Journal of the Egyptian Society of Haematology & Research, Vol. 15, No. 1, March: 23-28, 2019

Impact of TP53 Mutation on Induction Therapy in De Novo Egyptian Pediatric Acute Lymphoblastic Leukemia

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ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is malignant transformation and proliferation of lymphoid progenitor cells in bone marrow, blood and extramedullary sites.

TP53 is the guardian of genome and plays crucial role in regulation of cell cycle, apoptosis, Deoxyribonucleic acid (DNA) repair and angiogenesis.

In hematological malignancies TP53 mutations are not frequent. However, in these malignancies a strong correlation was found to be associated with resistance to chemotherapy.

Aim: This study was designed to investigate impact of TP53 mutation on response to induction therapy in newly diagnosed Egyptian pediatric ALL patients.

Methods: Forty de novo pediatric ALL patients coming to National Cancer Institute (NCI), Cairo University from May 2017 to February 2019, were included. Bone marrow aspirations (BMA) were obtained from all patients at diagnosis. Then, cultured, harvested and prepared for fluorescent in situ hybridization (FISH) to study TP53 gene using commercially available probe from Vysis LSI TP53 (17p 13.1) Spectrum Orange probe according to manufacturer's protocol Abbott/Vysis with small adjustments. All patients received total 15 induction therapy and assessed at end of phase 2 induction.

Results: Out of the 40 patients included in this study; Two copies of TP53 were expressed in 35 patients (87.5%), deletion of one copy was found in 2 patients (5%) and 3 copies were found in 3 patients (7.5 %). TP53 gene mutation had noimpact on response to induction therapy in our patients.

Conclusions: In this study we found that TP53 gene mutation had no impact on response to induction therapy inchildhood ALL, yet we found a significant relationship between high risk patients and both the deletion of one copy and the presence of an extra copy of TP53 gene. Further large prospective trials are needed to confirm these conclusions.

Key Words: *TP53 – Pediatric ALL – Induction therapy – Response – Risk status.*

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood and extramedullary sites **[1]**. It is the most common leukemia in children, accounting for up to 80% of leukemias in this group and 20% in adults **[2]**.

TP53, a tumor suppressor protein, mutations are among the most common genetic alterations observed in cancers and occur in about 50% of tumors **[3]**. Human TP53 is a nuclear phosphoprotein encoded by a 20-Kb gene containing 11 exons and 10 introns, which is located on the small arm of chromosome 17 **[4]**.

TP53 plays a crucial role in cell cycle regulation and apoptosis after deoxyribonucleic acid (DNA) damage, and its role in tumorigenesis is well-recognized in solid and hematologic malignancies, particularly acute myeloid leukemia and chronic myeloid leukemia, in which its deregulation represents an important predictor of poor outcome. In ALL, TP53 mutations have been poorly investigated, mainly in children, for whom the incidence is low at diagnosis, increases at relapse, and is associated with poor outcome **[5]**.

In ALL, genetic alterations affecting TP53 are uncommon at diagnosis, with their incidence being less than 5%. An exception is the rare low hypodiploid ALL subtype (32-39 chromo-

somes), in which there are almost invariably mutations or deletions affecting TP53 **[6]**.

Also in relapsed ALL, the chance of TP53 mutations or deletions rises to about 10% and represents a strong and independent predictor of treatment failure. TP53 alterations result in either a loss of protein expression or the generation of protein variants with (partly) impaired function. Regardless of whether a wildtype allele is still present, TP53 mutations or deletions in relapsed ALL predict a highly unfavorable response to therapy **[6]**.

MATERIAL AND METHODS

This study included 40 de novo Egyptian pediatric ALL patients attending National Cancer Institute (NCI), Cairo University; during the time period from May 2017 to February 2019.

The study was conducted after institutional ethical approval. Written informed consent was obtained from all patients and/or their parents.

Patients included ages between 2 and 18 years old, with confirmed diagnosis of ALL and were classified according to morphology, cytochemistry, immunophenotyping and cytogenetics.

Patients were monitored regularly in the oncology outpatient clinics and treated with the current chemotherapy protocols (Total XV induction therapy).

To confirm the diagnosis of ALL, BMA smears were stained with Leishman stain (Sigma -Aldrich-USA) and cytochemical stains, namely, myeloperoxidase (MPO) (Power stain 1.0 Poly HRP DAB kit for mouse + rabbit-Genemed Biotechnologies- USA) and Sudan black (SBB) (Sigma-Aldrich-USA).

BMA specimens from sodium heparin tubes were cultured, harvested, fixed, and placed on microscope slides utilizing standard cytogenetic method and prepared for fluorescence in situ hybridization (FISH) analysis of TP53 gene.

FISH analysis was done with commercially available probe from Vysis LSI TP53 (17p 13.1) Spectrum Orange probe according to manufacturer's protocol Abbott/Vysis with small adjustments. Slides were analyzed using an Imager fluorescence microscope (equipped with filter sets for DAPI and Spectrum Orange at a magnification of x1,000). Images were captured using Meta Systems digital camera and analyzed using Isis version 5.2, Meta Systems software for quantitative analysis of samples generated by FISH technique. For each subject hybridized signals were counted in 200 interphase nuclei.

Patients were classified to low, intermediate or high risk based on age, white blood count (WBC) count, immunophenotype, and central nervous system (CNS) involvement at diagnosis; in addition to their cytogenetic and molecular status **[7]**.

Response assessment:

Complete remission (CR) is defined as <5% marrow blasts with peripheral blood (PB) count recovery, evidence of normal hematopoiesis, and absence of extramedullary disease.

Statistical methods:

Data were analyzed using SPSS statistical package version 17. Qualitative variables are presented as proportions and quantitative variables are presented as mean ± standard deviation (SD) or median and range as appropriate. The comparison between qualitative variables were done using Chi-square test or Fisher's exact as appropriate and *p* less than 0.05 is considered significant. Qualitative data were expressed as frequency and percentage. Survival analysis was done using Kaplan-Meier method.

RESULTS

I- *Patients characteristics:*

Clinical and hematological characteristics of the 40 patients are listed in Table (1).

Risk stratification of the 40 patients included in the study:

In this study, 25 patients (62.5%) were standard risk, 12 patients (30%) showed low risk and 3 patients (7.5%) were high risk.

Treatment protocol:

All the 40 patients started their induction of remission therapy, 38 patients (95%) were given induction total XV therapy protocol, and the remaining 2 t(9; 22) (BCR-ABL fusion gene) positive patients were given an additional targeted therapy (Glivec), (total XV + Glivec).

Assessment of response:

Thirty one out of 40 patients (77.5%) were in CR at day 15 (early assessment) of induction of therapy, 28 patients (70%) continued induction till day 42 (late assessment) and were in CR. Four out of 28 patients who had been in CR1 (14.3%) had relapsed: 1 out of 4 patients had an isolated CNS relapse and received re-induction therapy with German protocol, the 3 other patients had bone marrow (BM) relapse and were given re-induction with FLAGM protocol. Three out of 4 relapsed patients were in second CR, and 1 patient died.

Table (1): Clinical and hematological findings at diagnosis of 40 ALL patients.

	N=40	%
Age:		
Median (range) (years)	8.5 (2.0-18.0)	
Sex:		
Male	31.00	77.50
Female	9.00	22.50
Initial blood count:		
TLC: $x10^9$/L: Median (range)	9.85 (1.4-109.0)	
Hb: gm/dl *Median (range)	8.6 (4.1-16.0)	
PLT: $x10^9$/L *Median (range)	53 (4-279)	
PB Blasts % *Median (range)	25.00% (0.0-88.0)	
Initial Bone Marrow (BM) Aspirate:		
BM blasts % *Median (range)	88.00% (17.00-99.0)	
BM Cellularity:		
Normocellular	9.00	22.50
Hypocellular	8.00	20.00
Hypercellular	23.00	57.50
TP53:		
Both copies of TP53	35.00	87.50
3 copies of TP53	3.00	7.50
del of TP53	2.00	5.00
Immunophenotype:		
Pre B-ALL	32.00	80.00
cALL	8.00	20.00

Table (2): Cytogenetic and molecular findings of 40 ALL patients at diagnosis.

Parameter	N=40	%
t(4;11)	0	0.0
t(1;19)	1	2.5
t(12;21)	2	5.0
t(9;22) p190	2	5.0
t(9;22) p210	0	0.0

Table (3): Conventional karyotyping and FISH of 40 ALL patients at diagnosis.

Conventional Karyotyping:		
45,XY,-21	1	2.5%
46 XX	6	15.0%
46 XY, t(12, 21)	1	2.5%
46 XY, t(9,22)	1	2.5%
46,XY	9	22.5%
47,XY, +17	1	2.5%
50 XY,	1	2.5%
51,XY	1	2.5%
52 XY,	1	2.5%
52,XY	1	2.5%
55XX, Hyper diploid	1	2.5%
No mitosis	10	25.0%
Not done	6	15.0%
FISH:		
Not done	37	92.5%
t(1, 19)	1	2.5%
t(12,21)	1	2.5%
t(9,22)	1	2.5%

II- *TP53 analysis:*

Out of the 40 patients included in this study, 35 patients (87.5%) were found to have wild type TP53 (expressing both copies of TP53), while only 2 patients (5%) were found to have deletion of one copy TP53 and 3 patients (7.5%) expressed 3 copies of TP53.

A- *Relation between TP53 gene expression and clinical and hematological parameters:*

We revealed no significant statistical relationship between different TP53 gene expression status and different clinical parameters of the patients and different cytogenetic categories, (Table 4).

After dividing patients total leucocytic count (TLC) into 2 groups above or below $50x10^9$/L we found a statistically significant relation between TLC $>50x10^9$/L and deletion of one copy of TP53 gene with a *p*-value 0.031. A statistically significant relationship was found between cases with high risk group and both the deletion of one copy and the presence of an extra copy (copy number alteration) of TP53 gene with a *p*-value of 0.042, as shown in Table (4).

B- *Relation between the response to induction therapy and TP53 gene mutation:*

In Table (5) we display the relation between response to induction therapy and different TP53 gene mutation status. There was no statistical significant relationship among all groups.

Table (4): Relation of TP53 gene expression and Clinical parameters of 40 ALL patients.

Parameter	Two copies of TP53		3 copies of TP53		del of TP53		*p*-value
	N	%	N	%	N	%	
Age group: Years:							
2-10	18	85.7	3	14.3	0	0.0	0.114
>10	17	89.5	0	0.0	2	10.5	
Sex:							
Male	26	74.3	3	100.0	2	100.0	1.000
Female	9	25.7	0	0.0	0	0.0	
Organomegaly:							
No	17	94.4	0	0.0	1	5.6	0.300
Yes	18	81.8	3	13.6	1	4.5	
Cerebro Spinal Fluid involvement:							
No	33	86.8	3	7.9	2	5.3	1.000
Yes	2	100.0	0	0.0	0	0.0	
Lymphadenopathy:							
No	18	90.0	1	5.0	1	5.0	1.000
Yes	17	85.0	2	10.0	1	5.0	
Total Leukocytic Count group:							
≤50000	31	91.2	3	8.8	0	0.0	0.031*
>50000	4	66.7	0	0.0	2	33.3	
Risk stratification:							
Standard Risk	23	92.0	1	4.0	1	4.0	0.042*
Low Risk	11	91.7	1	8.3	0	0.0	
High Risk	1	33.3	1	33.3	1	33.3	
Karyotype:							
Hyperdiploid	5	83.3	1	16.7	0	0.0	0.187
Translocation	3	100.0	0	0.0	0	0.0	
Normal	15	100.0	0	0.0	0	0.0	
No mitosis	7	70.0	2	20.0	1	10.0	

Table (5): Relation between the response to induction therapy and TP53 gene expression in 40 ALL patients.

Parameter	Two copies of TP53		3 copies of TP53		del of TP53		*p*-value
	N	%	N	%	N	%	
CR1:							
No	2	6.9	1	33.3	0	0.0	0.390
Yes	27	93.1	2	66.7	2	100.0	
CR2:							
No	0	0.0	0	0.0	0	0.0	0.30
Yes	2	100.0	0	0.0	1	100.0	
Relapse:							
No	20	87.0	1	100.0	0	0.0	0.30
Yes	3	13.0	0	0.0	1	100.0	
Status:							
Alive	20	57.1	1	33.3	0	0.0	0.31
Dead	15	42.9	2	66.7	2	100.0	
Death:							
Early	11	73.3	2	100.0	0	0.0	0.17
Late	4	26.7	0	0.0	2	100.0	

CR1: First complete remission. CR2: Second complete remission.

DISCUSSION

In this study, we evaluated the TP53 status in 40 newly diagnosed ALL patients.

Only 2 patients (5%) showed deletion of one copy of TP53 gene, while 3 patients (7.5%) had an extra copy, and the remaining 35 patients (87.5%) were expressing normally the two copies of TP53 gene. This is lower than the 5% and 14.5% previously reported **[8,9]**.

Hof et al. **[10]** studied children with ALL in relapse and recorded TP53 copy number alteration and sequence alterations in 12.4% (27 of 218) of patients with B-cell precursor ALL and 6.4% (three of 47) of patients with T-cell ALL Backtracking to initial ALL in 23 samples revealed that 54% of TP53 alterations were gained at relapse.

Regarding age, the 2 children with deletion of one copy of TP53 gene were older while the 3 having the extra copy of the gene were younger. Qian et al., **[11]**, reported that children with TP53 gene variants were older at diagnosis. A univariate analysis showed a clear relationship with a linear trend between the presence of TP53 mutations and increasing age **[8]**. Our results showed no significant statistical relation between age and TP53 gene mutation; this matches the finding of Klobusˇická et al., **[12]** who also found no correlation between age and TP53 gene expression.

In the current study, we found a statistically significant relationship between TP53 gene mutation and high initial WBCs (p=0.019) this is against other studies done by Klobusˇická et al., **[12]** and Moueden et al., **[8]** who stated that they did not find a significant relationship between TP53 abnormalities and WBC.

In our current study, we found a significant statistical relationship between TP53 gene mutation and high risk patients (p=0.042); to the best of our knowledge, no previous studies addressed the relation of the TP53 and risk stratification in ALL in children.

Regarding cytogenetics studies done for our 40 patients, our results were totally different from the large cohort study of Stengel et al., **[13]**, where they found that TP53 mutations are predominantly associated with ALL with low hypodiploidy and MYC-translocated ALL and with short survival independent of age and specific cytogenetic alterations. Comeaux and Mullighan, **[14]** also stated that TP53 alterations are present in almost all cases of ALL with low hypodiploidy and are associated with alterations of the lymphoid transcription factor IKZF2 and the tumor-suppressor gene loci CDKN2A and CDKN2B. Remarkably, more than half of TP53 mutations are found in low-hypodiploid ALL in children. The difference in our results from those of other studies may be due to difference in number of recruited patients and age groups.

In the current study, cases with TP53 gene deletion were associated with relapse and these while one of the cases who had an extra copy of the gene relapsed and the other two cases died early after induction therapy; these findings are in agreement with other studies stating that TP53 gene abnormalities are usually associated with poor outcome **[11]**.

In conclusion, we found that there was no impact on TP53 gene mutation or gene copy number alteration on response to induction therapy in newly diagnosed Egyptian pediatric ALL patients. Yet there was a relationship between risk status of the patients and TP53 gene mutation. The results should be cautiously interpreted as we are reporting only two cases with deletion and three with an extra p53 copy.

Further large studies are recommended to prove or deny this finding.

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