.10 | 0.005* |
| **Median (IQR)** | 0.90 (0.7 – 1) | 0.65 (0.6 – 0.8) | |
| **Urea (mg/dl)** | | | |
| **Min. – Max.** | 8.0 – 68.48 | 15.0 – 35.0 | <0.001* |
| **Mean ± SD.** | 31.40 ± 10.65 | 23.77 ± 5.75 | |

IQR: Inter quartile range
p: p value for comparing between the two studied groups
*: Statistically significant at $p \leq 0.05$

#### *3.1.2 AST and ALT Analysis in AML Patients and Healthy Control*

The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was examined in patients with acute myeloid leukemia (AML) as compared to healthy controls. The results revealed significant elevations in both liver enzymes among AML patients, highlighting potential liver involvement or dysfunction (Table 2).

For ALT levels, the median in AML patients (n = 60) was 31.50 U/L, with an interquartile range (IQR) of 24.50–44.0 U/L. In contrast, the healthy control group (n = 30) had a significantly lower median ALT level of 16 U/L, with an IQR of 13–41.0 U/L. Statistical analysis showed a highly significant difference, with a p-value of <0.001. These findings suggest that AML patients experience increased ALT levels, potentially indicating liver stress or damage related to disease progression, metabolic disturbances, or side effects of chemotherapy.

Similarly, AST levels were also elevated in AML patients. The median AST level in the AML group was 29.0 U/L (IQR: 22–33.5 U/L), whereas the control group had a median level of 13.5 U/L (IQR: 10–17.0 U/L). Again, the difference was statistically significant, with a p-value of <0.001. Elevated AST levels further support the notion of liver dysfunction, which could stem from leukemic cell infiltration, systemic effects of AML.

Table 2: ALT and AST in AML and control

| | AML patients (n = 60) | Control (n = 30) | P |
|---|---|---|---|
| **ALT (U/L)** | | | |
| **Min. – Max.** | 17.0 – 65.0 | 10.0 – 41.0 | <0.001* |
| **Median (IQR)** | 31.50 (24.50 – 44.0) | 16.0 (13.0 – 20.0) | |
| **AST (U/L)** | | | |
| **Min. – Max.** | 16.0 – 88.0 | 8.0 – 25.0 | <0.001* |
| **Median (IQR)** | 29.0 (22.0 – 33.50) | 13.50 (10.0 – 17.0) | |

IQR: Inter quartile range
p: p value for comparing between the two studied groups
*: Statistically significant at $p \leq 0.05$

### *3.2 Evaluating Plasma β-catenin* levels *in AML Patients vs. Healthy Controls*

The plasma level of β-catenin in AML patients ranged between 48.20 to 447.2 pg/ml with a median of 78.70 pg/ml, whereas in healthy control group it ranged from 65.70 to 682.2 pg/ml with a median of 87.60 pg/ml (Table 3).

Plasma β-catenin was statistically significantly lower in AML patients compared to the control group. (p=0.009).

Table 3: Plasma β-catenin levels in AML and control

| | AML patients (n = 60) | Control (n = 30) | P |
|---|---|---|---|
| **β-catenin (pg/ml)** | | | |
| **Min. – Max.** | 48.20 – 447.2 | 65.70 – 682.2 | 0.009* |
| **Median (IQR)** | 78.70 (74.0 – 85.25) | 87.60 (76.0 – 152.1) | |

IQR: **Inter quartile range**
p: p value for comparing between the two studied groups
*: Statistically significant at $p \leq 0.05$

### *3.3 Evaluating Relative expression of mRNA c-Myc in AML Patients vs. Healthy Controls*

Relative expression of circulating mRNA c-Myc in AML patients ranged from 0.12 to 2.55 with a median of 0.8, while in the control group it ranged from 0.43 to 1.83 with a median of 1.0 and there was no statistically significant difference between the two groups (p=0.089). (Table 4)

Table 4: ALT and AST in AML and control

| | AML patients (n = 60) | Control (n = 30) | p |
|---|---|---|---|
| **$2^{-\Delta\Delta ct}$ c-Myc** | | | |
| **Min. – Max.** | 0.12 – 2.55 | 0.43 – 1.83 | 0.089 |
| **Median (IQR)** | 0.80 (0.45 – 1.28) | 1.0 (0.78 – 1.37) | |

IQR: Inter quartile range
p: p value for comparing between the two studied groups

### *3.4 Evaluating Plasma β-Catenin Levels in AML Subtypes: Comparison with Controls*

The β-catenin plasma protein levels were measured in AML M0, M1, M2 vs. M4, M5 compared to controls. Data analysis showed alteration in β-catenin levels with some significant differences Table 5.

β-catenin in AML patients M0, M1, and M2, ranged 48.20-139.5 pg/ml, with a median of 81.95 pg/ml. β-catenin in the M4 and M5 AML patients was ranging from 62.4 to 447.2 pg/ml, with a median of 77.35 pg/ml. β-catenin plasma levels were distributed between 65.70 to 682.2 pg/ml, with a median of 87.60 pg/ml. Date analysis indicates that while β-catenin levels varied within each group, the median levels in AML patients were

mostly lower than in control group.

Statistical analysis revealed that plasma β-catenin levels were significantly lower in the M4 and M5 AML patients in comparison to control group (p = 0.002).

No significant difference was found between β-catenin levels of M0, M1, and M2 AML patients and the M4 and M5 patients (p = 0.121). Similarly, the comparison between the M0, M1, and M2 and controls showed no significant difference (p = 0.181). This suggests that in AML M0-M2, β-catenin levels may not deviate from healthy individuals, showing a potential pattern in β-catenin dysregulation.

Table 5: Plasma β-catenin levels in AML subtypes and control

| | AML patients (n = 60) | | Control (n = 30) | P |
|---|---|---|---|---|
| | M 0/1/2 (n = 26) | M 4/5 (n = 34) | | |
| **β-catenin (pg/ml)** | | | | |
| Min. – Max. | 48.20 – 139.5 | 62.40 – 447.2 | 65.70 – 682.2 | |
| Median (IQR) | 81.95 (74.10 – 92.50) | 77.35 (74.0 – 80.80) | 87.60 (76.0 – 152.1) | 0.010* |
| **Sig. bet. groups.** | $p_1$=0.121, $p_2$=0.181, $p_3$=0.002* | | | |

IQR: **Inter quartile range**
p: p value for comparing between the two studied groups
*: Statistically significant at $p \leq 0.05$

### *3.5 Relative c-Myc mRNA Expression Across AML Subtypes and Controls*

The relative expression of c-Myc mRNA, was evaluated in AML patients M0, M1, M2 vs. M4, M5 compared to controls. In AML patients M0, M1, and M2 subtypes. The expression of circulating c-Myc mRNA ranged from 0.12 to 2.44, with a median of 1.10. c-Myc expression ranged from 0.21 to 2.55 in the M4 and M5 AML patients, with a low median of 0.61 (Table 6).

On the other hand c-Myc expression exhibited a narrower range of 0.43 to 1.83 among control, with a median of 1.0. The median value of controls is close to that of the M0, M1, and M2 . AML subtypes is more comparable to normal levels.

c-Myc expression was significantly downregulated in M4 & M5 AML compared with M0-2 AML group ($p_1$<0.001) as well as with the controls ($p_3$=0.002). No significant difference could be detected between M0, M1 & M2 AML group with the control group ($p_2$=0.560) (Table 5).

Table 6: Comparison of c-Myc mRNA Expression Between AML Subtypes and Healthy Controls

| | **AML patients (n = 60)** | | **Control (n = 30)** | **p** |
|---|---|---|---|---|
| | **M 0/1/2 (n = 26)** | **M 4/5 (n = 34)** | | |
| **$2^{-\Delta\Delta CT}$ c-Myc** | | | | <0.001* |
| **Min. – Max.** | 0.12 – 2.44 | 0.21 – 2.55 | 0.43 – 1.83 | |
| **Median (IQR)** | 1.10 (0.81 – 1.52) | 0.61 (0.38 – 0.82) | 1.0 (0.78 – 1.37) | |
| **Sig. bet. groups.** | $P_1$<0.001*, $p_2$=0.560, $p_3$=0.002* | | | |

IQR: Inter quartile range
p: p value for comparing between the three studied groups
p1: p value for comparing between M 0/1/2 and M 4/5
p2: p value for comparing between M 0/1/2 and Control
p3: p value for comparing between M 4/5 and Control
*: Statistically significant at $p \leq 0.05$

### *3.6 Clinical Implications of β-Catenin Expression in AML Demographics and Subtypes*

The expression of β-catenin was analyzed in 30 AML patients categorized into low (≤78.70 pg/ml) and high (>78.70 pg/ml) levels. β-catenin expression was analyzed based on age and its potential implications for AML Table 7.

Significant variation across age categories exists in the group with low β-catenin levels (≤78.70 pg/ml). In (26.7%) In the youngest age group, 18–30 years, 8 patientsexhibited low expressions among the three age groups, suggesting that younger AML patients may be less likely to experience reduced β-catenin activity. The middle age group, 30–45 years, had the highest proportion of low β-catenin expression, with 15 patients (50%) indicating that β-catenin downregulation is most prevalent among middle-aged AML patients. On the other hand, the oldest age group, 45–65 years represents the lowest prevalence of reduced expression.

In the group with high β-catenin levels (>78.70 pg/ml), the distribution was more consistent across all age categories, with 10 patients (33.3%) in each group exhibiting elevated β-catenin expression.

β-catenin expression among AML patients revealed that younger patients (18–30 years) showed a higher prevalence of high β-catenin expression, which may be associated with enhanced Wnt signaling activity and potentially different disease mechanisms. Interetingly, middle-aged patients (30–45 years) predominantly exhibited low β-catenin levels, suggesting a greater likelihood of impaired signaling pathways in this group. Older patients (45–65 years) demonstrated a more consistent distribution of high β-catenin expression, indicating a potential shift in the disease's molecular characteristics with age.

These findings emphasize AML heterogeneity and its correlation with age. Age-specific therapeutic approaches targeting β-catenin or associated pathways is necessary to optimize treatment for AML patients and their implications for disease progression and treatment responses.

Gender Distribution of β-Catenin Expression Levels in AML Patients

Distinctive patterns of β-catenin expressionbetween male and female patients, highlight additional insights into potential gender-related differences in AML biology Table 7.

In the low β-catenin expression group, 14 males (46.7%) and 16 females (53.3%) exhibited reduced levels of β-catenin. This relatively balanced distribution suggests that both genders are similarly affected by low β-catenin expression, with a slightly higher proportion of females showing reduced levels.

Conversely, in the high β-catenin expression group, the gender distribution was notably different. Among the 30 patients with elevated β-catenin levels, 10 males (33.3%) and 20 females (66.7%) exhibited high expression. This indicates a predominance of females with high β-catenin levels, representing a significant shift in gender distribution compared to the low-expression group. A potential gender-related variation in β-catenin expression in AML patients is suggested. Females are more likely to exhibit elevated β-catenin levels than males indicating that biological or molecular

differences in how β-catenin-related pathways contribute to AML in male and female patients.

Distribution of FAB Subtypes in AML Patients by β-Catenin Expression

The distribution of AML patients across different French-American-British (FAB) subtypes was analyzed concerning their β-catenin expression levels. Patients were categorized into two groups: low expression (≤78.70 pg/ml) and high expression (>78.70 pg/ml), with 30 patients in each group. The results demonstrated distinct patterns in FAB subtype representation between the two expression groups table 6.

The majority of patients in the M5 subtype fall in the low β-catenin expression group, with 13 individuals (43.3%). This indicates a strong association between low β-catenin expression and the monocytic form of AML. The M1 subtype was the second most prevalent, comprising 8 patients (26.7%), suggesting that immature myeloblast-dominant forms are also frequently characterized by low β-catenin levels. The M4 subtype was present in 7 patients (23.3%), while the M0 and M2 subtypes were rare, each represented by only one patient (3.3%).

Conversely, in the high β-catenin expression group, the distribution shifted notably. The M1 subtype was the most prevalent, with 12 patients (40.0%), indicating that elevated β-catenin expression is more commonly associated with immature myeloblast forms of AML. The M5 subtype was the second most common, comprising 8 patients (26.7%), suggesting that the monocytic form can also exhibit high β-catenin levels but to a lesser extent compared to the low-expression group. The M4 subtype was represented by 6 patients (20.0%), while the M2 subtype included 3 patients (10.0%). The M0 subtype remained rare, with only one patient (3.3%) in this group as well.

A comparison of the two groups highlights important differences. In the low-expression group, the M5 subtype predominated, whereas in the high-expression group, the M1 subtype was the most common. This suggests a potential link between β-catenin expression levels and the molecular and biological characteristics of these specific FAB subtypes. The M4 subtype showed relatively consistent representation across both groups, while the M0 subtype was rare in both.

These findings highlight the potential role of β-catenin in the pathogenesis of AML subtypes. The predominance of M5 subtype in the low-expression group and M1 subtype in the high-expression group may indicate subtype-specific mechanisms of β-catenin regulation and its involvement in disease progression.

Table 7: Association Between β-Catenin Expression and AML Patients Demography

| | **β-catenin** | | | | | |
|---|---|---|---|---|---|---|
| | Low (≤78.70 pg/ml) (n = 30) | | High (>78.70 pg/ml) (n = 30) | | $\chi^2$ | p |
| | No. | % | No. | % | | |
| **Age (/years)** | | | | | | |
| **18 – 30** | 8 | 26.7 | 10 | 33.3 | 1.752 | 0.417 |
| **>30 – 45** | 15 | 50.0 | 10 | 33.3 | | |
| **>45 – 65** | 7 | 23.3 | 10 | 33.3 | | |
| **Sex** | | | | | | |
| **Male** | 14 | 46.7 | 10 | 33.3 | 1.111 | 0.292 |
| **Female** | 16 | 53.3 | 20 | 66.7 | | |
| **FAB** | | | | | | |
| **M0** | 1 | 3.3 | 1 | 3.3 | 3.238 | $^{MC}$n= 0.570 |
| **M1** | 8 | 26.7 | 12 | 40.0 | | |
| **M2** | 1 | 3.3 | 3 | 10.0 | | |
| **M4** | 7 | 23.3 | 6 | 20.0 | | |
| **M5** | 13 | 43.3 | 8 | 26.7 | | |

$\chi^2$: Chi square test MC: Monte Carlo
p: p value for comparing between Low (≤78.70) and High (>78.70)
*: Statistically significant at $p \leq 0.05$

### *3.7 Clinical Implications of c-Myc Relative Expression in AML Demographics and Subtypes*

The study was conducted on the c-Myc expression levels in the blood of 60 patients with acute myeloid leukemia who were then divided into two categories: high expression (n = 30, >80 pg/ml) or low expression (n = 30, ≤80 pg/ml) according to their level respective levels of the c-Myc proto-oncogene. The patients were further divided based on their age which was classified to the range of 18-30 years, 30-45 years, and 45-65 years Table 8.

In those with c-Myc low, the patients were evenly distributed among different age groups. In the youngest age category 18-30 years, there are 11 patients (36.7%) who continue to show low levels of c-Myc gene. There are also 11 patients aged between 30-45 years who present low levels of c-Myc (36.7%) and these characteristics are consistent with those observed in the younger age category. Though there were some 8 patients (26.7%) aged between 45-65 years showing low levels of c-Myc, the number depicting low levels of the c-Myc increased over age.

On the other hand, for the group characterized by high c-Myc expression, a completely different pattern emerged, the one with middle aged andolder groups being the predominant ones. Of the 18–30 years age group, only 23 percent, that is 7 patients had c-Myc high expression which was the least performing category in the high expression group. In contrast, the 30–45 years age group had the highest proportion of patients with c-Myc levels

over 14 individuals or 46.7 % of the total, with the implication that there is a significant increase of high level in this age bracket. In the age bracket of 45 – 65 years, 30 percent or 9 patients had high c, myc levels which also shows that there is moderate expression still in this group.

The two expression groups are able to show those significant differences regarding the age distribution of c-Myc c levels in this cohort. For the low expression group, there was fairly similar distribution across different age groups. For the high expression group there was a relative bulk of patients in the age category 30 years to 45 years, compared to the rest of the ages presented in the study which were younger patients aged 18-30 years, with the older category suffering more with quite low c-Myc levels.

Evaluating c-Myc s among the AML patients, it was noted that male and female patients were significantly different in the low and the high-expression groups. Of the 30 patients with low c-Myc levels (≤80 pg/ml), 14 were males (46.7%) while females were 16, slightly higher females in numbers with low expression levels. On the other hand, in the c-Myc over-expressing patients (>80 pg/ml), dysregulation showed: 10 males (33.3%) and 20 females (66.7%) had elevated c-Myc levels. It can thus be stated that there is a greater proportion of females with high c-Myc expression levels as compared to males which indicates that the distribution of c-Myc expression in AML may be influenced by gender substantiates further these differences need to be explored in the context of their clinical relevance Table 8.

The patients with AML were classified against the French-American-British (FAB) classification system to determine the germinal centers expression levels of c-Myc. It was observed that there exist some differences that marked differences between the low-expression (≤80 pg/ml) group and the high-expression (>80 pg/ml) group. Each group had a population of 30 patients.

Patients in the low c-Myc expression group 46.7% of 30 had low c-Myc expression levels, with 13 people belonging to the M4 subtype. This indicates a strong link between the c-Myc protein and levels of the M4 subtype. This was also true for the M5 subtype which consisted of monocytic AML patients constituting 11 patients and low levels of c-Myc expression although this group only accounted for 27.9% of the patients. M1 and M2 were present but accounted for a small percentage among the group accounting for only 10%. What is most notable is that this group reported no patients with M0 subtype, this may be attributed to the fact that the case has an immature c-Myc expression form.

On the other hand, there was a much different distribution of FAB subtypes when looking at the patients with high c-Myc expression. The M1 subtype was the most common subtype in the sample with 17 patients (56.7%) which shows a clear association between high c-Myc expression and this form of AML that is predominant in immature myeloblasts. The M5 subtype is ranked as the second most common type with 10 patients (33.3%) which suggests that this monocytic forms also has c-Myc overexpression, although to a lesser degree than M1. Of interest is that only 1 of the M2 patients (n = 1, 3.3%) had c-Myc overexpression and 2 patients (n = 2, 6.7%) had M0 type features which show that these types relatively have low levels. Strikingly, no patients were belonging to M4 subtype in the high-expression group and this further supports the specific relationship between this subtype and c-Myc expression levels Table 8.

The comparison between the two groups underscores significant differences in the association of c-Myc expression with FAB subtypes. The M4 subtype, which dominated the low-expression group, was absent from the high-expression group, suggesting a subtype-specific suppression of c-Myc expression. Conversely, the M1 subtype, which was relatively uncommon in the low-expression group, became the dominant subtype in the high-expression group, pointing to a potential role of c-Myc in the pathogenesis or progression of this subtype. The M5 subtype showed a notable presence in both groups, indicating that its association with c-Myc expression may vary depending on additional clinical or molecular factors.

Table 8: Association Between c-Myc Relative Expression and AML Patients Demography

| | $2^{-\Delta\Delta CT}$ c-Myc | | | | | |
|---|---|---|---|---|---|---|
| | Low (≤0.80) (n = 30) | | High (>0.80) (n = 30) | | $\chi^2$ | P |
| | No. | % | No. | % | | |
| **Age (/years)** | | | | | | |
| 18 – 30 | 11 | 36.7 | 7 | 23.3 | 1.308 | 0.520 |
| >30 – 45 | 11 | 36.7 | 14 | 46.7 | | |
| >45 – 65 | 8 | 26.7 | 9 | 30.0 | | |
| **Sex** | | | | | | |
| Male | 14 | 46.7 | 10 | 33.3 | 1.111 | 0.292 |
| Female | 16 | 53.3 | 20 | 66.7 | | |
| **FAB** | | | | | | |
| M0 | 0 | 0.0 | 2 | 6.7 | 27.830* | $^{MC}p$ <0.001* |
| M1 | 3 | 10.0 | 17 | 56.7 | | |
| M2 | 3 | 10.0 | 1 | 3.3 | | |
| M4 | 13 | 43.3 | 0 | 0.0 | | |
| M5 | 11 | 36.7 | 10 | 33.3 | | |

p: p value for comparing between Low (≤0.80) and High (>0.80)
*: Statistically significant at $p \leq 0.05$

## 4. Discussion

Acute Myeloid Leukemia is a disease of complex heterogeneity characterized by a wide range of genetic abnormalities and varying morphology, immunophenotypes and clinical outcomes. AML is regarded as the most prevalent form of acute leukemia in adults and the leading cause of leukemia-related mortality in adults [35-37].

Despite multiple factors present at the time of AML diagnosis that may help to predict the disease's clinical outcome; these factors are still far from being satisfactory to help with AML prognosis and prediction of long-term remission or relapse [38].

A Beta-catenin was shown to be significantly lower in AML patients compared to healthy controls. By further classifying patients according to FAB criteria; into the immature M0, M1 and M2 group and the more differentiated M4 and M5 with higher monocytic elements, β-catenin was statistically significantly lower in M4 and M5 AML subtypes compared to healthy controls.

A study by Ysebaert *et al*., 2006 [18] showed that beta-catenin mRNA was constantly expressed, irrespective of protein levels, which suggested a translational or post-translational regulation. It also showed that in normal hematopoiesis beta-catenin is strongly expressed in normal CD34+ cells, but is rapidly lost at a post-transcriptional level upon differentiation even if the chief activator of Wnt/beta-catenin pathway (Wnt3a or Wnt5a) is added to the medium.

However, their data showed that downregulation of β-catenin is achieved in CD34+/low 38+33+ immature progenitors and is also specific to myelomonocytic differention. Also, their results showed that beta-catenin expression is dependent on the type of cell maturation, example in erythroid differentiation, beta-catenin could be re-expressed. Moreover, Beta-catenin protein was expressed at varying levels and there was a significant difference in the expression of beta-catenin among AML FAB subtypes, where β-catenin was preferentially expressed in monocytic M4 & M5 rather than in mmature M0, M1 & M2 AML. Our findings was inconsistent with the previous data where β-catenin showed variable levels in AML patients, but there was no significance between AML FAB subtypes. However, β-catenin was found to be significantly lower in M4 & M5 AML patients (with higher monocytic element) compared to healthy controls.

In our study, expression of mRNA c-Myc, a well-known target of the WNT pathway, showed no statistically significant difference between AML patients and controls. However, there was a statistically significant difference in c-Myc expression between M0, M1 & M2 group and M4 & M5 and a statistically significant difference between M4 & M5 group and controls. However, there was no difference in c-Myc expression between AML patients with normal and abnormal cytogenetics.

Similar to our study, a study by Krygier *et al*.,(2020)[39] studied c-Myc expression in AML and their study found a difference in c-Myc expression among FAB subtypes. Our results was in concordance with the study regarding no relation was found between patient mortality and c-Myc expression. However, their study found a relatively high expression was often noted in the M5 subtype, whereas in our study, low expression level was noted in M4 and M5 groups, which is in agreement to a study by Ohanian *et al*., (2019) [40] which observed low c-Myc expression levels in acute monoblastic/monocytic leukemia.

## 5. Conclusion

This study underscores the heterogeneity of AML and the subtype-specific roles of β-catenin and *c-Myc* in disease progression. The observed low β-catenin levels in middle-aged and M5-subtype patients may reflect impaired Wnt signaling, while variability in *c-Myc* expression highlights its complex role in AML pathogenesis. These findings suggest that therapeutic strategies targeting β-catenin and *c-Myc* should account for differences in FAB subtypes, age, and gender. Further researchinto these molecular pathways is essential to improve diagnostic and prognostic approaches for AML.

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