Journal of the Egyptian Society of Haematology & Research, Vol. 14, No. 1, March: 17-24, 2018

Expression of Insulin-Like Growth Factor-Binding Protein-2 and 7(*IGFBP2* and *IGFBP7*) Genes in Acute Myeloid Leukemia

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ABSTRACT

Background: Variations of the insulin-like growth factor (IGF) receptors and ligands are well defined in different hematological and solid neoplasms, providing a possible anti-neoplastic molecular target.

Objectives: This study aims to determine the expression level of human *insulin-like growth factor binding protein2* and *7 (IGFBP2* and *IGFBP7)* genes in adult AML patients and to asess outcome accordingly.

Patients and Methods: A case control study carried out on 35 adult De novo AML patients who were managed at the National Cancer Institute, Cairo University (NCI), as well as 20 age and gender matched healthy controls. Detection of *IGFBP2* and *IGFBP7* genes was done using real time RT-PCR. Expression of both genes was correlated with the clinical and Laboratory data, complete remission rate and overall survival.

Results: There was a higher *IGFBP2* and *IGFBP7* genes expression in AML patients compared to control. This difference in expression was statistically significant for *IGFBP7* (p=0.036) but not for *IGFBP2* (p=0.1). There was a fare positive correlation between *IGFBP2* and *IGFBP7* gene, (r=0.478, p=0.004). High expression of both *IGFBP2* and *IGFBP7* was significantly associated with lower complete remission rate (p=0.008) and shorter overall survival (p=0.001 and p=0.012 respectively).

Conclusion: High expression of *IGFBP2* and *IGFBP7* genes was associated with poor prognosis in AML cases. Moreover, both genes may present a potential molecular target or a new therapeutic modality for treatment strategy and management of AML.

Key Words: *IGFBP-2 – IGFBP-7 – Acute Myeloid Leukemia (AML).*

INTRODUCTION

Acute myeloid leukemia (AML) is a complex heterogeneous disease in clinical course, cytogenetic and molecular genetics. Understanding the molecular patho-biology in acute leukemia has dramatically improved over the last years [1].

The identification of aberrant signaling pathways in AML and cross-talks has clarified mechanisms of disease pathogenesis and has directed to the development of new targets. Further characterization of prognostically relevant signaling pathways might improve risk-assessment and development of therapeutic strategies for AML patients [2].

Despite being part of the physiological machinery for growth and development, Insulin-like growth factor (IGF) gained special interest over the last decade because of its oncogenic potential [3].

Variations of the insulin-like growth factor (IGF) receptors and ligands are well defined in different hematological and solid neoplasms, providing a possible anti-neoplastic molecular target [4].

The IGF pathway has 2 growth factors ligands (IGF-I and IGF-II), their 2 corresponding receptors (IGF-IR and IGF-IIR) and a group of IGFBPs (IGFBP1-7) [5].

The functional activity of IGF-I and IGF-II is regulated by 7 high-affinity IGF binding proteins (IGFBP1-7), which act as carrier proteins modulating bioavailability and half-life of the IGFs. In addition, some authors suggested that IGFBPs promote cell growth independently of IGFs [2].

IGFBP2, one of the major IGFBPs, is aberrantly expressed in a variety of solid tumors, lymphomas and in patients with acute lymphoblastic leukemia (ALL) and AML [6,7]. Although the functional role of IGFBP2 in acute leukemia and other types of cancer is still unknown, overexpression of IGFBP2 has been associated with higher tumorigenicity and therapy resistance [6]. In preclinical studies, IGFBP-2 administration could improve tumor metabolism (inhibition of adipogenesis and enhanced insulin sensitivity), while blocking of IGFBP-2 blockade could prevent neoplastic growth and development of metastasis [8]. Unlike IGFBP1 to IGFBP6, IGFBP7 binds insulin with 500-folds higher affinity than insulin-like growth factor I (IGF-I) [9].

The study of *IGFBP7* in a variety of solid malignancies, including breast, lung, prostate, colorectal, and liver cancer has suggested a role of a tumour suppressor gene [10-14]. High IGFBP7 was also associated with induction chemotherapy failure in acute lymphoblastic leukemia [1]. In this study we assessed the expression of human *insulin-like growth factor binding protein 2 and 7* (*IGFBP2* and *IGFBP7*), as potential oncogenes implicated in AML and correlated the results with the different clinical and laboratory features as well as response to treatment and overall survival.

PATIENTS AND METHODS

Patients:

A case-control study was conducted on 35 consecutive newly diagnosed AML patients who presented to the Medical Oncology Department, National Cancer Institute (NCI), Cairo University over a period of 6 months. The age ranged from18-68 with a median of 45 years. Nineteen out of 35 (54%) were males and 16/35 (46%) were females.

Twenty age and gender-matched healthy individuals were included as control group. The study was approved by the ethical committee of NCI [Institutional Research Board (IRB)], Cairo University. All subjects were aware by the nature of the study and gave a written informed consent.

Methods:

Pre-treated BM samples were available from 35 AML patients and 20 healthy controls. Bone marrow mononuclear cells (BMMCs) were extracted from 1-2ml EDTA samples, (they were obtained by gradient density centrifugation using Ficoll-Hypaque 1077 (Sigma). Cell pellets were preserved at –80°C until RNA extraction was performed. Extraction of total RNA from mononuclear cells was carried out using Prep Ease RNA Spin kit 78766, 50 preps, (Affymetrix), following the manufacturer's instructions. The concentration and purity of RNA was measured at 260 & 280 & 230nm using Nano Drop 2000/ 2000c spectrophotometer (Thermo Scientific, USA). Ratio of A260/A280=1.8-2.1 and A260/ A230=1.8-2.1 indicates highly pure RNA.

Extracted RNA was reverse transcribed into complementary DNA (cDNA) using TaqMan® RNA reverse Transcription Kit (Applied Biosystem, USA) according to the TaqMan RNA Assay protocol. The PCR reactions were carried out in a total volume of 20ul (10ulmix+10ul extracted RNA), the thermal cycle reaction program was as follows: 10 minutes at 25ºC (incubation), 37ºC for 2 hours for reverse transcription, 85ºC for 5 seconds to stop the reaction. The main PCR cycles were repeated 40 times (denaturation at 95ºC for 15 seconds, annealing extension at 60ºC for 1m).

The Primers and Probes sequences used were as follows:

- *IGFBP2* Forward primer: 5'CATCACCTT-GGCCTGGAG
- *IGFBP2* Reverse primer: 5'GGATGTGCA GGGAGTAGAGG
- *IGFBP2* probe: 5'-FAM- CCTGCCAGGACT CCCTGCCAAC-TAMRA
- *IGFBP7* Forward primer: 5' CATCACCCAG-GTCAGCAAG
- *IGFBP7* Reverse primer: 5'TCACAGCTC AAGTACACCTG
- *IGFBP7* probe:6-FAM-TGCGAGCAA GGTCCTTCCATAGT-TAMRA-30

The "comparative threshold method" ($2^{-\Delta\Delta}$ CT method) was used to calculate the relative expression levels of a target (*IGFBP2* and *7*) relative to a reference control using the Ct data. The amount of target was normalized to the endogenous housekeeping gene *GAPDH* and

evaluated relative to the calibrator (healthy control). The formula used was as follow:

$2^{-\Delta\Delta}$ CT, where $\Delta\Delta$ Ct = Δ Ct (sample) - Δ Ct (calibrator), and Δ Ct is the Ct of the target gene subtracted from the Ct of the housekeeping gene.

$$\Delta\Delta \text{ CT} = \Delta\text{CT sample} - \Delta\text{CT calibrator}$$
$$\text{Fold Change} = 2^{-\Delta\Delta\text{CT}}$$

Response rate at day 28:

Complete remission (CR) in our patients detected on day 28 post induction is defined as morphological recovery of the BM and blood counts (i.e., neutrophils ≥1,500/L and platelets ≥100,000/L), and no circulating leukemic blasts, BM cellularity more than 20% with maturation of all cell lines, no Auer rods, <5% BM blasts and without extramedullary leukemia. Relapse was defined by ≥5% BM blasts, reappearance of circulating leukemic blasts, or development of extramedullary leukemia **[15]**.

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. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative not normally distributed 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. A *p*-value <0.05 was considered significant.

RESULTS

We evaluated the expression of *IGFBP2* and *IGFBP7* genes in 35 de novo AML patients. They included 19 males and 16 females with an age range of 18-68, a mean of 41.5±15.6 and a median of 45 years. Twenty healthy age and gender matched subjects were included as a control.

Taking the median value as cut off for both *IGFBP2* and *IGFBP7* genes, 18/35 (51%) were high expressers as compared to 17/35, 49% low expressers.

Correlation between IGFBP2 and IGFBP7 genes:

Good positive correlation was found between *IGFBP2* and *IGFBP7* gene, (r=0.478, p=0.004, Fig. 3).

Both genes had higher expression level in AML patients compared to control that achieved statistical significance for *IGFBP7* (p=0.036) but not for *IGFBP2* (p=0.1).

As regards *IGFBP2* gene, we found that the gene expression level in AML patients (median value=2.05) was higher than gene expression level of control group (median value=1.0), however this difference was not statistically significant (*p*-value=0.1). While in *IGFBP7* gene, we found a statistically significant difference in *IGFBP7* gene expression level between the AML group and control group (median values were 2.11 and 0.97, respectively, *p*-value =0.036).

Categorization of patients according to gene expression levels:

The median value of gene expression was taken as a cut off (2.05 for *IGFBP2* and 2.11 for *IGFBP7*). Within each category 17 patients (49%) had values below (low expressers) and 18 (51%) had values above the median (high expressers).

High and low *IGFBP2* and *IGFBP7* expressers showed comparable clinical and haematological characteristics (Tables 1,2).

Follow-up of patients:

In case of *IGFBP7*, 6/35 patients died before day 14 of treatment. A significant positive association between blast count at day 14 with *IGFBP2* and *IGFBP7* gene expression was encountered (p=0.008 and 0.002, respectively, Table 3).

Response rate at day 28:

After the first course of induction, CR was achieved in 11/35 (31%) patients and CR was more associated with low expression of both genes (p=0.008, Table 4, Fig. 1a,b).

Overall survival:

In our study, the median follow-up period was 1.9 month (range 0.1-11.1 month). Overall cumulative survival was 13.3% with a median of 1.9 month (95% CI: 1-2.8m).

Low expressers of both genes showed significantly higher survival rate (p=0.001 and 0.012, respectively; Fig. 2a,b).

Table (1): *IGFBP2* expression in 35 Adult AML cases in relation to clinical and haematological characteristics.

Parameter	Low expressers No. 17 (%)	High expressers No. 18 (%)	p-value
Sex:			
Male	7 (41.2%)	12 (66.6%)	0.130
Female	10 (58.8%)	6 (33.3%)	
TLC x10⁹/L:			
Median (range)	26 (3.5-181)	20.5 (1.3-265)	0.732
Hb: gm/dl*	7.2 (4.4-10)	7.4 (3.8-10.4)	0.660
PLTx10⁹/L*	37 (11-315)	26.5 (6-257)	0.232
Splenomegaly:			
Yes	4 (23.5%)	3 (16.7%)	0.691
Hepatomegaly:			
Yes	4 (23.5%)	1 (5.6%)	0.177
CD34 expression:			
+Ve	6 (35.3%)	10 (55.5%)	0.229
CD117:			
+Ve	11 (64.7%)	6 (33.3%)	0.063
Aberrant lymphoid markers:			
+Ve	6 (35.3%)	3 (16.7%)	0.264
FAB classification:			
M0, M1&M2	15 (88%)	14 (77%)	0.658
M4	2 (12%)	4 (22%)	

TLC: Total leukocyte count.
Hb : Haemoglobin.
Plt : Platelet count.
* Median (range).

Table (2): *IGFBP7* expression in 35 Adult AML cases in relation to clinical and haematological characteristics.

Parameter	Low expressers	High expressers	p-value
Gender:			
Male	11 (64.7%)	8 (44.4%)	0.229
Female	6 (35.3%)	10 (55.5%)	
TLCx10⁹/L:*			
Median (range)	13.3 (3.5-119)	27.5 (1.3-265)	0.732
Hb: gm/dl:*			
Median (range)	7 (4.4-10.4)	7.3 (3.8-10)	0.660
PLTx10⁹/L:*			
Median (range)	25 (12-315)	29 (6-257)	0.546
PB blasts:			
Median (range)	55 (28-94)	72.5 (6-94)	0.351
BM blasts:			
Median (range)	73 (30-95)	72 (30-93)	0.782
Splenomegaly:			
Yes	4 (23.5%)	3 (16.6%)	0.691
Hepatomegaly:			
Yes	4 (23.5%)	1 (5.9%)	0.177
CD34:			
+Ve	6 (35.3%)	10 (55.5%)	0.229
CD117:			
+Ve	11 (64.7%)	6 (33.3%)	0.063
Aberrant lymphoid markers:			
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M0, M1&M2	15 (88%)	14 (77%)	0.658
M4	2 (12%)	4 (22%)	

TLC: Total leukocyte count. Plt: Platelet count.
Hb : Haemoglobin. * Median (range)

Table (3): *IGFBP2* and *IGFBP7* expression in relation to response to treatment; day 14 blasts in 35 Adult AML patients.

	Low expressers	High expressers	p-value
Day 14 blasts IGFBP2:			
No.	16/35	13/35	0.008
Median (range)	4 (1-42)	15 (4-60)	
Day 14 blasts IGFBP7:			
Median (range)	6 (1-42)	22 (2-60)	0.002

Table (4): *IGFBP2* and *IGFBP7* expression rate in relation to response to treatment at day 28.

IGFBP2	Complete response (n=11)	No response (n=24)	*p*-value
Low expression	9/11 (81.8%)	8/24 (33.3%)	
High Expression	2/11 (18.2%)	16/24 (66.7%)	
IGFBP7	Complete response (n=11)	No response (n=24)	0.008
Low expression	9/11 (81.8%)	8/24 (33.3%)	0.008
High Expression	2/11 (18.2%)	16/24 (66.7%)	

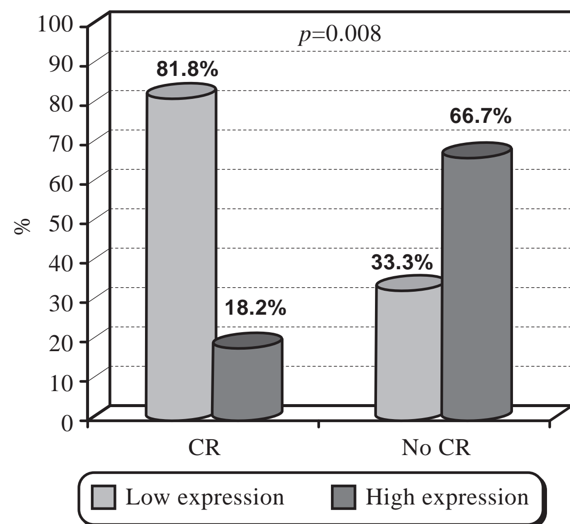

Fig. (1-A): IGFBP2 expression in relation to response to treatment at day 28.

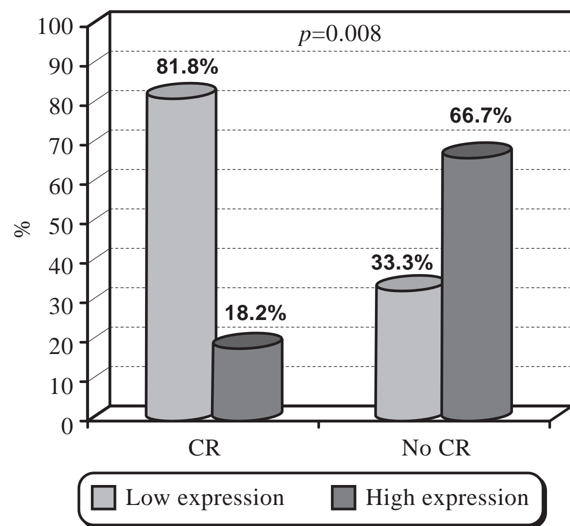

Fig. (1-B): IGFBP7 expression in relation to response to treatment at day 28.

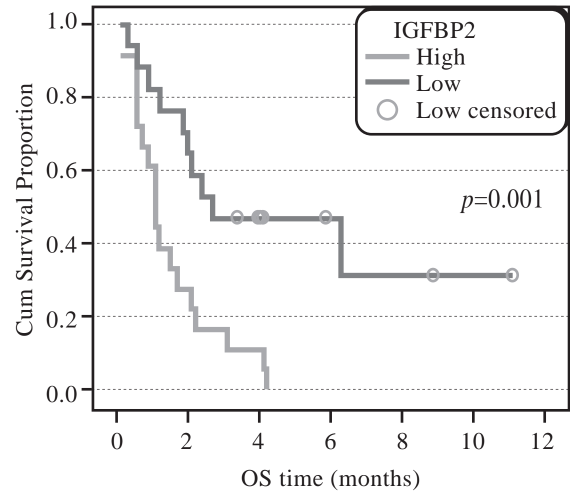

Fig. (2-A): Kaplan Meier curve of overall survival for high and low IGFBP2 expressers.

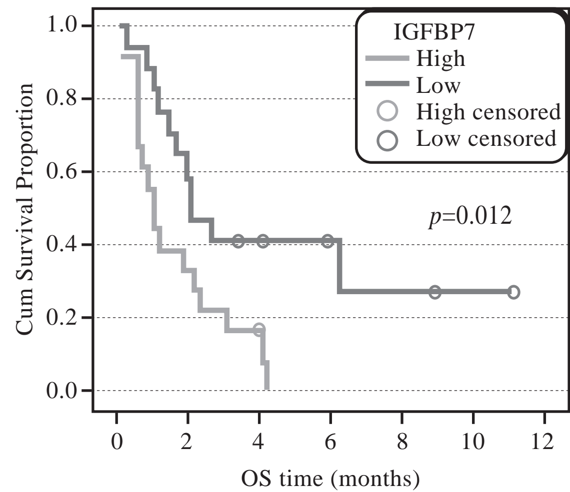

Fig. (2-B): Kaplan Meier curve of overall survival for high and low IGFBP7 expressers.

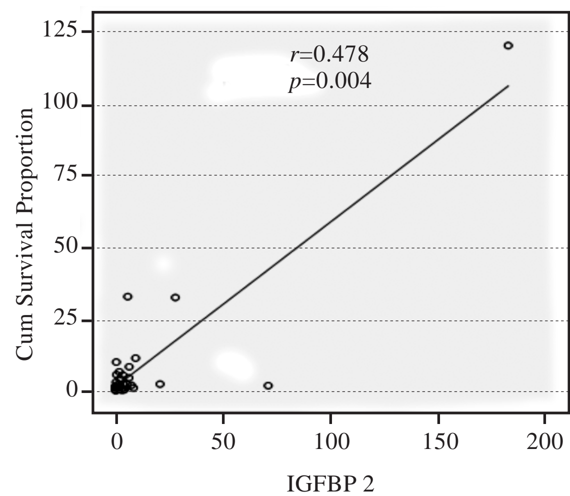

Fig. (3): Correlation between IGFBP2 and IGFBP7 genes.

DISCUSSION

In our study, we measured the level of mRNA expression of both *IGFBP2* and *IGFBP7* genes by real time RT-PCR in BM samples of 35 de novo adult AML patients and 20 age and gender matched healthy controls. We evaluated their level of expression in relation to clinical and hematological parameters, response to treatment and survival.

The frequency of *IGFBP2* gene expression was higher in AML patients than the control group. However, this difference could not reach a statistically significant level (median: 2.05 vs. 1; p=0.1). In line with our results, significantly higher expression levels of both genes were encountered in AML patients **[2,7]**.

Moreover, IGFBP2 serum protein levels were significantly higher in AML patients compared to controls **[2]**. In the current study, a level of significance might be reached by increasing sample size.

In our study, there was no association between *IGFBP2* expression and either age or initial TLC. This is in accordance with Kühnl et al. **[2]**.

We did not either found any association of *IGFBP2* expression with other hematological or clinical parameters including FAB subtypes. In contrast to the latter, Kühnl et al. **[2]** reported an association between high expression and M4/M5 FAB subtype.

Differences in sample size and differences in relative frequency of FAB subtypes may explain that differences in results.

In the current study high *IGFBP2* expression was significantly associated with a lower CR rate (p=0.008). This is in agreement with Kühnl et al. **[2]** who reported significant association of high *IGFBP2* expression with a higher incidence of primary resistant disease (p=0.02) and a lower CR rate (p=0.01). This is also in line with others **[16]** who reported that serum IGFBP-2 level was significantly lower in responders than in non-responders both at diagnosis and after induction cycle of chemotherapy. On the same line another study **[7]** reported that high *IGFBP2* expression was a predictor of higher relapse rate in pediatric AML.

In the current study, lower *IGFBP2* expression was significantly associated with better overall survival (p=0.0013). This is in contrast to Kühnl et al. **[2]** who reported no relationship between *IGFBP2* expression and OS (p=0.53).

On the other hand, Huang et al. **[17]** and Lin et al. **[18]** concluded from their study on newly diagnosed glioma patients using an IGFBP-2 ELISA kit that IGFBP-2 levels are significantly correlated with recurrence and DFS in patients with astrocytoma grade IV. Low *IGFBP2* expression had an obvious benfit in overall and disease-free survival, whereas those with high *IGFBP2* expression have worse median survival.

This controversy in results is likely due to different ethnic population, different sample size and/or exposure to different carcinogens in different environments (gene environmental interaction) and also gene to gene interaction in cancer.

IGFBP2 has strong oncogenic potential promoting proliferation, survival, and migration of leukemic cells. Chen et al. **[19]** found that deletion of *IGFBP2* decreased extramedullary leukemic infiltration, suggesting that IGFBP2 is required for the migration of AML cells out of bone marrow. For this reason, the development of IGFBP2 therapy that affects leukemic stem cells with minimal effect on normal stem cells is a potentially promising therapeutic cancer treatment **[20]**.

In the current study, *IGFBP7* gene expression showed significantly higher frequency in AML patients as compared to the control group (p=0.036). This is in agreement with previous reports both in adult **[1]** and paediatric AML **[21]**.

In our study, no association was encountered between *IGFBP7* gene expression and any hematological or clinical parameters at diagnosis.

However, high *IGFBP7* expression was significantly associated with a lower CR rate (p=0.008) and worse overall survival (p=0.012). In contrast, Verhagen et al. **[22]** reported the association of high *IGFBP7* expression with a better outcome in AML patients. Heesch et al. **[1]** concluded that high expression of *IGFBP7* was found to be associated with a more immature phenotype of early T-ALL, inferior survival, and resistance against chemotherapy in T-ALL.

Also, Tomimaru et al. [23] reported improved OS rate in patients with *IGFBP7* expression was significantly lower than that in patients with positive *IGFBP7* expression in hepatocellular carcinomas (p=0.0063). Benassi and colleagues [24] concluded that not only tissue expression of *IGFBP7* is highly prognostic in poor metastasis-free survival, but in parallel, the determination of serum protein levels might contribute to soft tissue sarcoma diagnosis. Jiang et al. [25] found that expression of *IGFBP7* correlated inversely with overall Glioblastoma survival rates. This controversy in *IGFBP7* expression in leukemia and glioblastoma cell lines is in disagreement with other solid tumors findings. This may be due to different mechanisms of action of IGFBP7. One of these possibilities is that cell signaling pathways that result in senescence or apoptosis due to IGFBP7 are not present or functioning in hematopoietic or glioma cells. Another proposed mechanism by Cao et al., [26] is that deletion of IGFBP7 leads to activation of IGF1 which in turn stimulates the FGF4-FGFR1-ETS2 pathway in tumor-associated endothelial cells and induce chemo-resistance and malignant stem cells formation.

In the current study, we found a good positive correlation between the expression of both *IGFBP2* and *IGFBP7* genes. High expression of one gene is accompanied by high expression of the other. *IGFBP2* and *IGFBP7* genes have functions both dependant and independent of IGF/IGF receptor signalling. An appropriate understanding of their mechanism of function is required. Moreover, *IGFBP2* and *IGFBP7* may present a potential molecular target or a new therapeutic modality for treatment strategy and management of AML.

In conclusion, more studies with larger sample size are needed to establish and confirm the function and clinical significance of *IGFBP2* and *7*. The role of *IGFBP2* and *IGFBP7* as prognostic molecular markers and potential therapeutic tool in management of AML needs to be confirmed.

REFERENCES

1- Heesch S, Schlee C, Neumann M, et al. BAALC-associated gene expression profiles define IGFBP7 as a novel molecular marker in acute leukemia. Leukemia. 2010; 24: 1429-36.

2- Kühnl A, Kaiser M, Neumann M, et al. High expression of IGFBP2 is associated with chemoresistance in adult acute myeloid leukemia. Leuk. Res. 2011; 35: 1585-90. doi:10.1016/j.leukres.2011.08.006.

3- Brahmkhatri V, Prasanna C, Atreya HS. Insulin-Like Growth Factor System in Cancer: Novel Targeted Therapies. BioMed Research International. 2015; doi:10.1155/2015/538019.

4- LeRoith D, Roberts CT. The insulin-like growth factor system and cancer. Cancer Lett. 2003; 195: 127-37.

5- Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat. Rev. Cancer. 2008; 8: 915-28.

6- Hoeflich A, Reisinger R, Lahm H, et al. Insulin-like growth factor-binding protein 2 in tumorigenesis: Protector or promoter? Cancer Res. 2001; 61: 8601-10.

7- Dawczynski K, Steinbach D, Wittig S, et al. Expression of components of the IGF axis in childhood acute myelogenous leukemia. Pediatr Blood Cancer. 2008; 50: 24-8.

8- Yau S, Azar W, Sabin M, et al. IGFBP-2 - taking the lead in growth, metabolism and cancer. J. Cell Commun Signal. 2015; 9: 125-42. doi:10.1007/s12079-015-0261-2.

9- Hwa V, Oh Y, Rosenfeld RG. The insulin-like growth factor-binding protein (IGFBP) superfamily. Endocr Rev. 1999; 20: 761-87.

10- Burger A, Leyland-Jones B, Banerjee K, et al. Essential roles of IGFBP-3 and IGFBP-rP1 in breast cancer. Eur. J. Cancer Oxf. Engl. 1990, 2005; 41: 1515-27.

11- Chen Y, Pacyna-Gengelbach M, Ye F, et al. Insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) has potential tumour-suppressive activity in human lung cancer. J. Pathol. 2007; 211: 431-8.

12- Ruan W, Lin J, Xu E, et al. IGFBP7 plays a potential tumor suppressor role against colorectal carcinogenesis with its expression associated with DNA hypomethylation of exon 1. J. Zhejiang Univ. Sci. B. 2006; 7: 929-32. doi:10.1631/jzus.2006.B0929.

13- Sprenger C, Damon S, Hwa V, et al. Insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1) is a potential tumor suppressor protein for prostate cancer. Cancer Res. 1999; 59: 2370-5.

14- Chen D, Yoo B, Santhekadur P, et al. Insulin-like growth factor-binding protein-7 functions as a potential tumor suppressor in hepatocellular carcinoma. Clin. Cancer Res. Off J. Am. Assoc. Cancer Res. 2011; 17: 6693-701.

15- Cheson B, Bennett J, Kopecky K, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J. Clin. Oncol. Off J. Am. Soc. Clin. Oncol. 2003; 21: 4642-9.

16- El-Naggar A, Mahmoud B, Abou Shamaa L, et al. Changes of serum growth factors (IGF-I & IGFBP-2) and prediction of response to chemotherapy in patients with acute myeloid leukemia. Egypt J. Immunol. 2008; 5: 73-80.

17- Huang L, Cohen A, Colman H, et al. IGFBP2 expression predicts IDH-mutant glioma patient survival. Oncotarget. 2017; 8: 191-202.

18- Lin Y, Jiang T, Zhou K, et al. Plasma IGFBP-2 levels predict clinical outcomes of patients with high-grade gliomas. Neuro-Oncol. 2009; 11: 468-76. doi:10.1215/15228517-2008-114.

19- Chen X, Zheng J, Zou Y, et al. IGF binding protein 2 is a cell-autonomous factor supporting survival and migration of acute leukemia cells. J. Hematol. Oncol. J. Hematol. Oncol. 2013; 6: 72.

20- Yao X, Sun S, Zhou X, et al. IGF-binding protein 2 is a candidate target of therapeutic potential in cancer. Tumour Biol. J. Int. Soc. Onco. Developmental Biol. Med. 2016; 37: 1451-9.

21- Hu S, Chen R, Man X, et al. Function and Expression of Insulin-Like Growth Factor-Binding Protein 7 (IGFBP7) Gene in Childhood Acute Myeloid Leukemia. Pediatr. Hematol. Oncol. 2011; 28: 279-287.

22- Verhagen H, de Leeuw D, Roemer M, et al. IGFBP7 induces apoptosis of acute myeloid leukemia cells and synergizes with chemotherapy in suppression of leukemia cell survival. Cell Death Dis. 2014; 5: e1300.

23- Tomimaru Y, Eguchi H, Wada H, et al. IGFBP7 down-regulation is associated with tumor progression and clinical outcome in hepatocellular carcinoma. Int. J. Cancer. 2012; 130: 319-27.

24- Benassi M, Pazzaglia L, Novello C, et al. Tissue and serum IGFBP7 protein as biomarker in high-grade soft tissue sarcoma. Am. J. Cancer Res. 2015; 5: 3446-54.

25. Jiang W, Xiang C, Cazacu S, et al. Insulin-like growth factor binding protein 7 mediates glioma cell growth and migration. Neoplasia N Y N. 2008; 10: 1335-42.

26- Cao Z, Scandura J, Inghirami G, et al. Molecular Checkpoint Decisions Made by Subverted Vascular Niche Transform Indolent Tumor Cells into Chemoresistant Cancer Stem Cells. Cancer Cell. 2017; 31: 110-26.