

Quantitative expression of the eukaryotic translation initiation factor 4E (eIF4E) in Egyptian acute leukemia patients and its clinical significance

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Original Article

Abstract

Purpose: The eukaryotic translation initiation factor eIF4E is part of the eIF4F protein complex, which includes, in addition to eIF4E, eIF4G (a scaffolding protein) and eIF4A (an ATP-dependent RNA helicase). The eukaryotic translation initiation factor eIF4E is a potent oncogene elevated in many cancers including leukemias. **Methods:** In this study, the expression level of eIF4E gene was analyzed in 20 normal healthy controls and 64 patients with de novo acute leukemia (33 Acute myeloid leukemia (AML) and 31 Acute lymphoblastic leukemia (ALL)) using a real-time quantitative reverse-transcriptase polymerase chain reaction (RTQ-PCR) to investigate a possible relation, association or correlation with the clinical features at diagnosis, such as age, gender, lineage, hemoglobin (Hb), total leucocytic count (TLC), platelet count and bone marrow (BM) blast cell infiltration as well as its effect on patients' outcome. **Results:** Comparing AML and ALL patients as regards their clinical and laboratory data showed no statistical significance for TLC and hemoglobin ($p = 0.838$ and 0.920 , respectively) but was of statistically significant difference for platelets ($p = 0.022$) and bone marrow blasts percentage ($p = 0.007$). Comparison between the 2 groups as regards eIF4E level was of no statistically significant difference, p -value being ($p = 0.257$) but there was statistically significant difference between eIF4E expression level in AML/Control ($p = 0.002$) and ALL/Controls ($p = 0.025$). Analysis of overall survival (OS) time and disease free survival (DFS) in each group and its relation to eIF4E gene showed no statistical significance ($p = 0.843$ and 0.310 , respectively) in AML group and ($p = 0.971$ and no p -value for DFS in ALL as all cases remained alive except for one case while 3 cases were relapsed) in ALL group. Correlation studies showed no significant correlation between AML group and eIF4E gene level as regards age, TLC, hemoglobin and platelets ($r = -0.064$, $p = 0.722$; $r = 0.062$, $p = 0.732$; $r = 0.068$, $p = 0.712$; and $r = -0.318$, $p = 0.071$) respectively, while there was significant positive moderate correlation on comparing bone marrow blast% and eIF4E gene level ($r = 0.545$ and $p = 0.001$). There was no significant correlation between ALL group and eIF4E gene level as regards age, TLC, hemoglobin, platelets and bone marrow blasts% ($r = -0.214$, $p = 0.248$; $r = 0.175$, $p = 0.347$; $r = -0.056$, $p = 0.766$; $r = -0.072$, $p = 0.700$; and $r = -0.0004$, $p = 0.983$) respectively. **Conclusion:** eIF4E was found to be elevated in acute leukemia patients in relation to normal controls and its levels were more in myeloid than lymphoid leukemia and positively correlated with the blast percentage in AML thus its level may contribute to leukemogenesis. eIF4E levels and translation initiation may be an attractive target for anticancer therapeutics.

Keywords: eIF4E, AML, ALL, RTQ-PCR

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1. Introduction

The eukaryotic translation initiation factor 4E (eIF4E) is frequently overexpressed in human cancers in relation to disease progression and drives cellular transformation, metastatic progression and tumorigenesis in experimental models. Enhanced eIF4E function results from eIF4E overexpression and/or activation of the ras and phosphatidylinositol 3-kinase/AKT pathways and selectively increases the translation of key mRNAs involved in tumor growth, angiogenesis, and cell survival. Targeting eIF4E for inhibition may provide an attractive therapy for many different tumor types.¹

eIF4E overexpression has been demonstrated in human tumors and has been related to disease progression. Overexpression of eIF4E in experimental models changes cellular morphology, enhances proliferation and induces cellular transformation, tumorigenesis and metastasis. On the contrary, blocking eIF4E function by expression of antisense RNA, or overexpression of the inhibitory eIF4E binding proteins (4E-BPs), inhibits cellular transformation, tumor growth, invasiveness and metastasis.²

eIF4E is a well-established proto-oncogene; whose expression and activation is associated with transformation and tumorigenesis. MnKs (MAP (mitogen activated protein) kinase interacting kinases) phosphorylate Eif4E at a single site. This phosphorylation is implicated in cell transformation, tumorigenesis and tumor progression.³

Abnormal translation of mRNAs frequently occurring during carcinogenesis is among the mechanisms that can affect the expression of proteins involved in tumor development and progression. eIF4E is a key regulator of translation of many cancer-related transcripts and its expression is altered in various cancers and was associated with worse survival.⁴

Regulation of mRNA translation is an important regulatory step in gene expression. During embryonic development, mRNA translation is tightly regulated to produce the protein at the right place and time. eIF4E is a major target for the regulation of cap-dependent translation, that plays a key role during embryogenesis including gametogenesis, fertilization and establishment of embryonic axes.⁵

Protein synthesis is a complex, tightly regulated process in eukaryotic cells and its deregulation is a hallmark of many cancers. Translational control occurs primarily at the rate-limiting initiation step, where ribosomal subunits are recruited to template mRNAs through the concerted action of several eukaryotic initiation factors (eIFs). One factor that interacts with both the mRNA and ribosomes, and appears limiting for translation is eIF4F, a complex composed of the cap-binding protein, eIF4E;

the scaffold protein, eIF4G; and the ATP-dependent DEAD-box helicase, eIF4A.⁶

Many studies aimed to compare the expression level of eIF4E in patients with leukemia and normal controls, and to explore its role in leukemogenesis. The mRNA and protein expressions of eIF4E were detected by QT-PCR and the absolute expression level of eIF4E mRNA and its protein expression were up regulated in most leukemia patients, which may play an important role in leukemogenesis, so the eIF4E may be a promising target for leukemia therapy and eIF4E-targeted therapy may be an option.⁷

This study aimed to investigate the expression level of eIF4E gene in acute leukemia patients using QRT-PCR to elucidate any possible relation or correlation with patients' clinical and laboratory data and its effect on patients' outcome.

2. Methods and Materials

2.1. Patients and controls series

Sixty-four Egyptian patients with acute leukemia were included in this study. Patients were recruited from Beni-suef University hospital, Health Insurance hospital, Beni-Suef governorate and Cairo university hospitals, Egypt between December 2013 and December 2014. Informed consent was taken from all contributors prior to their inclusion in the study. All work was performed in accordance with the ethical standards of the 2008 declaration of Helsinki.⁸

Patients were 33 acute myeloid leukemia (AML) and 31 acute lymphoblastic leukemia (ALL), 39 male and 25 females. All the patients were newly diagnosed and did not receive any treatment.

Twenty age and sex matched healthy individuals with normal laboratory findings were included as a control group. They were 9 male and 11 females. Their age ranged between 10-45 years with a mean of 23.2±12.4 years. Diagnosis of acute leukemia was based on (1) morphologic findings from Giemsa stained smears of bone marrow (BM) aspirates, (2) cytochemical stains criteria such as negativity for myeloperoxidase (MPO) and Sudan Black B (SBB) in cases of acute lymphoblastic leukemia [ALL] or their positivity in cases of acute myeloid leukemia [AML] and positivity for acid phosphatase in T-cell acute lymphoblastic leukemia [T-ALL] and (3) immunophenotyping criteria as CD10+/- CD19+, CD20+, CD22+ for B-ALL, CD2+/-, CD3+, CD5+/-, CD7+ for T-ALL, and positivity of CD13 and CD33 for AML cases. A complete blood count and a differential count including blast cell percentage were done for all patients. Peripheral blood (PB) samples and bone marrow (BM) aspiration samples were collected at diagnosis from the 64 Egyptian acute leukemia patients,

while peripheral blood samples were obtained from the control group.

eIF4E gene was analyzed in patients and controls using real-time quantitative reverse transcriptase polymerase chain reaction (RTQ-PCR) to study mRNA expression levels.

2.2. RNA isolation and real-time quantitative RT-PCR for eIF4E

PB and BM mononuclear cells (MNCS) were isolated at diagnosis by Ficoll density gradient centrifugation. Total RNA was extracted from MNCs using a QIAamp RNA Blood Kit (Qiagen, Germany) according to manufacturer's instructions. Complementary DNA (cDNA) was synthesized using (dt) 15-mer primer by Superscript III Reverse Transcriptase and stored at -20°C till use.

The mRNA expression levels of eIF4E gene and GAPDH (endogenous control) were measured by quantitative RT-PCR using an ABI PRISM 7000 Sequence Detector System (Applied Biosystems, Foster City, CA). The quantitative RT-PCR amplification was performed using the predeveloped assays-on-demand Gene Expression Set for the eIF4E (no 4532182, ID: NCT 00559091) and TaqMan GAPDH control reagents (Applied Biosystems) with the TaqMan Universal PCR Master Mix (Applied Biosystems).

All reactions were performed in duplicate using 20 µl samples containing 50 ng cDNA. The reaction protocol used involved heating for 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of amplification (15 s at 95°C and 1 min at 60°C). Analysis was performed using ABI PRISM 7000 Sequence Detection Software (Applied Biosystems).

The expression levels of eIF4E gene in tested samples were expressed in the form of CT (cycle threshold) level; then normalized copy number (relative quantitation) was calculated using the $\Delta\Delta CT$ equation as follows: $\Delta\Delta CT = \Delta CT$ of case - ΔCT of control, then the normalized copy number (relative quantitation) = $2^{-\Delta\Delta CT}$. A negative control without template was included in each experiment.

Expression level of eIF4E was correlated with the clinical and laboratory features of the studied patients at diagnosis including: age, sex, organomegaly, lymphadenopathy, total leucocytic count (TLC), hemoglobin (Hb), platelet count, lineage and blast cell percentage at time of diagnosis, as well as the overall survival, disease free survival and complete remission rate after treatment.

2.3. Treatment of AML patients

AML patients were treated according to Department of Oncology, Cairo University. All patients received

induction chemotherapy 7-3 protocol consisted of a course of 12mg/m² novantrone on day 1,3 and 5; Ara-C 100mg/m² continuously every 12 hours from day 1through 7.If the patient did not enter into remission, this protocol was repeated. If no or minimal response, patients were shifted to high dose chemotherapy. Patients who entered into remission received 4 courses of high dose Ara C as consolidation. This was Ara-C 2g/m² on 2 hours infusion every 12 hours day 1, 3 and 5.⁹

2.4. Treatment of ALL patients:

ALL patients were treated according to the treatment protocol adopted from the total therapy study XV for standard/high risk ALL of St. Jude Children's Research Hospital (SJCRH). It included 6 weeks of induction, 8 weeks of consolidation, and continuation therapy of 120 weeks for girls and 146 for boys.¹⁰

2.5. Assessment of the response to induction chemotherapy

By the end of induction therapy, complete remission (CR) status was defined as a normocellular bone marrow (BM) containing less than 5% blasts and showing evidence of normal maturation of other marrow elements. Peripheral blood regeneration was not a requirement, but 97% of cases defined as CR achieved a neutrophil count of $1 \times 10^9/L$ and a platelet count of $100 \times 10^9/L$.

Remission failures were classified as either partial remission (defined as 5-15% blasts or <5% blasts but a hypocellular BM), resistant disease (RD) >15% blasts in the BM, or induction death (ID) (i.e: related to treatment or hypoplasia). Overall survival (OS) end points were measured from the date of diagnosis to death or last follow up.¹¹

2.6. Statistical methods

Data was analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Pearson and Spearman-rho methods were used to test correlation between gene and numerical variables. Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. Relation of survival with the gene was done using Cox-regression method. All tests were two-tailed. A *p*-value < 0.05 was considered significant.

3. Results

The present study was conducted on 64 acute leukemia patients (33 AML and 31 ALL), clinical and laboratory

characteristics of which were presented in Table 1. Twenty normal healthy volunteers age and sex matched served as a control group.

They were 39 male patients (21 AML&18 ALL) and 25 female patients (12 AML &13 ALL), while controls were 9 males and 11 females. The AML cases were classified according to FAB classification : 2 case M0 (6%), 8 M1(24.2%), 15 M2 (45.5%), 4 M3(12.1%), 1 M4(3%),1 M5(3%), 2 M7 (6.1%) while the ALL cases were 3 cases pre B (9.7%), 1 pro B (3.2%), 1 C ALL (3.2%), 3 TALL

(9.7%), 7 L1 (22.6%), 14 L2 (45.2%), 2 early T (6.5%). AML and ALL patients characteristics in comparison to eif4 gene expression levels and its significance were presented in Tables 2 and 3, respectively.

Comparing AML and ALL patients as regards their clinical and laboratory data showed no statistical significance for TLC and hemoglobin ($p=0.838$ and 0.920) respectively, but was of statistically significant difference for platelets ($p = 0.022$) and bone marrow blasts percentage ($p = 0.007$).

Table 1: Clinical and Laboratory data of the AML &ALL patients.

	AML n = 33	ALL n = 31
Age(years)		
mean±SD	31.4±15.5	17.6±17.7
median	35	12
range	8-66	7.0-64
Sex (n,%)		
Male	21 (63.6%)	18 (58.1%)
Female	12 (36.4%)	13 (41.9%)
TLCX10 ³		
mean±SD	33.4±32.9	44.2±52.3
median	24.4	22.9
range	1.0-119.0	1.3-213.0
Haemoglobin(gm/dl)		
mean±SD	7.7±2.4	7.4±2.6
median	7.4	7.3
range	3.5-13.9	2.3-14.0
PlateletsX 10 ³		
mean±SD	43.8±48	75.9±65.3
median	2.0	62.0
range	2-244	6.0-296.0
B.M Blast (%)		
mean±SD	72.18±29.7	79.6±25.9
median	90	92
range	15-98	20-100
CSF (%)		
Negative	33(100%)	26(83.9%)
positive	0(0%)	5(16.1%)
Hepatomegaly (n%)		
Negative	18(54.5%)	13(41.9%)
Positive	15(45.5%)	18(58.1%)
Splenomegaly (n%)		
Negative	13(39.4%)	5(16.1%)
Positive	20(60.6%)	26(83.9%)
Lymphadenopathy		
Negative	19(57.6%)	5(16.1%)
Positive	14(42.4%)	26(83.9%)
Response		
CR (n %)	18 (54.5%)	30(96.8%)
No CR (n %)	15 (45.5%)	1(3.2%)
OS	24(72.7%)	30(96.8%)
DFS	18(54.4%)	27(87.1%)
Eif4e level		
mean±SD	74.407±195.103	27.741±96.739
median	0.206	0.0032
range	0.003-930.847	0.-504.951

Table 2: AML Patient characteristics and eif4e expression levels in different clinical groups.

Group	No. of patients	Eif4e level (mean ± SD)	Range	p-value
Sex (n,)				
Male	21	113.208±237.497	0.005-930.84	*0.016
Female	12	6.506±16.637	0.003-57.900	
B.M Blast (%)				
<90	11	0.064±0.129	0.003-0.409	*<0.001
≥90	22	111.579±231.650	0.022-930.84	
Hepatomegaly (n%)				
Negative	18	102.129±234.497	0.004-930.84	0.343
Positive	15	41.141±134.798	0.003-525.48	
splenomegaly (n%)				
Negative	13	89.490±255.776	0.004-930.84	0.730
Positive	20	64.603±150.114	0.003-525.48	
Lymphadenopathy				
Negative	19	16.328±34.298	0.004-133.06	0.483
Positive	14	153.229±283.636	0.003-930.84	

*p-values are significant <0.05

Table 3: ALL Patient characteristics and eif4e expression levels in different clinical groups.

Group	No. of patients	Eif4e level (mean ± SD)	Range	p-value
Sex (n,)				
Male	18	19.255±50.259	0.00-166.572	0.622
Female	13	39.490±139.857	0.001-504.95	
B.M Blast (%)				
<90	8	0.137±0.366	0.00-1.043	
≥90	23	37.342± 111.3011	0.003-504.95	0.010
CSF (%)				
Negative	26	13.086±42.438	0.00-166.572	0.280
positive	5	103.947±224.225	0.007-504.95	
Hepatomegaly (n%)				
Negative	13	0.825±1.797	0.00-6.195	0.242
Positive	18	47.180±124.734	0.00-504.951	
splenomegaly (n%)				
Negative	5	102.730±229.861	0.033-504.95	0.071
Positive	26	13.320±42.416	0.00-166.572	
Lymphadenopathy				
Negative	5	102.446±225.020	0.033-504.95	0.103
Positive	26	13.374±42.401	0.00-166.572	

*p-values are significant <0.05

Table 4: Eif4e gene expression levels in the 3 studied groups and statistical comparison between them.

Eif4e gene level	Control N=20	AML n=33	ALL n=31	p-Value
Level (RQ)				
mean±SD	0.0984±0.222	74.407±195.103	27.741±96.739	P1=0.257
median	0.0101	0.206	0.0032	*P2=0.002
range	0.01-1.0	0.003-930.847	0.-504.951	*P3=0.025

*p-values are significant <0.05

Table 5: Relationship between patient outcome and Eif4e gene expression.

Group	AML n=33	ALL n=31	p-value
Overall survival (event)	24(72.7%)	30(96.8%)	P1 =0.971
Disease free survival (event)	18(54.4%)	27(87.1%)	P2=0.843
			P3=0.310
			P4= no p-value

*p-values are significant <0.05

P1: Eif4e gene/OS in AML

P2: Eif4e gene/OS in ALL

P3: Eif4e gene/DFS in AML

P4: Eif4e gene/DFS in ALL (no p-value as all cases remain free except the case who died early and 3 relapsed cases)

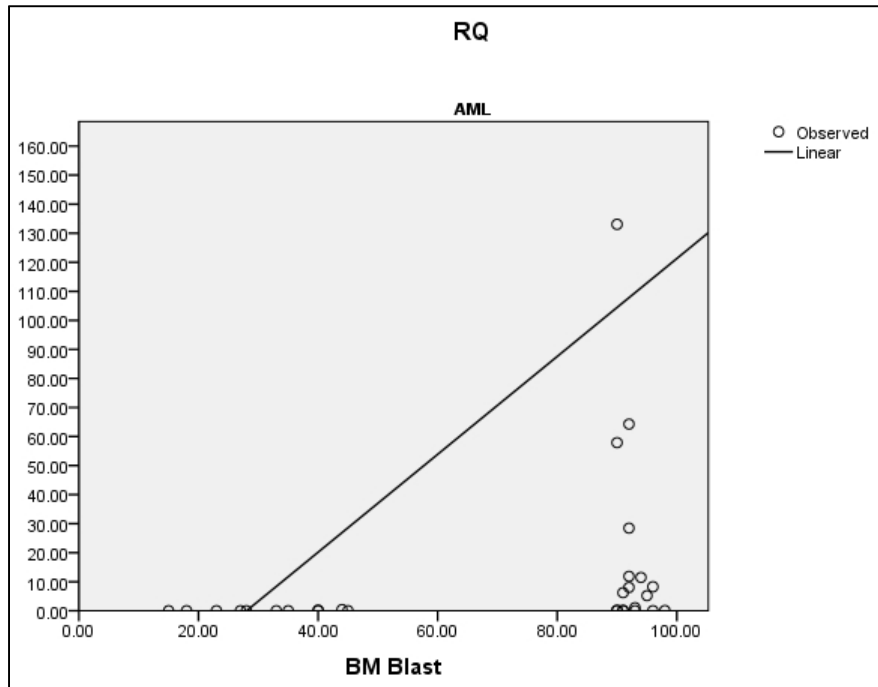


Figure 1: Correlation between Eif4e expression level and bone marrow blasts in AML patients.

Comparison between the 2 groups as regards Eif4e level was of no statistically significant difference, p -value being ($p = 0.257$) but there was statistically significant difference between Eif4e expression level in AML/Control ($p = 0.002$), ALL/Controls ($p = 0.025$) (Table 4). Response to treatment of AML cases showed that post induction chemotherapy 18/33 (54.5%) entered complete remission (CR) while 14/33 (45.5%) did not enter CR overall survival (OS) time was of mean 11.43 ± 7.83 months, with median 13.03 and range (0.66-22.86) months, While the disease free survival (DFS) time was of mean 11.01 ± 7.83 months with median 13.09 and range (0.3-22.34) months in AML.

While response to treatment of ALL cases showed that 30/31 (96.8%) entered in CR and only 1/31 (3.2%) did not enter CR. Overall survival (OS) time was of mean 23.47 ± 9.09 months, with median 23.06 and range (3.39-52.37) months. While the disease free survival (DFS) time was of mean 23.90 ± 8.50 months with median 24.29 and range (9.38-51.74) months in ALL.

Also analysis of OS and DFS in each group and its relation to Eif4e gene showed no statistical significance ($p = 0.843$ and 0.310 , respectively) in AML group and ($p = 0.971$ and no p -value for DFS in ALL as all cases remained alive except for one case who died and 3 relapsed cases) in ALL group (Table 5).

Correlation studies showed no significant correlation between AML group and Eif4e gene level as regards age, TLC, hemoglobin and platelets ($r = -0.064$ $p=0.722$, $r = 0.062$ $p = 0.732$, $r = 0.068$ $p = 0.712$ and $r = -0.318$ $p = 0.071$) respectively, while there was significant positive moderate correlation on comparing bone marrow blast% and Eif4e gene level ($r = 0.545$ and $p = 0.001$) (Figure 1)

There was no significant correlation between ALL group and Eif4e gene level as regards age, TLC, hemoglobin, platelets and bone marrow blasts% ($r = -0.214$ $p=0.248$, $r = 0.175$ $p = 0.347$, $r = -0.056$ $p = 0.766$, $r = -0.072$ $p = 0.700$ and $r=-0.0004$ $p= 0.983$) respectively.

4. Discussion

The eukaryotic translation initiation factor 4E (eIF4E) is a potent oncogene which is elevated in about 30% of human cancers including the M4 and M5 subtypes of Acute Myelogenous Leukemia (AML) and in blast crisis, but not chronic phase of chronic myeloid leukemia (CML).¹² eIF4E overexpression leads to increased proliferation, oncogenic transformation, evasion of apoptosis, tumor invasion and metastases.¹²⁻¹⁴ eIF4E interacts with the methyl-7-guanosine cap moiety on the 5' end of mRNAs and via this activity plays a central role in cap dependent translation and in nucleo-cytoplasmic export of a subset of transcripts encoding proteins involved in cellular growth, survival and transformation such as Cyclin D1, vascular endothelial growth factor (VEGF), Mcl1, c-myc, and Pim1.¹⁵ Both the export and translation activities of eIF4E contribute to its transformation potential.¹⁶ Depletion of eIF4E in cancer cells using anti-sense oligonucleotides, siRNA or pharmacological inhibitors leads to cell cycle arrest and decreased tumorigenicity.¹⁷⁻²⁰ eIF4E is a downstream eukaryotic translation initiation factor of the PI3K/AKT/mTOR pathway, and is highly elevated in the M4 and M5 subset of AML at both the RNA and protein levels.²¹ eIF4E acts as both a key translation factor and as a promoter of nucleocytoplasmic transport of specific transcripts, its transformation capacity in vivo is attributed to its role in translation initiation in the cytoplasm.²²

In the present study the expression of eIF4E mRNA was measured by QRT-PCR in 64 de novo AL patients in comparison with 20 healthy control subjects, to find a possible relation or correlation with the clinical and laboratory features of AL patients at diagnosis, such as age, gender, lineage (AML or ALL), Hb, TLC, platelets count and bone marrow blast cell infiltration as well as patients outcome.

Comparing AML and ALL patients as regards their clinical and laboratory data showed no statistical significance for TLC and hemoglobin with p value being (0.838 and 0.920) respectively, but was of statistically significant difference for platelets ($p = 0.022$) and bone marrow blasts ($p = 0.007$).

Comparison between the 2 groups as regards Eif4e level was of no statistically significant difference, p-value being ($p = 0.257$) but there was statistically significant difference between Eif4e expression level in AML/Control ($p = 0.002$), ALL/Controls ($p = 0.025$) (Table 4). Also analysis of OS and DFS in each group and its relation to Eif4e gene showed no statistical significance ($p = 0.843$ and 0.310) respectively in AML group and ($p = 0.971$) in ALL group (Table 5).

Correlation studies showed no significant correlation between AML group and Eif4e gene level as regards age,

TLC, hemoglobin and platelets ($p = 0.722, 0.732, 0.712,$ and 0.071 , respectively), while there was significant positive moderate correlation on comparing bone marrow blast% and Eif4e gene level ($r = 0.545$ and $p = 0.001$) (Figure 1).

There was no significant correlation between ALL group and Eif4e gene level as regards age, TLC, hemoglobin, platelets and bone marrow blasts % ($p = 0.248, 0.347, 0.766, 0.700,$ and 0.983 , respectively).

In accordance with our study; Zhu *et al.*⁷ compared the expression level of eIF4E in patients with leukemia and normal controls, and explored its role in leukemogenesis. They found that compared with normal controls, the absolute expression levels of eIF4E mRNA increased in patients with AML, ALL and CML in blastic phase ($p < 0.05$), but had no significant change between groups of CML in chronic and accelerated phase although some increasing in group of CML in accelerated phase. They found that the relative expression level of eIF4E mRNA had no significant change in AML, ALL, CML groups except the two subtypes of leukemia M4 and M5. Furthermore, they found that the protein expression level in group of CML in accelerated phase and blastic phase and all acute leukemia patients including AML and ALL were higher than that in normal controls ($P < 0.05$).

In our study we couldn't reach the same outcome concerning M4 and M5 because we only had 1 case of each FAB subgroup.

Jiang *et al.*²³ detected in their study the expression level of eIF4E gene in patients with non-treated, remission and non-remission/relapse acute myeloid leukemia (AML), and other non-malignant haematologic diseases to analyze and reveal the relationship of eIF4E gene expression with AML progression using SYBR Green I RT-PCR. The results showed that the eIF4E expression level in patients with non-treated and non-remitted/relapsed AML was significantly higher than that in patients with remission ($P < 0.01$) and non-malignant hematologic diseases ($P < 0.01$). There was no difference between latter two group patients, even though the expression level of eIF4E gene in patients with M4 and M5 was higher. As compared with non-malignant hematologic diseases, the expression level of eIF4E gene of patients with remission patients showed no significant difference. They concluded that the over-expression of eIF4E gene has been found in patients with AML, and its level obviously decreases along with remission of disease, thus they concluded that eIF4E gene may be an indicator for disease progression.

This was in agreement with our study as we showed that AML cases expressed higher levels of the gene than normal controls and this was significantly positively correlated to the blast percentage. We did not

demonstrate difference in levels of gene expression of FAB subtypes M4 and M5 and this probably is due to the small number of cases of these subtypes (1 case each).

In their study; Ivan, *et al.*²² demonstrated that elevated eIF4E blocks monocytic and granulocytic differentiation. Their subsequent mutagenesis studies indicate that this block is a result of dysregulated eIF4E-dependent mRNA transport. These studies indicate that the RNA transport function of eIF4E could contribute to leukemogenesis. They extended their studies to provide evidence that the nuclear transport function of eIF4E contributes to human malignancy, specifically in a subset of acute and chronic myelogenous leukemia patients. They observed an increase in eIF4E-dependent cyclin D1 mRNA transport and a concomitant increase in cyclin D1 protein levels. Thus, their findings indicate that this nuclear function of eIF4E can contribute to leukemogenesis by promoting growth and by blocking differentiation, which was in agreement with our findings.

5. Conclusion

In conclusion, eIF4E was found to be elevated in acute leukemia patients compare to normal controls and its levels were more in myeloid than lymphoid leukemia and positively correlated with the blast percentage in AML thus its level may contribute to leukemogenesis.

Eif4e levels and translation initiation may be an attractive target for anticancer therapeutics. Though there are different treatment protocols for ALL and AML between adult and Pediatric age groups, yet in our study we tried to assess the clinical significance of eIF4E in ALL and AML regardless the different biologic behavior in both groups. Future studies correlating different drug regimens with the gene level may be of great benefit.

Conflict of interest

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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