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Assessment of INPP4B Expression Level in Acute Myeloid Leukemia Patients and Its Prognostic Significance. An Egyptian Study

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ABSTRACT

Background: Acute myeloid leukemia (AML) is a complex and heterogeneous hematopoietic tissue neoplasm caused by gene mutations, chromosomal rearrangement and deregulation of gene expression. Inositol polyphosphate 4-phosphatase type II (INPP4B) is a clinically relevant factor in the phosphoinositide 3 kinase (PI3K) pathway-associated cancers, has been found to have a bad prognostic role in AML. However, the exact mechanism is still unclear. **Objective:** We aimed in this study to investigate the prognostic role of INPP4B overexpression in Egyptian AML patients. **Patients and methods:** A total of 80 patients with newly diagnosed AML were included in the study. In addition, 40 apparently healthy, age and sex matched subjects served as control group. Immunophenotyping, cytogenetic analysis and quantitative assessment of INPP4B gene transcript were performed using real time PCR. **Results:** INPP4B overexpression was detected in 27.5 % of newly diagnosed AML patients. There was a statistically significant decrease in the probability of achieving complete remission (CR) with shorter overall survival (OS), event free survival (EFS) and disease-free survival (DFS) in the INPP4B high expression group compared with the low expression group (p=0.003, 0.03, 0.02 and <0.001; respectively).

Conclusion: We can conclude that INPP4B overexpression is associated with poor response to therapy with poor outcome, and add prognostic value in patients with AML. INPP4B overexpression could be a valuable tool for making therapeutic decisions.

Keywords: Acute myeloid leukemia; INPP4B expression; Prognosis; Survival.

INTRODUCTION

Acute myeloid leukemia (AML) is a complex hematopoietic cellular neoplasm characterized by clonal expansion of immature myeloid cells in the bone marrow, and peripheral blood with uncontrolled proliferation and impaired differentiation program of the affected cells. Although 50–75% patients with AML have a response to chemotherapy, relapse represents the major cause of treatment failure [(1)].

Activation of tyrosine kinase receptors and intracellular signal transducer molecules, such as phosphoinositide-3 kinase (PI3K), plays an important role in the regulation of hematopoiesis [(2)].

Constitutive activation of tyrosine kinase receptors with abnormal activation of PI3K signaling pathway has been reported in more than 50% of AML cases [(3)]. Activated PI3K subsequently phosphorylates the phosphatidyl-inositol biphosphate (PIP2) to generate phosphatidyl-inositol triphosphate (PIP3) and that subsequently enhance activation of AKT protein, which is a critical oncogene in various cancers that increasing cellular proliferation [(4)].

PI3K/AKT signaling can be terminated by several phosphoinositide phosphatases that dephosphorylate PIP species, such as phosphatase and tensin homolog (PTEN) that hydrolyse ptdIns(3,4,5)P3 to generate ptdIns(4,5)P2[(5)] and inositol polyphosphate 4-phosphatase type II (INPP4B) which dephosphorylate ptdIns(3,4)P2 to produce ptdIns(3)P, which in turn terminate PI3K/AKT signaling [(6,7)]. So, INPP4B was initially shown to play a tumor suppressor role in variety of cancers. This tumor suppressive mechanism of INPP4B has been attributed to its negative regulatory role in PI3K/AKT signaling [(8)].

However, recent studies reported that INPP4B role is more complicated than previously expected [(9)] and seems to have an oncogene role. INPP4B is highly expressed in some of AML patients and this is predictive of poor prognosis and resistance to chemotherapy [(10,11)]. INPP4B high expression is not associated with changes in phosphorylation status of AKT in leukemia patients, indicating that AKT-independent mechanisms are expected to play a role [(11)]. Serum and glucocorticoid-regulated kinase-3 (SGK3), which has functional and structural similarities with the AKT protein, is a PI3K-dependent serine/threonine kinase [(12)].

SGK3 contains a unique N-terminal phox homology domain that binds to PI(3)P, thus targeting early endosomes where SGK3 is fully activated [(13-14)]. In addition, SGK3 seems to be a downstream effector of INPP4B [(15)]. previous studies reported a relation between high INPP4B expression and SGK3 phosphorylation status in some cancers, in which INPP4B enhances the activation of SGK3, which in turn promotes cell proliferation and growth [(16)]. Also, ***Jin et al.*** [(17)] reported that INPP4B is frequently highly expressed in NPM1-mutated AML, promoting cellular proliferation and survival in a SGK3-dependent and

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AKT-independent pathway and associated with poor outcome in NPM1- mutated patients. So, these findings may indicate that INPP4B could be a potential target for the treatment of NPM1-mutated AML patients [17].

Another possible explanation for the oncogenic role of INPP4B in AML patients is mediated by inducing autophagy and inhibiting apoptosis with involvement of interferon regulatory factor. Autophagy is involved in some biological processes as cell survival, and death [18]. At present, autophagy is considered as an essential mechanism of defense and a survival response to stress to save the injured cells [19-22]. While, apoptosis, which is a form of programmed cell death, is an important mechanism for inhibiting cellular overgrowth and discarding unsalvageable cells. So, an imbalance between both autophagy and apoptosis might leads to tumorigenesis. **Qiang *et al.*** [23] reported that autophagy-related gene 5 (Atg5)-dependent autophagy promotes development of AML. Also, **Folkerts *et al*.** [24] demonstrated that loss of Atg5 leads to an identical hematopoietic stem and progenitor population (HSPC) phenotype and inhibit leukemia maintenance. Thus, targeting autophagy could provide a novel therapeutic options for AML treatment.

The interferon regulatory factor (IRF) proteins family are the essential factors in regulation of cell proliferation, and cellular response which is involved in carcinogenesis [25]. A previous study [26] reported that knockdown of IRF2 inhibited cellular growth and colony formation, with downregulation of anti-apoptotic proteins as Bcl-2 and up-regulation of apoptotic proteins as Bax. Further studies reported that IRF2 upregulated INPP4B expression via binding to INPP4B promoter, which in turn inhibited cell apoptosis in AML cells. However, the exact mechanism by which INPP4B inhibited apoptosis of AML cells still unclear. Also, IRF2 might regulate cell autophagy by interacting with INPP4B, and facilitating the development of AML. So, IRF2–INPP4B axis inhibited the apoptosis of AML cells via inducing autophagy in vitro, and thus could be a new target for gene therapy in AML [27]. Accordingly, we assessed the effect of INPP4B overexpression on the treatment outcome and survival in denovo AML patients.

PATIENTS AND METHODS

This study was carried out in Departments of Clinical Pathology, Hematology Unit of Internal Medicine, and Biochemistry, Faculty of Human Medicine, Zagazig University during the period from August 2017 to July 2020.

A total of 80 newly diagnosed AML patients were included in the study. They were 42 males and 38 females. Their ages ranged from 18 to 59 years. In addition, 40 apparently healthy, age and sex matched subjects served as control group were investigated. They were 18 males and 22 females, their ages ranged from 17 to 57 years.

Samples: Peripheral blood and bone marrow samples were collected from all patients, samples were collected at the time of presentation before therapy was initiated.

Treatment plan: Patients received induction chemotherapy, consisting of continuous intravenous infusion of cytarabine (100 mg/m^2/day) for 7 consecutive days with 3 days of doxorubicin (25 mg/m^2/day). Patients who achieved CR received consolidation therapy, which included three to four courses of high-dose cytosine arabinoside (2 g/m^2 every 12 h on days 1, 3 and 5; total, 12 g/m^2)

Criteria for therapy outcomes: Response to induction therapy was assessed on day 28 by bone marrow aspiration to assess morphological remission. Complete remission (CR) was defined according to standard criteria as less than 5% blasts in bone marrow aspirates with recovery of peripheral blood counts and no evidence of extramedullary leukemia. Hematological relapse was defined as more than 5% blasts in bone marrow aspirates or appearance of extramedullary leukemia.

Regarding survival, overall survival (OS) was measured from the time of initial diagnosis to the date of death, Disease-free survival (DFS) was measured from the time of CR to the time of relapse or death, and Event free survival (EFS) was measured from the time of diagnosis to the time of treatment failure, disease relapse or death by any causes. Patients who didn't reach the endpoint of follow up as being lost, or didn't express the event were considered as censored.

Patients follow-up: Patients were followed up once every 3 months with complete blood cell counts and blood smear. Bone marrow aspiration and examination was done only if there was any doubt of a relapse on blood smear as recommended by the National Comprehensive Cancer Network (NCCN) guidelines. The patients were followed up for two years to evaluate OS, EFS and DFS.

Methods:

All the study patients were subjected to the following: full history taking, clinical examination, complete blood count, bone marrow aspiration and examination, immunophenotyping by flow cytometry using Becton Dickenson FacsCalibar device **(Franklin Lakes, New Jersey, USA)** using acute panel of monoclonal antibodies to detect the following markers: MPO, CD13, CD33, HLADR, TDT, CD14, CD64, CD34, CD3, CD20 and CD22; conventional cytogenetic analysis by G banding technique; and karyotyping according to International System for Human Chromosomes Nomenclature. A minimum of 20 metaphases were required to be examined for a patient to be classified and evaluated[28].

INPP4B gene expression analyses: It included the following steps: RNA extraction from whole blood, cDNA synthesis, and RQ-PCR.

RNA extraction and cDNA synthesis:

Total RNA was isolated using pureLink RNA minikit (Invitrogen, Carlsbad, California, USA) and transcribed into cDNA using High-Capacity cDNA Reverse transcription Kit for RNA reverse transcription according to the manufacturer's protocol (Applied Biosystems, Foster City, California, USA) on a PCR thermocycler (Verti; Applied Biosystems, Foster City, California, USA). Quantitative real time PCR (RQ-PCR) analysis was performed using TaqMan real-time PCR methods. A house keeping gene B-actin was used as an internal control for calibration. The operation was performed on the Strata gene Mx3005P platform (Agilent Technologies, Santa Clara, California, USA).

The relative quantification of INPP4B mRNA expression levels were determined using TaqMan universal master mix II, (Applied Biosystems, Foster City, California, USA), INPP4B readymade TaqMan gene expression assays, and β-actin readymade TaqMan gene expression assays. The cycle threshold values were obtained for INPP4B and then normalized to B-actin. Finally, fold changes were calculated by the 2^-ΔΔCt method [(29)].

Ethical approval

The study groups were informed about the nature and purpose of the study and an informed written consent was taken from all the patients for the required investigations including bone marrow aspiration. The study groups were not exposed to any harm or risk and the patient's data were confidential. **Also, approval of Ethical Committee in Faculty of Medicine of Zagazig University was obtained.**

Statistical analysis

Analysis of data was performed using SPSS computer program (version 20; SPSS Inc. Chicago, Illinois, USA). Qualitative data were expressed as frequency and percentage and quantitative data were expressed as median and range and they were compared by X^2-test and Mann–Whitney tests respectively. Kaplan-Meier method was used to estimate survival and the difference between groups was analysed by the log rank test. Hazard ratio (HR) with its 95% confidence interval (CI) was used for risk estimation. A P value less than 0.05 was considered statistically significant, and P<0.001was highly significant.

RESULTS

INPP4B expression levels and laboratory characteristics at diagnosis:

The expression value of INPP4B transcript in AML group was statistically significantly higher than the control group (P<0.001) (Table 1).

Table (1): Level of INPP4B gene expression among the studied AML patients and control groups

INPP4B expression	AML group (no.=80)	Control group (no.=40)	P value
Median Range	1.6 0.6-29.9	0.8 0.5-1.8	**<0.001**

The cutoff value above which the sample should be considered as INPP4B high expression was determined using the receiver operating characteristic curve. The optimal cut off value we used was 1.9 (Table 2 and Figure 1). Then, our patients were divided into INPP4B high expression and INPP4B low expression groups by using this cutoff value. At diagnosis, 22 (27.5 %) out of 80 newly diagnosed AML patients had INPP4B high expression. While, 58 (72.5 %) patients had INPP4B low expression. All subjects of the control group had INPP4B low expression.

Table (2): Cutoff value for INPP4B gene expression among the studied AML patients:

AUC	95% CI	Sensitivity	Specificity	Optimal cutoff level of fold change
0.87	0.81-0.94	94%	60%	1.9

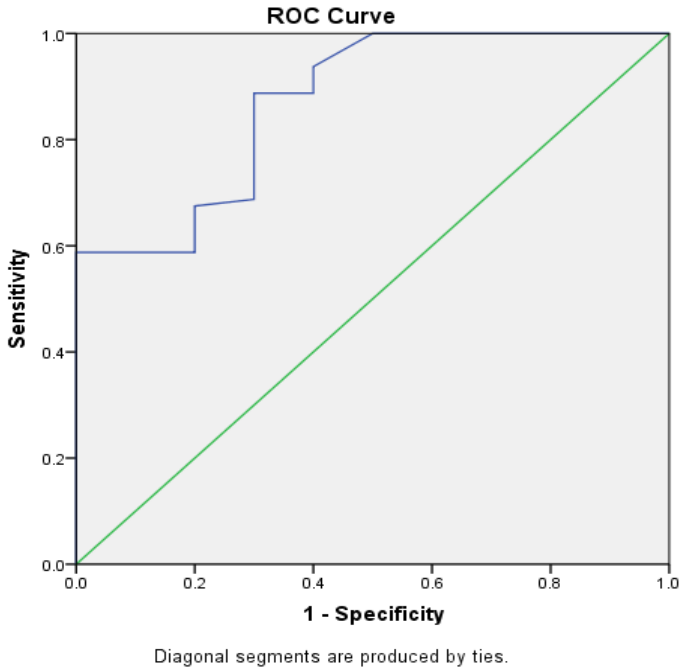


Figure (1): ROC curve for the level of INPP4B gene expression among the studied AML patients

Regarding laboratory characteristics of the patients, total leukocyte count was statistically significantly higher in the INPP4B high expression group than INPP4B low expression group (P<0.001). While, no statistically significant differences were detected between both groups as regards age, sex, platelets and BM blasts (Table 3).

INPP4B expression demonstrated non-significant heterogeneity among FAB subtypes of AML between both groups (P=0.17), except for M2, which was statistically significantly higher in the high expression group (P=0.01). While, there was a statistically significant heterogeneity of its expression as regards cytogenetic risk stratification of AML patients being either favorable, intermediate or adverse risk (P=0.014). Favorable and intermediate risk were higher in the INPP4B low expression group, but with no statistically significant difference. While, adverse risk was statistically significantly higher in INPP4B high expression group (P=0.001) (Table 3).

Table (3): Demographic and laboratory characteristics of INPP4B expression groups

Characteristics	Low INPP4B expression group (no.=58)	High INPP4B expression group (no.=22)	P value
Sex (no.): Male	30	12	0.8
Female	28	10	
Age (years): Median	45	49	0.42
Range	18-55	22-59	
WBCs: (x10^9/L)			**0.001**
Median	9	35	
Range	1-38	2-80	
Platelet: (x10^9/L)			0.54
Median	45	30	
Range	7-200	10-198	
BM blasts (%):			0.25
Median	70	62	
Range	35-95	40-93	
AML FAB subtypes:			0.17
M1	2	1	0.8
M2	13	10	**0.01**
M3	8	2	0.52
M4	22	7	0.61
M5	13	2	0.17
Karyptypes:			
Normal	24	5	0.12
Abnormal	32	16	
Unknown	2	1	
Cytogenetic risk:			**0.014**
Favorable	23	6	0.3
Intermediate	30	8	0.2
Adverse	3	7	**0.001**
Unknown	2	1	---

WBCs, white blood cells; BM, bone marrow

Prognostic significance of INPP4B high expression at diagnosis:

We studied the role of INPP4B high expression in predicting the treatment outcome of AML patients. CR rate was statistically significantly lower in the high

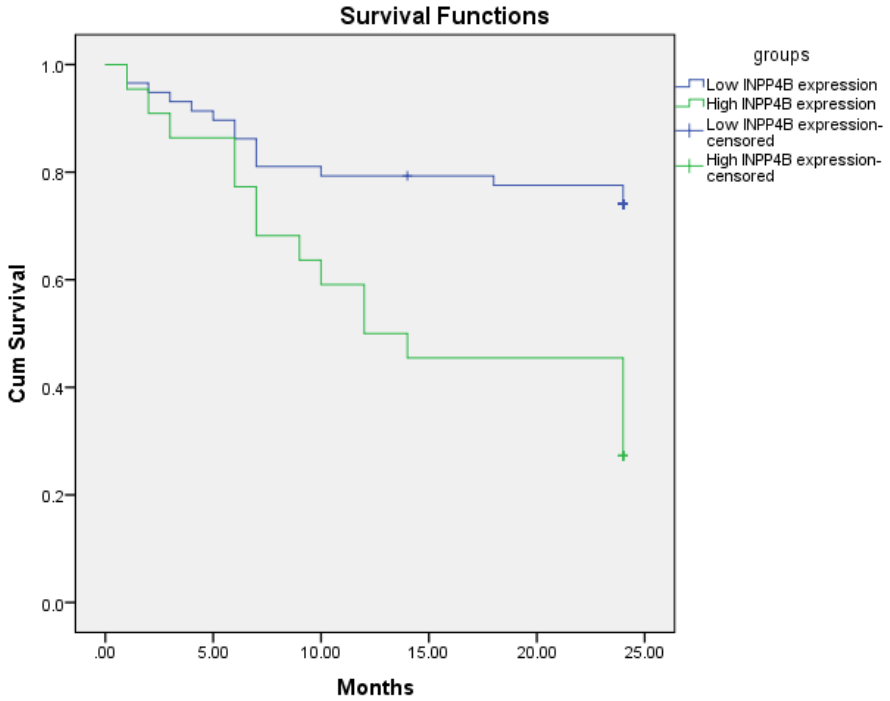
expression group than the low expression group (P=0.003) (Table 4).

Regarding survival rates, including OS, EFS and DFS, we applied Kaplan-meier survival analysis to evaluate them. INPP4B high expression group had a statistically significant shorter OS and EFS than the low expression group (P=0.03 and 0.02; respectively). Finally, a highly significant shorter DFS in the high expression group was also reported (P<0.001) (Table 4 and Figure 2).

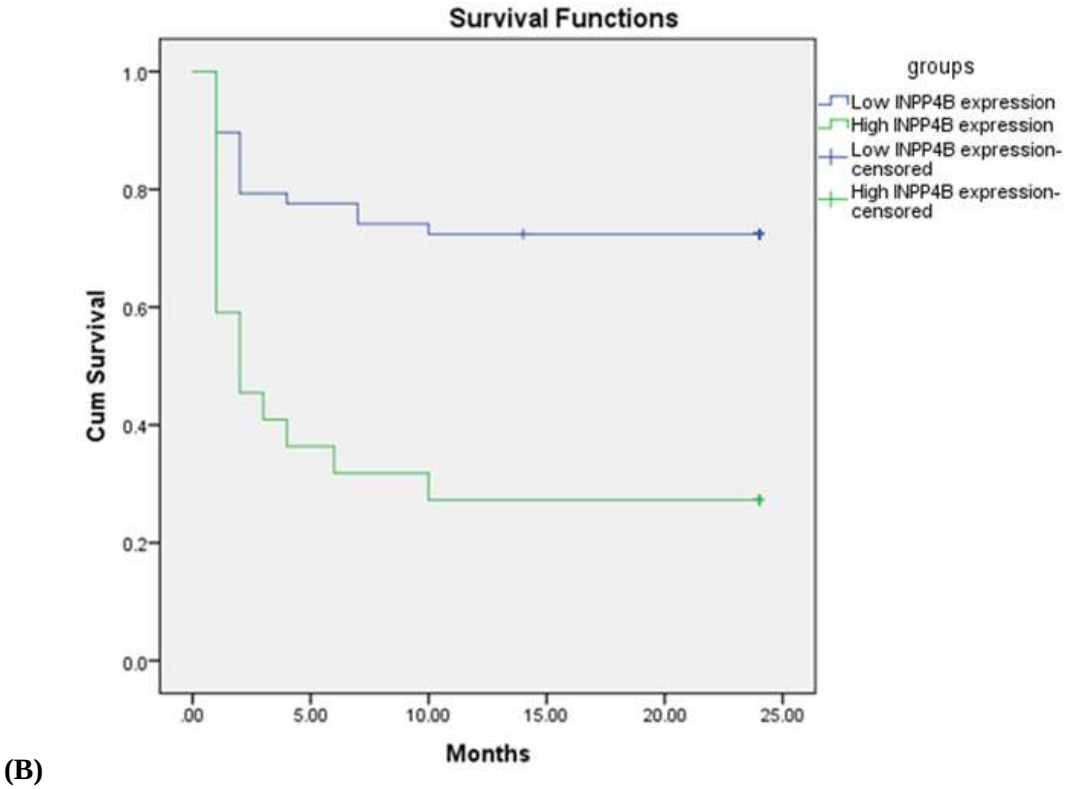
Table (4): Comparison between INPP4B low and high expression groups regarding survival rates

	Low INPP4B expression group (no.=58)	**High INPP4B expression group (no.=22)**	**P value**
CR [no. (%)]	46/58 (79.3%)	10/22 (45.4%)	**0.003**
Relapse [no. (%)]	4/46 (8.8%)	4/10 (40%)	**0.01**
2 years OS			**0.03**
Mean (95% CI) (Months)	19.9 (17.8-22)	14.9 (11.1-18.7)	
Percent probability	77.6 %	45.5 %	
HR	2.4		
2 years EFS			**0.02**
Mean (95% CI) (Months)	18.2 (15.7-20.6)	8.3 (4.2-12.4)	
Percent probability	72.4 %	27.3 %	
HR	2.6		
2 years DFS			**<0.001**
Mean (95% CI) (Months)	22.4 (20.9-23.9)	16.3 (10.4-22.2)	
Percent probability	91.3%	60 %	
HR	4.5		

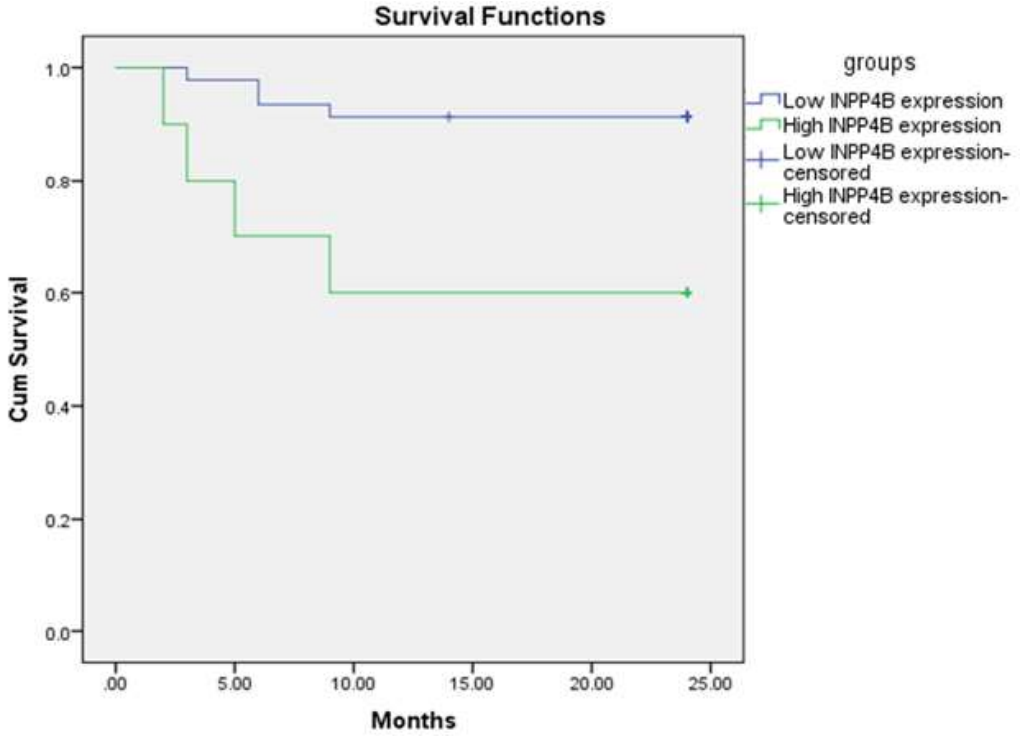
HR, Hazard ratio



(A)



(B)



(C)

Figure (2): Kaplan–Meier curve shows probability of (a) Overall survival, (b) Event-free survival, and (c) Disease-free survival for the INPP4B high expression and INPP4B low expression groups

Multivariate analysis using Cox regression model (HR) for survival analysis was done. Multivariate modeling including INPP4B expression, total leukocyte count, BM blasts and cytogenetic risk was designed. INPP4B overexpression was the only independent prognostic factor which significantly affects OS, EFS and DFS in the AML group (P= 0.002, 0.03 and 0.003; respectively). (Table 5).

Table (5): Multivariate analysis of prognostic factors for OS, EFS and DFS

Variants	OS		EFS		DFS	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
INPP4B expression	2.2 (1.3-3.7)	**0.002**	1.9 (1.2-3.1)	**0.03**	2.1 (1.1-3)	**0.003**
TLC	1.5 (1.1-2.0)	0.2	1.3 (1-1.7)	0.1	1.3 (1-1.8)	0.2
BM blasts	1.4 (1-2.1)	0.1	1.2 (0.9-1.6)	0.1	1.2 (0.8-1.6)	0.3
Favorable cytogenetic	0.6 (0.5- 1.1)	0.5	0.8 (0.5-1.1)	0.4	0.7 (0.4-1)	0.6
Adverse cytogenetic	1.2 (0.9-1.8)	0.2	1.0 (0.7-1.5)	0.3	1.1 (0.6-1.5)	0.2

DISCUSSION

In this study, the incidence of INPP4B high expression was 27.5% of AML patients, which corresponds with study of **Dzneladze *et al*.** [10] (24.9%), but it was higher than that reported by **Rijal *et al.*** [11] (12.2%) and lower than that detected by **Song *et al.*** [30] (44.1%). This great discrepancy between studies could be attributed to the use of different reference genes in determining the level of INPP4B expression. Also, INPP4B gene expression ultimately may have different level in different ethnic populations. Different cutoff values for normal INPP4B expression had been reported in different studies.

The INPP4B high expression was associated with a statistically significant increase in total leukocyte count compared with patients with low expression, which is in agreement with that reported by **Dzneladze *et al.*** [10]. On contrary, **Rijal *et al.*** [11], reported no significant difference between both groups.

No statistically significant difference was observed between INPP4B high expression and low expression groups as regards age and sex, which came in accordance with **Dzneladze *et al.*** [10] and **Rijal *et al.*** [11]**.** Also, there was no significant difference observed between both groups regarding platelet count and BM blasts at the time of diagnosis, which was in agreement with other studies [10-11].

No statistically significant difference regarding FAB subtypes of AML patients was observed between both groups. Although, we found an association between M2 FAB subtype and INPP4B high expression with a statistically significant difference. While, M4 subtype was more associated with INPP4B low expression, but with no statistically significant difference. This was in agreement with study reported by **Dzneladze *et al.*** [10], who reported that the highest INPP4B high expression was observed in M2 subtype with a statistically significant difference. While, M4 subtype was dominated in the INPP4B low expression group but this was statistically non-significant.

Evaluation of INPP4B expression in association with karyotype as being normal or abnormal showed non-significant results. This was in agreement with other studies reported by **Dzneladze *et al.*** [10] and **Rijal *et al.*** [11]. Although, regarding cytogenetic risk stratification, there was a statistically significant difference between INPP4B expression groups among the three cytogenetic risk groups and this came in accordance with study reported by **Dzneladze *et al.*** [10]. On contrary to our results, **Rijal *et al.*** [11] reported no statistically significant difference as regard cytogenetic risk in relation to INPP4B expression level.

In our research, we studied the possible impact of INPP4B high expression in AML patients in response to induction chemotherapy and survival outcomes. INPP4B high expression seems to add prognostic information in patients with AML. Examination of response to induction chemotherapy revealed that patients with high expression of INPP4B gene had significantly lower CR rates compared to those with low expression. This was in agreement with reports of **Dzneladze *et al.*** [10] with CR rates of (57% vs. 74%) being lower among high INPP4B expression group. While, **Rijal *et al.*** [11] reported that patients with high INPP4B expression had lower CR rates than those with low expression (48% vs. 64%; respectively) but with no statistically significant difference.

A possible explanation for decreased CR of patients with high INPP4B expression is that overexpression leads to increased colony forming potential and increased proliferation in AML cell lines[10]. INPP4B overexpression provide the cell a growth advantage, in part by decreased basal apoptotic activity [27]. Also, overexpression in AML cells leads to decreased sensitivity to DNA and ionizing radiation because INPP4B has a direct role in chemotherapy response and is important in mediating cellular response to DNA damaging agents as reported by **Dzneladze *et al.*** [10].

In our study, high expression of INPP4B in leukemic blasts was associated with poor survival rates in AML patients as 2 years OS was shortened for patients with high INPP4B expression compared with those with low expression. This finding was also reported by **Dzneladze *et al.*** [10] who reported that INPP4B high expression AML patients had significantly shorter OS than low expression patients with 3 years percent probability of (21.4% vs. 41.6%; respectively).

Also, **Rijal *et al.*** [11] reported that high INPP4B expression was an independent predictor of poor OS outcomes among AML patients at diagnosis with median 3 years OS of (11.5 vs. 26.6 months; respectively) and **Song *et al.*** [30] reported that INPP4B positive subjects had significant decreased OS time. Clinical analysis suggests that the associated decreased survival may be attributed to reduced likelihood of achieving remission due to decreased response to therapy.

In the current study, other survival analysis revealed that INPP4B high expression group had significantly shorter EFS and DFS compared with low expression group. This was in agreement with **Song *et al.*** [30] who reported that EFS and DFS time of INPP4B positive patients were significantly shorter compared with INPP4B negative AML patients. Also, **Dzneladze *et al.*** [10] reported significantly shorter 3 years EFS in the high expression group than low expression group with percent probability of (18.6% vs. 33.2%; respectively). While, reported no statistically significant difference between both groups regarding RFS with percent probability of (58.4% vs. 55.1%; respectively). Also, in agreement with our results, **Rijal *et al.*** [11] reported that RFS was statistically significant lower in the INPP4B high expression group than low expression group with median survival of (6.2 vs. 11.8 months; respectively).

So, overexpression of INPP4B is associated with poor outcome in AML patients and INPP4B high expression is an independent prognostic marker in AML patients, which can improve AML prognostication model, predicting poor prognosis[11]. Also, INPP4B becomes a possible target for directed therapy due to the proposed oncogenic role of INPP4B in AML. Additional work is still needed to investigate the role of INPP4B in promoting AML and its potential and effectiveness as a target for therapy in AML patients [10].

CONCLUSION

INPP4B overexpression would expect a high risk AML patients characterized by reduced response to chemotherapy, and shorter OS survival outcomes. If this will be validated that INPP4B overexpression is an important prognostic marker in AML patients, they could be considered as candidates for alternative and experimental treatment trials. A better understanding on how INPP4B overexpression induce drug resistance could stimulate the development of INPP4B-directed target therapies in the future.

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REFERENCES:

1. **Papaemmanuil E, Gerstung M, Bullinger L *et al.* (2016):** Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med., 374: 2209–2221.
2. **Park S, Chapuis N, Tamburini J *et al.* (2010):** Role of the PI3K/AKT and mTOR signaling pathways in acute myeloid leukemia. Haematologica., 95: 819–828.
3. **Fransecky L, Mochmann L, Baldus C (2015):** Outlook on PI3K/AKT/mTOR inhibition in acute leukemia. Mol Cell Ther., 3: 2-9.
4. **Faes S, Dormond O (2015):** PI3K and AKT: unfaithful partners in cancer. Int J Mol Sci., 16:21138–21152.
5. **Eramo M, Mitchell C (2016):** Regulation of PtdIns (3,4,5) P3/Akt signalling by inositol polyphosphate 5-phosphatases. Biochem Soc Trans., 44: 240–252.
6. **Gewinner C, Wang Z, Richardson A *et al.* (2009):** Evidence that inositol polyphosphate 4-phosphatase type II is a tumor suppressor that inhibits PI3K signaling. Cancer Cell, 16: 115–125.
7. **Vo T and Fruman D (2015):** INPP4B is a tumor suppressor in the context of PTEN deficiency. Cancer Discov., 5: 697–700.
8. **Zhang L, Zeng D, Chen Y *et al.* (2016):** miR-937 contributes to the lung cancer cell proliferation by targeting INPP4B. Life Sci., 155: 110-115.
9. **Woolley J, Dzneladze I, Salmena L (2015):** Phosphoinositide signaling in cancer: INPP4B Akt (s) out. Trends Mol Med., 21: 530–539.
10. **Dzneladze I, He R, Woolley J *et al.* (2016):** INPP4B overexpression is associated with poor clinical outcome and therapy resistance in acute myeloid leukemia. Leukemia, 29: 1485-1495.
11. **Rijal S, Fleming S, Cummings N *et al.* (2015):** Inositol polyphosphate 4- phosphatase II (INPP4B) is associated with chemoresistance and poor outcome in AML. Blood., 125:2815–2824.
12. **Bruhn M, Pearson R, Hannan R *et al.* (2010):** Second AKT: the rise of SGK in cancer signalling. Growth Factors, 28: 394–408.
13. **Lien E, Dibble C, Toker A (2017):** PI3K signaling in cancer: beyond AKT. Curr Opin Cell Biol., 45: 62–71.
14. **Tessier M, Woodgett J (2006):** Role of the Phox homology domain and phosphorylation in activation of serum and glucocorticoid-regulated kinase- 3. J Biol Chem., 281: 23978–23989.
15. **Vasudevan K, Barbie D, Davies M *et al.* (2009):** AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. Cancer Cell, 16: 21–32.
16. **Gasser J, Inuzuka H, Lau A *et al.* (2014):** SGK3 mediates INPP4B-dependent PI3K signaling in breast cancer. Mol Cell, 56: 595–607.
17. **Jin H, Yang L, Wang L *et al.* (2018):** INPP4B promotes cell survival via SGK3 activation in NPM1-mutated leukemia. Journal of Experimental and Clinical Cancer Research., 37: 8-18.
18. **Deretic V, Klionsky D (2018):** Autophagy and inflammation: a special review issue. Autophagy, 14: 1–4.

19. **Tooze S, Yoshimori T (2010):** The origin of the autophagosomal membrane. Nat Cell Biol., 12:831–835.
20. **Hasan M, Majid N, Mustafa M (2018):** Activation of autophagy by stress-activated signals as a cellular self-defense mechanism against the cytotoxic effects of MBIC in human breast cancer cells in vitro. Biochem Pharmacol., 152: 174–186.
21. **Song S, Tan J, Miao Y *et al.* (2017):** Crosstalk of autophagy and apoptosis: involvement of the dual role of autophagy under ER stress. J Cell Physiol., 232: 2977-2986.
22. **Yang J, Moon H, Chettimada S *et al.* (2014):** Cross-talk between apoptosis and autophagy in lung epithelial cell death. J Biochem Pharmacol Res., 2: 99-108.
23. **Qiang L, Chen L, Atkinson J *et al.* (2016):** Atg5-dependent autophagy contributes to the development of acute myeloid leukemia in an MLL-AF9-driven mouse model. Cell Death Dis., 7:2361-6.
24. **Folkerts H, Hilgendorf S, Wierenga A *et al.* (2017):** Inhibition of autophagy as a treatment strategy for p53 wild-type acute myeloid leukemia. Cell Death Dis., 8:2927-35.
25. **Manzella L, Tirrò E, Pennisi M *et al.* (2016):** Roles of interferon regulatory factors in chronic myeloid leukemia. Current Cancer Drug Targets, 16: 594–605.
26. **Zhang F, Zhu J, Li J *et al.* (2017):** IRF2–INPP4B axis participates in the development of acute myeloid leukemia by regulating cell growth and survival. Gene, 12: 627:629.
27. **Zhang F, Li J, Zhu J *et al.* (2019):** IRF2–INPP4B-mediated autophagy suppresses apoptosis in acute myeloid leukemia cells. Biol Res., 52: 11-17.
28. **Swansbury J (2003):** Cancer cytogenetics: methods and protocols; cytogenetic techniques for myeloid disorders. Mol Biol., 220: 43–57.
29. **Livak K, Schmittgen T (2001):** Analysis of relative gene expression data using real time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods, 25: 402–408.
30. **Song H, Zhang Y, Liu Y *et al.* (2019):** The expression of PTEN and INPP4B and their clinical significance in patients with acute myeloid leukemia. European Journal of Inflammation, 7: 1-5.