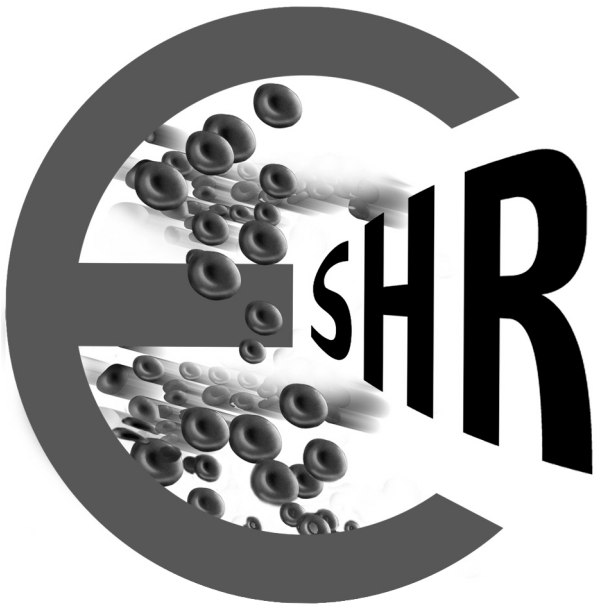
The Journal of the Egyptian Society of Haematology & Research



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Iron Deficiency Anemia in Egypt: Impact on Growth and Relation to Serum Level of Interlukein-2

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ABSTRACT

Background: Iron deficiency is the most common cause of anemia in the developing countries. Late diagnosis and treatment in school aged children lead to stunting and affect neurocognitive development. Iron deficiency may reduce the level of serum interleukin 2 and increase the susceptibility to infections.

Objectives: To study the iron status in children aged 6 to 12 years of both sexes and to investigate the relation of iron deficiency to growth and of serum level of IL2.

Patients and Methods: Three hundred sixty nine children 6 to 12 years were enrolled in the study, 242 (65.6%) were males and 127 (34.4%) were females; M/F ratio 1.9. Anthropometric measures (weight, height and BMI) were studied in all cases and analyzed by growth curves according to their age and sex. Red cell indices and iron profile were measured in all participants. IL2 was measured in the serum of 100 patients with iron deficiency and 50 patients with normal iron profile.

Results: The prevalence of iron deficiency anemia (IDA) among the studied children was 40.4%. Up to 70.5% (n=105) of IDA patients were <10 years, 47.7% (n=71) were short, and one third (n=50) were underweight. Patients with IDA had statistically significant decrease in the level of RBCs count, MCV, MCHC, serum iron, transferrin saturation and raised TIBC when compared to normal subjects (p<0.05). Serum ferritin was lower in IDA group but the difference from normal subjects was marginally insignificant (p=0.058). Serum IL2 was significantly low in the serum of the IDA patients when compared to cases with normal iron status (p=0.001).

Conclusion: Iron deficiency anemia is common in Egyptian children younger than 10 years. Children with IDA looked predominantly short rather than underweight. Low serum iron, low transferrin saturation, and raised total iron-binding capacity might be the reliable markers in diagnosis of IDA. IDA patients had decreased levels of serum IL2 which may increase the risk of infections.

Key Words: *Iron deficiency anemia – Growth – IL2.*

INTRODUCTION

Iron deficiency is the most common cause of anemia worldwide. It has been identified as a modifiable risk factor for the poor development of more than 200 million children in developing countries **[1]**. Failure to investigate iron deficiency anemia (IDA) appropriately in primary care can cause significant delay in the final diagnosis of associated morbidities **[2]**. Malnutrition with underlying multiple vitamin and mineral deficiencies is found to be common among young children and is usually coupled with iron deficiency anemia **[3]**. Among the well described consequences of anemia are impaired physical growth, immune alterations and increased susceptibility to infections **[4]**.

The relation between recurrent infections and iron deficiency is not well studied. Few studies found that mild iron deficiency might be protective against infections; yet other studies showed the opposite results **[5,6]**. They reported that inflammatory cytokines like IL-2 are reduced in children with iron deficiency anemia **[7-9]**; this may cause disturbance of the cell mediated immunity function **[10]**. IL2 is a growth factor secreted from T cells and results into the formation of effector T cells as well as antibody formation hence its important relation to immunity **[11]**. Children with iron deficiency might have abnormally low levels of IL-2. The aim of the study was to assess the iron status in children 6 to 12 years, and to investigate the association of iron deficiency with growth of children and serum level of IL2.

PATIENTS AND METHODS

This was a prospective cross-sectional study carried out on 369 children aged 6-12 years. They were recruited from the outpatient clinics of Abou El-Reesh Hospital, Cairo University during the period from January to December 2017. The study received Institutional Review Board (IRB) approval and was conducted in accordance with the University bylaws for human research and Helsenki declaration for studies on human subjects. A written informed consent was obtained from the legal guardians and assent from children in accordance with the IRB guidelines. A sample size of 369 children was estimated by using anemia prevalence of 20% at 95% confidence level. Patients with chronic diseases, genetic and chromosomal anomalies and active infection proven by elevated CRP were excluded. A detailed clinical history and relevant physical examination were done. The criteria for IDA were combination of hypochromic microcytic anemia with low ferritin, low transferrin saturation, low iron, and raised total iron-binding capacity **[12]**.

Specimen collection and evaluation: Whole blood samples were collected into EDTA tubes for determining the Red blood cell indices automatically using the blood counter (Diagon Ltd D-cell 60). Serum samples were separated and frozen for estimation of serum levels of iron, transferrin saturation, TIBC and IL2. Serum iron and total iron binding capacity (TIBC) were measured using Olympus 400 auto-analyzer. Serum ferritin was measured for all cases by Microparticle Enzyme Immunoassay (AxSYM, Abbott, USA). IL2 was measured by enzyme-linked immune assay (ELISA) technique **[11]**. Transferrin saturation was calculated by following the equation: [Total iron] / [TIBC] x 100 **[13]**.

Statistical analysis: Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 23. Data was summarized using mean, standard deviation, and range in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. The quantitative variables were compared using paired *t*-test. The comparison of qualitative variables was performed using chi-square test or Fisher's exact test. *p*-values less than 0.05 were considered as statistically significant.

RESULTS

Demographics and clinical data: The study included 369 children from 6 to 12 years of age. They were 242 (65.6%) males and 127 (34.4%) females; M/F ratio 1.9. The most common clinical presentation included pallor (85%), fatigue (75%), exertional dyspnea (67%), decreased attention span (45%), lack of concentration (42%), headache (30%), glossitis and angular stomatitis (10%). The prevalence of iron deficiency anemia (IDA) among the studied children was 40.4%. Table (1) illustrates a comparison between patients with IDA deficiency and cases with normal iron profile. Up to 70.5% (n=105) of IDA patients were aged below 10 years, 47.7% (n=71) were short, and one third (n=50) were underweight. The frequency of IDA among males and females was comparable (42.15% and 37.0% respectively; p= 0.339).

Table (1): Comparison between patients with non-IDA and IDA regarding demographic data and anthropometric measures.

Parameter	Non-IDA (n=220)		IDA (n=149)		*p*-value
	No.	%	No.	%	
Age group:					
Age <10 yrs	167	75.9	105	70.5	0.244
Age >10 yrs	53	24.1	44	29.5	
Sex:					
Female	80	63	47	37.0	0.339
Male	140	57.85	102	42.15	
Height for age:					
Normal	142	64.5	78	52.3	0.019*
Stunted	78	35.5	71	47.7	
BMI for age:					
Normal	159	72.3	115	77.2	0.290
Wasted	61	27.7	34	22.8	
Weight for height:					
Normal	107	48.6	99	66.3	0.066
Wasted	113	51.4	50	33.7	
Consanguinity:					
Positive	61	27.7	49	32.9	0.288
Negative	159	72.3	100	67.1	
Family History:					
Positive	31	14.1	13	8.7	0.119
Negative					

*Statistically significant.

Table (2) shows that there was statistically significant decrease in the level of RBCs count, MCV, MCHC, iron, transferring saturation and

raised TIBC in patients with IDA when compared to normal subjects (p<0.05). Patients with IDA had lower serum ferritin in comparison to normal subjects but the difference was marginally insignificant (p=0.058).

Table (2): Comparison of CBC parameters, reticulocyte count and iron profile between IDA patients and non-IDA groups.

Variables	Non-IDA (n=220)	IDA (n=149)	*p*-value
	Mean ± SD	Mean ± SD	
RBC (millions/cmm)	4.20±0.54	3.01±0.49	<0.001*
MCV (fl)	77.47±6.37	60.09±6.53	0.015*
MCH (pg)	27.32±3.53	26.03±3.37	0.190
MCHC (g/dl)	33.56±4.48	31.03±4.15	0.045*
RDW (%)	14.45±1.03	14.52±0.96	0.077
Retics (%)	1.69±1.18	0.9±0.15	0.080
Serum Iron (mg/dl)	75.07±21.86	27.05±10.82	<0.001*
Serum Ferritin (ng/ml)	62.07±19.99	19.80±11.02	0.058
TIBC	240.08±56.87	329.19±42.74	<0.001*
Transferrin saturation (%)	23.06±6.84	14.02±9.41	<0.001*

*Statistically significant.

Serum IL-2 test was carried out to observe the level of cytokine concentration in 100 children with documented IDA in addition to 50 children of non-IDA as a control group. IL2 was significantly lower in the IDA group as compared to the non-IDA group (8.76±0.6, range 3.5-13.9 vs. 31±1.2, range 20.5-195.9 pg/ml respectively; p=0.001).

DISCUSSION

The present study included 369 children aged between 6 and 12 years, none of them had a history of chronic illness or medication. Their main presenting symptoms were pallor (85%), fatigue (75%), and exertional dyspnea (67%). It was reported that nine out of ten anemia sufferers live in developing countries and the most vulnerable, the poorest and the least educated are disproportionately affected by iron deficiency and anemia **[14]**. Poor dietary habits and decreased intake of iron containing food in the study group was reported in up to 65.9% which increased to 95% in IDA group. This was in line with previous reports as Al Ghwass et al., study highlighted the ingestion of low iron containing foods as a significant predictor of IDA **[15]**, which agrees with previous similar studies **[16,17]**.

Detailed interpretation of the iron profile of the studied patients showed that 40.4% had IDA. This is similar to results of a study reporting that anemia with depleted iron stores was identified in 47.1% of children **[18]**. Our rate is lower than that reported in a previous study including 245 5-11 years old children with 58% prevalence of anemia **[19]**. However, a lower rate was reported by Legason et al., who studied 342 children and results revealed that anemia prevalence was 34.4% **[20]**.

Among our patients, the frequency of IDA among males and females was comparable (p=0.339). This was in harmony with results of Murila and associates, studied 403 children aged 6 months to 6 years and found no association between IDA and sex **[20]**. Our results disagree with an Egyptian study carried out by Al-Gawas et al., who reported male predominance (58.3%) in studied patients with a significantly higher prevalence of ID (with or without anemia) among males (85.33%) compared to females (p=0.004) **[15]**.

In our study, nearly half (47.7%) of IDA patients showed decreased height for age and 33.7% had decreased weight for height. This is in line with a study carried out on young Egyptian children which showed that stunting, wasting and underweight were associated with iron deficiency anemia, but the underweight only was statistically significant **[15]**. Similarly, Gosdin et al., reported that the prevalence of decreased height for age in their patients with IDA was 44.2% **[21]**. Moreover, Kishawi et al., who carried out a study on 357 children found that anemia in children was significantly associated with underweight **[22]**. Also Luo et al., mentioned that children with anemia were shorter for their age, and a higher percentage of them had stunted growth **[23]**. Lower rates of abnormal weight or height parameters among anemic children were reported in previous studies. In a study of 184 children with anemia, Gwetu and colleagues reported that the prevalence of decreased height for age was 7.6%, and decreased BMI for age was 1.1% **[24]**.

In our study, we found significantly lower levels of MCV, MCHC, iron level, transferrin saturation and raised TIBC in the IDA patients

when compared to Non-IDA, but no statistical difference found in other parameters (MCH and Ferritin). Another study, El Baroudi et al., **[19]**. reported that ferritin is an inflammatory marker that we should not consider as a reliable marker for diagnosis of iron deficiency anemia. On the other hand, they confirmed that MCV and MCH are the most sensitive parameters for IDA diagnosis. This is not shown in our study, as MCH was not significantly different between the 2 groups and need further studies to be confirmed.

In our present study, we found significantly lower levels of serum IL2 in IDA patients when compared to children with normal iron values. Our study is in agreement with several studies which reported the important role of iron in the process of activation and proliferation of T cells which is needed for the maintenance of the cellular immune response **[8,9]**. In their study, Suegaand and Bakta reported the significant increase in plasma IL2 of iron deficiency patients after treatment with iron tablets for 8 weeks, which confirms the key roles of iron in T cell proliferation, activation and function **[11]**.

In conclusion, iron deficiency anemia is common in Egyptian children younger than 10 years. Children with IDA looked predominantly short rather than underweight. Low serum iron, low transferrin saturation, and raised total iron-binding capacity might be the reliable markers for diagnosis of IDA. IDA patients had decreased levels of serum IL2 which may increase susceptibility to infections.

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Characteristics and Outcomes of Patients with Myelodysplastic Syndrome: First Report from Upper Egypt

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ABSTRACT

Background and Objectives: Myelodysplastic syndromes (MDS) are a group of myeloid neoplasms with significant clinical heterogeneity and variable overall survival (OS). This was the first study that aimed to assess characteristics and outcomes of MDS patients at Upper Egypt.

Patients and Methods: Seventy six MDS patients were prospectively enrolled in the study; they were recruited in the period Jan. 2017 – Jan. 2019. Data were collected at enrollment and after 3-months to assess overall response rate (ORR).

Results: Patients' median age at diagnosis was 44.5 years, 50% of them were in age range 18-45 years. Female predominance 46 (60.5%) was obvious with M:F ratio 1:1.5. Rural residency and exposure to fertilizers were reported in 62% and 15.8%, respectively. Eastern co-operative group (ECOG) performance status of most patients was good and only 40% have co-morbid diseases. Hematologically, anemia was normocytic normochromic in 40 (52.6%) and BM hypercellular in 43 (56.6%). ORR and complete remission (CR) were reported in 55% and 1.3%, respectively. Median OS was 13 and the longest 168 months, without significant gender differences.

Conclusion: Compared with other studies, this study showed a younger age and female predominance of MDS at Upper Egypt. It reports normocytosis, rather than macrocytosis, to be the salient feature of MDS in our region. Moreover, the study concludes lower ORR and CR of our patients and the hematologic improvement at level of erythrocyte is the most achievable therapeutic response. Finally, the study concludes that MDS could be an indolent disease with long OS up to 14-years.

Key Words: *Myelodysplastic syndrome – Upper Egypt – Survival.*

INTRODUCTION

Myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid neoplasms characterized by peripheral cytopenias, bone marrow (BM) failure, morphologic dysplasia in one or more hematopoietic lineage, and genetic instability with an increased risk to transform to acute myeloid leukemia (AML) **[1-4]**. The disease progression of MDS is multistep with a series of genetic events that reduce the ability of the proliferating clone to differentiate and mature. Hematopoiesis is ineffective, due to premature apoptosis, with the apparent paradox of peripheral cytopenia in one or more cell lines associated with hypercellular BM. The risk of transformation to acute myeloid leukemia (AML) is variable and the clinical outcome is greatly heterogeneous. Therefore, MDS constitutes a complex hematological problem that gives rise to difficulties in diagnosis and therapeutic decision-making **[5-7]**.

The incidence of MDS has appeared to be increasing. The apparent rise was presumed to reflect improvements in recognition and criteria for the diagnosis **[8]**. MDS can be classified as primary (de novo) or secondary. Eighty% of patients with MDS do not have an obvious cause as being secondary to aggressive treatment of other cancers with exposure to radiation, alkylating agents, or topoisomerase II inhibitors. It also occurs in heavily pretreated patients who underwent autologous bone marrow transplants. In certain patients, MDS is an indolent disorder; serious cytopenias occur in other patients and

in the remainder of cases, the disease follows an aggressive course and transforms into secondary AML **[9,10]**.

The standard care for patients with MDS and decreased blood counts is constantly evolving. Supportive therapy is the main component of care. The therapeutic strategy for MDS is based on the revised International Prognostic Scoring System (IPSS-R) score, patient's age, co-morbidities, patient's expectations and personal goals. The more toxic and aggressive forms of therapy, such as stem cell transplantation and aggressive chemotherapy, are reserved for fit and young patients with high-risk disease. The hypomethylating agent azacytidine has been shown to improve survival compared with either supportive or aggressive therapy **[11-13]**.

Numerous researchers reported differences in incidence of MDS, disease presentation, progression and outcome, among different countries and regions **[14-16]**. Upper Egypt is a region in South Egypt and far away from Mediterranean basin. It has different demographic characteristics regarding gender (male to female ratio), age (life expectancy), occupation, level of education, ethnicity, income, geographical location, social class, and various other aspects of the population which differ from lower Egypt. These differences affect risk factors and characteristics of many diseases particularly hematological diseases. To our knowledge, this is the first study of MDS in Upper Egypt. The study aimed to explore demographic and clinical characteristics, hematologic, morphologic, and BM features of patients with MDS residing in Upper Egypt. Moreover, the study assessed ORR to various treatment modalities, OS, PFS and clinical outcomes in those patients. The overall study objectives were to provide a full scenario of MDS in Upper Egypt territory; thus could help hematologists, oncologists and clinical pathologists when dealing with those patients.

PATIENTS AND METHODS

Study design, patients and settings:

This prospective longitudinal study was conducted at the Clinical Hematology Unit of the Internal Medicine Department and the Hematology Laboratory at Assiut University Hospital and the Medical Oncology Department, South Egypt Cancer Institute, in the period from Jan. 2017 to Jan. 2019. The study included MDS patients who were attending/admitted in those departments during the study period. Both newly diagnosed patients and follow-up patients were recruited. Patients' data were collected at enrollment in the study and after 8-12 weeks follow-up to assess patients' response to treatment.

Methods:

Patients' demographic and clinical data were collected through medical history taking and clinical examination.

Diagnosis of MDS patients:

MDS was diagnosed, in the study patients by clinical suspicion in those presented with manifestations of cytopenias of one or more of the affected myeloid lineage cells. Next diagnosis was proved with laboratory investigations as following:

- Complete blood count shows refractory cytopenia (s) in one or more lineage.
- Morphological examination of blood smear, bone marrow aspirate and biopsy for dysplasia in one or more lineage; the dysplasia is considered significant if ≥10% in the erythroid precursors and granulocytes, ≥10% dysplastic megakaryocytes based on evaluation of at least 30 megakaryocytes on smears or sections.
- Dyserythropoiesis: Megaloblastoid changes, cytoblasmic vacuolization and Periodic Acid Schiff (PAS) positivity, internuclear bridging, karyorrhexis, multinuclarity and/or nuclear budding.
- Dysgranulopoiesis: Nuclear hpolobation (pseudo Pelger Huet), hypersegementation and/ or hypogranulation.
- Megakaryocytic dysplasia: Micromegakaryocytes, hpolobated or non-lobated nuclei or widely separated nuclei.
- BM aspirate and/or biopsy provides definitive diagnosis where evidence of dyserythropoiesis, dysmyelopoiesis or dysplastic megakaryocytes could be present.
- Cytogenetic studies: Normal cytogenetics do not exclude MDS.

Morphological classification of MDS was done by counting the blast cells in a 500 cell differential of all nucleated cells in a bone marrow aspirate, or a 200 cells in PB. MDS subtypes were categorized according to the 2016 World Health Organization (WHO) criteria **[17]**.

Treatment and Response criteria:

- Treatment varied from symptomatic therapy for cytopenias, especially transfusions and hematopoietic growth factors, immunosuppressive therapy (IST), chemotherapy, to hypomethylating agents (Decitabine).
- Patients were investigated during their regular follow-up visits, every 2-4 weeks, at the outpatient clinic with clinical examination and peripheral hemogram.
- Response to treatment was assessed after 8-12 weeks and response criteria were based on recommendations of an international working group (IWG 2006) **[18]**.
- OS and PFS were estimated from the time of disease diagnosis till death or disease progression, respectively.

Statistical analysis:

Numerical data were expressed as mean ± SD, median and range, whereas categorical variables were presented as percentages. Kaplan-Meier survival analysis was used to calculate survival outcomes. All analyses were done using SPSS statistical package V. 20 (IBM; corporation, New York, USA). Graph pad Prism V.5 was used for creation of figures.

RESULTS

Socio-demographic and clinical characteristics of MDS patients included in the study (Table 1):

The study included 76 MDS patients who were admitted to our Institutions in the period from Jan. 2017 to Jan. 2019. Female predominance was apparent among the study patients where females represented 46 (60.5%) compared with 30 (39.5%) for males and a 1:1.5 male to female ratio. The median age at diagnosis of our MDS patients was 44.5 years, and the majority of them (50%) were diagnosed at an age range of 18-45 years. MDS was diagnosed in those older than 60-years in one fifth only of the study patients. Housewife, unemployment and farming were the most common occupational status in the study patients, 55.3%, 17.1% and 15.8% in order. Residency in Urban areas was reported in only 36.8% of the study patients Nearly one fifth of MDS patients were smokers and exposure to fertilizers was reported in 15.8%.

Table (1): Demographic characteristics of patients with myelodysplastic syndrome included in the study (n=76).

Variable	Results
Demographics:	
Age (years):	
- Mean± SD	45.80±16.4
- Median (range)	44.5 (18-80)
Age groups [n (%)]:	
- 18-45	38 (50%)
- 46-60	23 (30.3%)
- 61-80	15 (19.7%)
Gender:	
- Males	30 (39.5%)
- Females	46 (60.5%)
Urban area	28 (36.8%)
Occupation:	
- Farmer	12 (15.8%)
- Housewife	42 (55.3%)
- Employed	7 (9.2%)
- Student	2 (2.6%)
- Unemployed	13 (17.1%)
Environmental exposure:	
- Alcohol	2 (2.6%)
- Smoking	15 (19.7%)
- Fertilizers	12 (15.8%)
- Insecticides	5 (6.6%)
- Familial	0 (0%)
- t-MDS	0 (0)
- Hair dyes	1 (1.3%)

N.B.: SD: Standard deviation.
t-MDS: Therapy related MDS.

Fig. (1) Illustrates the distribution of the study patients over 8-Governorates of Upper Egypt including Assiut, Sohag, El Menia, Qena, Luxor, Aswan, Al Wady El Jadeed and Red Sea. The highest prevalence was at Assiut followed by Sohag 53.9% and 21.1% respectively.

Clinically the ECOG PS was grade 0 in 57.6% of the study MDS cases. Anemic manifestations followed by fever were the predominant presenting complaints, 29 (38.2%) and 19 (25%) respectively. However, pallor and purpura were the predominant physical signs 22 (28.9%) and 18 (23.7%) respectively (Table 2). Comorbid illnesses were detected in 40.8%, and secondary MDS in 22.4% of cases.

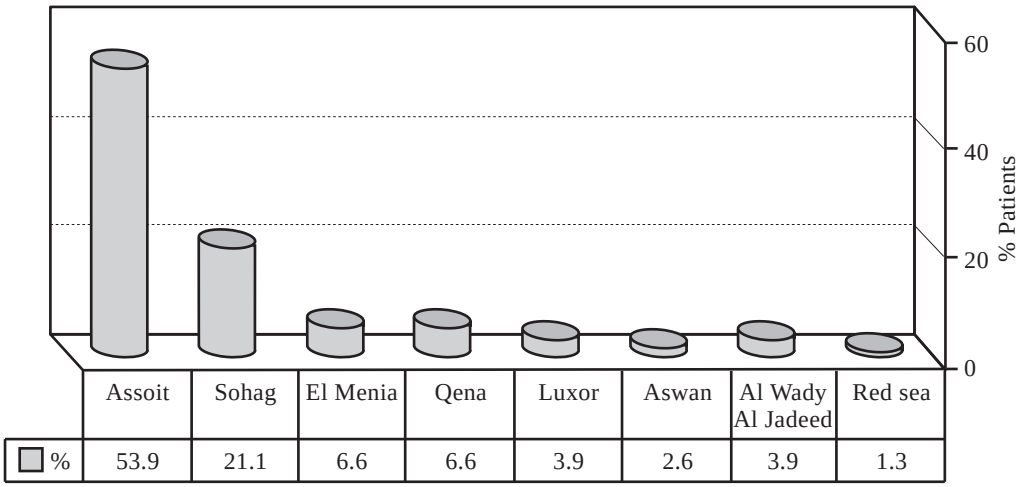

Fig. (1): Distribution of 76 myelodysplastic syndrome patients over 8-Governorates of Upper Egypt in the period from Jan. 2017 to Jan. 2019.

Table (2): Clinical characteristics of 76 myelodysplastic syndrome patients.

Variable	No. (%)
Clinical characteristics:	
ECOG PS:	
- Grade 0	44 (57.9%)
- Grade 1	22 (28.9%)
- Grade 2	9 (11.8%)
- Grade 3	1 (1.3%)
Symptoms:	
- Symptoms of anemia	29 (38.2%)
- Fever	19 (25%)
- Anemia and fever	17 (22.4%)
- Anemia and bleeding	11 (14.5%)
Signs:	
- Pallor	22 (28.9%)
- Purpura	18 (23.7%)
- Pallor and fever	17 (22.4%)
- Fever	8 (10.5%)
- Pallor, purpura and fever	5 (6.6%)
Co-morbidity:	
- Present	31 (40.8%)
- Absent	45 (59.2%)
Clinical MDS subtype:	
- De novo	59 (77.6%)
- Secondary	17 (22.4%)

ECOG PS: Eastern Co-operative Group performance status.

Hematologic, morphologic, bone marrow features and subtypes of MDS in the study patients:

Table (3) shows the hematological profile of patients with MDS included in the study at their first presentation. Table (4) reveals morphologic and bone marrow features of MDS patients included in the study, at diagnosis. Normocytic normochromic anemia was predominant among cases 40 (52.6%) while macrocytosis was detected in only 13.2% of cases. BM cellularity was hyper-cellular in 43 (56.6%), Hypocellular in 20 (26.3%), normocellular in 8 (11.3%) and heterogeneous cellularity in 4 (5.3%) of cases. Different MDS subtypes in the study patients are shown in Fig. (2), half (50%) of cases were MDS with multilineage dysplasia.

Treatment modalities, response to treatment and clinical outcome of the study patients (Table 5):

Various treatment plans were applied according to patients' age, performance status, treatment availability and financial issues. Supportive treatment was the mainstay of therapy in most patients 37 (48.7%) followed by immunosuppressive therapy and growth factors in 27 (35.5%). Hematologic improvement at the level of erythrocyte was the most noticeable treatment response in 24 (31.6%) of the study patients. CR and marrow CR were observed in one patient only (1.3%), each. Progression to AML occurred in 12 (15.8%) cases, and 5.3% died during the period of the study.

Survival studies of MDS patients included in the study:

Figs. (3,4) show OS and PFS survival curves of MDS patients included in the study. Patient's median PFS and OS were 12 and 13 months respectively. The longest OS was 14-years. Remarkably, there was no gender effect on OS (log Rank=0.34, p=0.55) or PFS (log Rank= 1.15, p=0.28).

Table (3): Hematological profile of 76 patients with myelodysplastic syndrome.

Statistics	TLCx10^9/L	Neut. x10^9/L	Hb g/dl	Plts.x10^9/L	Retics. %	Blast %	MCV fl	MCHC g/dl
Mean	5.20	2.45	6.19	175.08	1.65	1.46	86.21	31.61
SE	.50	.27	.25	23.54	.231	.368	1.06	.231
Median	3.15	1.45	6.00	98.50	.80	.00	85.50	32.00
SD	4.43	2.41	2.25	205.28	2.02	3.206	9.29	2.019
Minimum	1.1	.01	2.5	6	.10	0	64.00	23.00
Maximum	20.0	9.10	14.0	833	9.00	15	108.00	36.00

N.B.: SE: Standard error, SD: Standard deviation, TLC: Total leucocytic count, Neut.: Neutrophil, Retics.: Reticulocytes, Hb: Hemoglobin, Plts.: Paltelets, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration.

Table (4): Erythrocyte morphology and bone marrow (B.M.) cellularity of patients with myelodysplastic syndrome included in the study.

Variable	Frequency	Percent	Cumulative Percent
Erythrocyte morphology:			
- Normocytic normochromic	40	52.6	52.6
- Normocytic hypochromic	12	15.8	68.4
- Macrocytic normochromic	10	13.2	81.6
- Microcytic hypochromic	13	17.1	98.7
- Microcytic normochromic	1	1.3	100.0
B.M. cellularity:			
- Hypercellular	43	56.6	56.6
- Hypocellular	20	26.3	82.9
- Normocellular	9	11.8	94.7
- Heterogenous cellularity	4	5.3	100.0

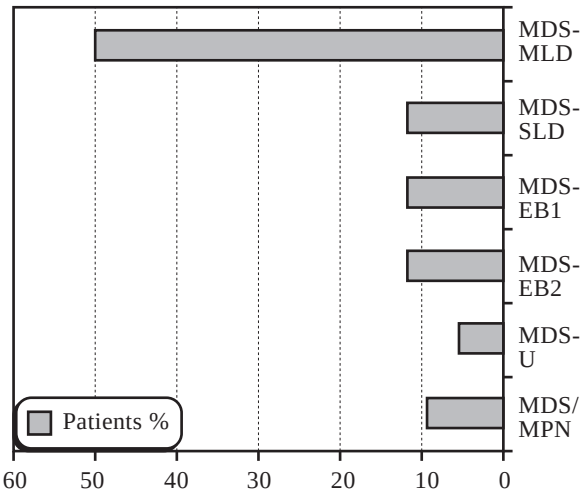

Fig. (2): Types of myelodysplastic syndrome (MDS) in the study patients.

N.B.: MDS-MLD: MDS with multilineage dysplasia, MDS-SLD: MDS with single lineage dysplasia, MDS-EB1: MDS with excess blast1, MDS-EB2: MDS with excess blast2, MDS-U: MDS-unclassifiable, MDS/MPN: MDS Myeloproliferative Neoplasm overlap.

Table (5): Treatment modalities, treatment responses and outcome of 76 myelodysplastic syndrome patients.

Treatment modalities:			
Decitabine & GF	5	6.6	6.6
Supportive treatment	37	48.7	55.3
IST & GF	27	35.5	90.8
IST and thrompoietin mimetics	7	9.2	100.0
Treatment response:			
CR	1	1.3	1.3
PR	6	7.9	9.2
HI-E	24	31.6	40.8
HI-N	1	1.3	42.1
HI-p	8	10.5	52.6
SD	15	19.7	72.4
Failure	8	10.5	82.9
Marrow CR	1	1.3	84.2
Disease progression	12	15.8	100.0
Outcome:			
Living	53	69.7	69.7
Died	4	5.3	75.0
Progression to AML	12	15.8	90.8
Discharge on request	1	1.3	92.1
Stopped follow-up	6	7.9	100.0
Total	76	100.0	

IST: Immunosuppressive therapy; GF: Growth factors; CR: Complete remission; PR: Partial remission;
HI-E, HI-N and HI-P: Hematologic improvement erythrocyte, neutrophil and platelet; SD: Stable disease; AML: Acute myeloid leukemia

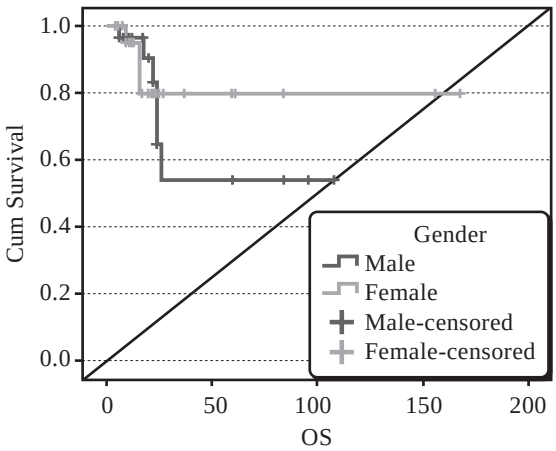

Fig. (3): Overall survival (OS) of 76 myelodysplastic syndrome (MDS) patients included in the study; Log Rank (Mantel-Cox) = 0.35, *p*=0.55.

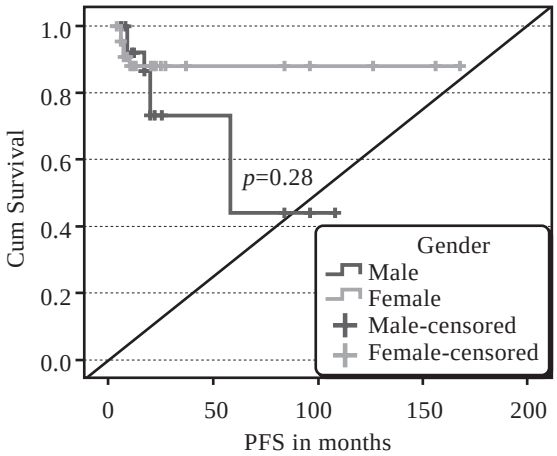

Fig. (4): Progression free survival (PFS) of 76 myelodysplastic syndrome (MDS) patients included in the study; Log Rank (Mantel-Cox) = 1.15, *p*=0.28.

DISCUSSION

In the last few years, our knowledge about MDS diagnosis and treatment improved. Patients with MDS have various medical problems that affect their quality and duration of life **[19]**. However, research about MDS whether primary or secondary is rare in our region. Here we prospectively analyzed 76 MDS cases aiming to investigate their disease characteristics and outcomes. Cases were collected among those admitted at our Institutions in the period from Jan. 2017 to Jan. 2019, over 8-different Governorates of Upper Egypt, of which the higher prevalence was at Assiut, the largest medical Governorate in Upper Egypt.

It is universally agreed that MDS is a disease of elderly subjects with frequent incidence of other co-morbid conditions, however it may occur in persons of any age including children **[16]**. This was not the case in the current study where the median age at diagnosis was 44.5 years and half of cases were diagnosed in the age group 18-45 years. In one study, the annual incidence per 100,000 was estimated to be 0.5, 5.3, 15, 49, and 89 for individuals <50, 50 to 59, 60 to 69, 70 to 79, and ≥80 years of age, respectively **[14]**. Based on the November 2018 SEER data submission, posted to the SEER web site, April 2019, only 4-7% of all MDS cases were below 50 years **[20]**. Both studies are not consistent with our results. Nevertheless, similar to our results, a retrospective Egyptian study of 69 MDS patients that was conducted at the Clinical Hematology Unit Of Cairo University in the period from 2007 to 2010, in which the median age at diagnosis was 55 years **[21]**. The younger age of our patients compared to other Eastern and Western countries could be explained by socio demographic differences as shorter life span or could denote ethnic differences in incidence of MDS.

It is widely agreed that, there is a male predominance in most categories of MDS, with the exception of MDS with isolated del (5q) which is more common in females **[22]**. Based on SEER data from 2001-2003, the incidence rate was significantly higher in men than in women (4.5 vs 2.7 per 100,000 population) **[15]**. This is not the case of our study as the proportion of MDS was higher among females 60.5% compared with that of males 39.5%. Moreover, urbanization was detected in only 36.8% of patients, this may explain, to some extent the increased contact with chemical substances in rural areas. Again, our findings of female predominance and rural residence were albeit consistent with the previously mentioned Egyptian study **[21]**. The female predominance in our patients could be also explained with higher exposure to environmental hazards as most of them were housewives living in rural areas **[23]**.

Clinically, most of our patients have good ECOG-PS and lower incidence of co-morbid conditions compared to other studies; this may be due to the younger age of our patients. Nevertheless, consistent with others the vast majority of them have primary MDS **[24]**. Furthermore, exposure to chemical fertilizers and insecticides were the most important risk factors for secondary MDS; t-MDS was not reported in this study.

Anemia is the most common cytopenias occurring with MDS, and this was the case in our study. Anemia in MDS is generally associated with an inappropriately low reticulocyte response and the red blood cells are usually normocytic or macrocytic, but some patients may have hypochromic microcytic red cells; ovalomacrocytosis is the most common morphologic abnormality **[25,26]**. Normocytic normochromic anemia was predominant among our cases 52.6%. Why most of our patients have normocytic normochromic anemia? This could be explained by the concomitant iron deficiency, the most prevalent nutritional deficiency in our region **[27]**.

MDS-MLD accounts for approximately 30 percent of all cases of MDS **[10]**. In our series, it accounted for half of the patients. This may denote late presentation of our patients, where patients start to complain in advanced stages of the disease.

MDS is usually associated with a normo- or hypercellular bone marrow (BM) whereas 10-20% of cases present with hypocelluar BM **[28]**. This was concordant with our findings in this study where BM was hypercellular in 56.6%, normocellular in 11.3% and hypocellular in 26.3% of cases.

The mainstay of treatment for MDS aims to deal with symptoms and potential morbidity associated with the disease **[29,30]**. Supportive treatment was the mainstay in most of our patients, immunosuppressive therapy and growth factors applied in one third of cases. Therapy that is more intensive was applied in a smaller portion of our patients due to profound cytopenias, availability and financial issues **[31,32]**. Hematologic improvement at the level of erythrocyte was the most noticeable treatment results among our patients, as packed red blood cells transfusion and hematopoietic growth factors were the most accessible treatment lines used. ORR (55%) and CR were lower in our study compared with other studies **[29-32]**. This could be due to shorter follow-up, 3-months, in our study, besides profound cytopenias at presentation in our patients.

OS is extremely variable in MDS ranging from few months to almost a decade with significant clinical heterogeneity and various treatment options. Gender is considered an important predictive factor for survival in MDS patients but is not stated until now as a prognostic factor in the scoring system **[33]**. In a study analyzed 897 MDS patients, further demonstrated gender as a potential prognostic factor **[34]**. Our patients' median PFS and OS was 12 and 13 moths respectively without significant overall survival difference based on gender, this may be due to small sample size of our study with predominance of female gender. Evolution to AML occurs in approximately 10% and 70% of lower- and higher-risk (HR) patients respectively **[35]**. This was albeit consistent with our findings where progression to AML occurred in 12 (15.8%) of our cases.

In Conclusion, this is the first study that assessed characteristics and outcomes of MDS at Upper Egypt. It assessed various aspects of the disease including, demographic, clinical, hematologic and bone marrow features. MDS subtypes, treatment responses, OS, PFS and clinical outcomes. Although it is widely accepted that MDS is a disease of elderly males with co-morbid conditions, poor general health and shorter OS than females. This study concluded obvious differences in disease description in our region, compared to other studies as following:

- Younger age at presentation, female predominance.
- Rural residency.
- No link of disease to previous treatment (therapy related MDS).
- Lower incidence of co-morbid conditions.
- Normocytosis was the hallmark of erythrocyte morphology in our patients.
- MDS-MLD was the commonest MDS subtypes in our region.
- Lower ORR and poor outcome.
- The disease was indolent in some patients with long OS up to 14-years.

These socio-demographic differences in MDS incidence among different regions may be attributed to ethnic differences and late presentation in our patients. Furthermore, these different ORR and outcome results may be due to lack of accessibility of advanced therapeutic options in our patients.

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Increased Angiogenesis and Response to Induction Therapy in De Novo Egyptian Pediatric Acute Lymphoblastic Leukemia Patients

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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. It is the most common leukemia in children.

Angiogenesis is the formation of new blood vessels from preexisting vessels and is a normal and highly regulated physiological process throughout the body. Tumors have traditionally been the most extensively studied angiogenic-dependent diseases.

Purpose: This study was designed to investigate impact of increased angiogenesis measured by microvascular density (MVD) using anti-CD34 marker on the response to induction therapy in newly diagnosed Egyptian pediatric acute lymphoblastic leukemia patients.

Methods: Forty de novo pediatric ALL patients coming to National Cancer Institute (NCI), Cairo University from May 2017 to June 2019, were included. Bone marrow aspirations (BMA) and bone marrow biopsy (BMB) specimens were obtained from all patients at diagnosis: BMA smears were stained with Leishman stain and cytochemical stains and BMB were processed then stained with haematoxylin and eosin, reticulin and CD34 immunohistochemical (IHC) stain for assessment of MVD.

All patients received total 15 induction therapy and assessed at end of phase 2 induction.

Results: Angiogenesis ranged from 1 to 20 vessels per high power field (HPF), with median of 6 vessels per high power field. From our experience in NCI, Cairo University, cases with count above 6 vessels/HPF are considered having increased angiogenesis.

Out of the 40 patients included in the study, 19 patients (47.5%) were having increased angiogenesis (>6 vessels/HPF) while 21 patients (52.5%) were having normal vessel count (≤6 vessels/HPF).

Conclusions: In this study we found that increased angiogenesis had no impact on response to induction therapy with Total XV protocol in childhood ALL. Still large prospective trials are needed to confirm or deny this conclusion.

Key Words: *Angiogenesis – Childhood ALL – Induction therapy – Response – Total XV protocol.*

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a malignant clonal disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow, blood and extramedullary sites **[1]**. It is the most common type of cancer and leukemia in children accounting for up to 80% of leukemias in this age group and 20% of leukemias in adults **[2]**.

Angiogenesis is the process through which novel blood vessels are formed from pre-existing ones and it is involved in both physiological and pathological processes of the body. Tumor angiogenesis is a crucial factor associated with tumor growth, progression, and metastasis. That is why there were great interestsfor the development of anti-angiogenic strategies that could inhibit tumor vascularization. Approaches comprise either the administration of anti-angiogenic drugs that target and block the activity of proangiogenic factors or combining anti-angiogenic agents with chemotherapy or immunotherapy **[3]**.

PATIENTS AND METHODS

This study included 40 de novo Egyptian pediatric ALL patients attending National Can-

cer Institute (NCI), Cairo University; during the time period from May 2017 to June 2019.

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

Patients included aged between 2 to 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).

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 their cytogenetic and molecular status **[4]**.

Bone marrow aspiration (BMA) and Bone marrow biopsy (BMB) were obtained from all patients at diagnosis. The BMA smears were stained with Leishman stain and cytochemical stains, namely, myeloperoxidase (MPO) and Sudan black (SBB).

BMB were formalin fixed and paraffin embedded and cut into 3-4μm thick sections then stained with haematoxylin and eosin staining, as well as reticulin and CD34 immunohistochemical (IHC) stain.

Microvessel Density (MVD) Calculation:

Estimation of MVD was accomplished through the following steps done for BM sections as follows:

Using a research binocular light microscope [Leica, DM – 750], the tested immuno-stained slides were initially scanned at 100 x magnification to identify the section area of the slide, check the staining quality, verify the distribution pattern of the highlighted microvessels and to locate regions of higher vascular concentrations (i.e. hot spots) **[5]**.

Ten hotspots were chosen and numbers of microvessels were counted in each of these hotspots at 400x magnification. To ensure the accuracy of the method, each stained sample was reviewed by 2 separate hematopathologist, in a blinded fashion. Morphologic analysis was performed carefully to ensure vessel specificity of the CD 34-stained stroma considered for analysis. The MVD was expressed as the mean number of microvessels per field **[6]**.

Quantification of microvessels was performed according to the following rules [5]:

1- Any IHC-stained (CD34 +ve) individually scattered endothelial cell was considered as a single distinct countable microvessel.

2- Any IHC-stained endothelial cell cluster (whether arranged in a complete or incomplete vascular structure, with or without a lumen and clearly separated from adjacent microvessels, blasts, and other marrow elements) was considered also as a single distinct countable microvessel.

3- The presence of a lumen (with or without RBCs) was not necessary for microvessels morphological identification but considered only as a helpful feature.

4- All immuno-stained highlighted microvessels (whether crowded within the “Hot spots” or randomly individually dispersed among the intertrabecular hemopoietic areas) were included into the count. Those encountered within the BM trabeculae were not included into the count.

5- All immuno-stained microvessels (whether located among the malignant hemopoietic infiltrates or among the non-infiltrated normal hemopoietic areas) were included into the count.

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 with statistical package version 17. Qualitative variables will be presented as proportions and quantitative variables will be presented as mean ± standard deviation (SD) or median and range as appropriate. The comparison between qualitative variables will be done using Chi-square test or Fisher's exact as appropriate and *p* less than 0.05 will be considered. 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 had died.

II- *Relation between angiogenesis and clinical parameters in our 40 patients:*

Statistical relation between angiogenesis and clinical parameters of patients included in the study at diagnosis is shown in Table (2).

III- *Relation of angiogenesis to the response to induction therapy:*

Among our 40 patients 31 patients achieved CR1 at day 42 of induction therapy of whom 17 patients were having increased MVD, and 3 patients did not achieve CR1, all the 3 were having normal MVD. Three patients (1 patient with normal MVD and 2 were having increased MVD) achieved CR2 after changing therapy protocol and 4 patients (2 patients were having increased MVD and the other 2 were having normal MVD) relapsed after induction therapy; still no statistically significant relationship was found neither between response to induction therapy nor relapse to angiogenesis as shown in Table (3).

Table (1): Clinical and hematological findings at diagnosis.

	N=40	%
Age:		
Median (range) (years)	8.5 (2.0-18.0)	
Sex:		
Male	31.00	77.50
Female	9.00	22.50
Initial CBC:		
TLC:		
Median (range) ($x10^9$/L)	9850 (1400-109000)	
HB:		
Median (range) (mg/dl)	8.6 (4.1-16.0)	
PLT:		
Median (range) ($x10^9$/L)	53000 (4000-279000)	
PB Blasts:		
Median (range) (%)	25.00% (0.0-88.0)	
Initial BMA:		
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
IPT:		
Pre B-ALL	32.00	80.00
c ALL	8.00	20.00

Table (2): Relation between angiogenesis and clinical parameters of study patients.

	Angiogenesis ≤6		Angiogenesis >6		*p*-value
	N	%	N	%	
Age group (yrs):					
2-10	11	52.4	10	47.6	0.987
>10	10	52.6	9	47.4	
Sex:					
Male	15	71.4	16	84.2	0.457
Female	6	28.6	3	15.8	
Organomegaly:					
No	8	44.4	10	55.6	0.356
Yes	13	59.1	9	40.9	
CSF:					
No	19	50.0	19	50.0	0.488
Yes	2	100.0	0	0.0	
LNs:					
No	11	55.0	9	45.0	1.000
Yes	10	50.0	10	50.0	

Table (3): Relation between angiogenesis and response to induction therapy.

	Angiogenesis ≤6		Angiogenesis >6		*p*-value
	N	%	N	%	
CR1:					
No	3	17.6	0	0.0	0.227
Yes	14	82.4	17	100.0	
CR2:					
No	0	0.0	0	0.0	0.341
Yes	1	100.0	2	100.0	
Relapse:					
No	7	77.8	14	87.5	0.60
Yes	2	22.2	2	12.5	
Status:					
Alive	7	33.3	14	73.7	0.011
Dead	14	66.7	5	26.3	
Death:					
Early	10	71.4	3	60.0	1.00
Late	4	28.6	2	40.0	

DISCUSSION

In this study, detection of BM microvessels was performed by IHC staining using the mouse antihuman CD34 which highlights the endothelial cells. Babarovic et al., 2012 **[5]** noticed that the best IHC results were obtained with anti-CD34 monoclonal antibody as CD31 and factor VIII are expressed in a big population of bone marrow cells, including megakaryocytes and myeloid cells.

In the current study, we found no association between angiogenesis (MVD) and both age and sex of the patients, this goes well with other statistical analysis reported by Pule et al., 2002 **[7]** and Noren–Nystrom et al., 2009 **[8]** and who also did not reveal any significant association between MVD and age or sex of the patients in ALL.

This work did not also reveal any significant difference in angiogenesis (MVD) in relation to the presence of organomegaly and lymphadenopathy. To the best of our knowledge, no previous studies addressed the relation of these parameters with MVD in acute leukaemia.

The increase in cellularity and MVD were noticed in our ALL patients, since the majority of cellularity is composed of blasts, a correlation between MVD and cellularity may be expected. However, the increased angiogenesis was not related to neither BM cellularity nor to BM blast cell percentage in ALL patients included in our study. These results may suggest that the increase in BM vascularity is not related to BM degree of cellularity; to our knowledge no published studies were available discussing this relation in ALL. But, this goes well with Kuzu et al., 2004 **[9]** who found no statistical relation between MVD and percentage of BM blast cells in AML.

This study shows no relation between increased angiogenesis and WBC count in ALL, this is consistent with Noren-Nystrom et al., 2008 **[8]** who also found no correlation between microvascular density MVD and WBC count in ALL.

In newly diagnosed ALL patients we found that there is no relation between increased BM angiogenesis (MVD) and response to induction therapy nor relapse or patient survival, this was in accordance with Pule et al., 2002 **[7]** who also demonstrated no association between high MVD and prediction of relapse. In contrast to a study done by Todorovic et al., 2011 **[10]** found that the initial values of MVD had a positive correlation with OS and leukaemia free survival.

Kuzu et al., 2004 **[9]** and Shih et al., 2009 **[11]** found a significant increase in MVD in the BM of AML patients, this is particularly significant when considering the strong positive correlation between increased BM vasculature and OS in AML. A high MVD predicted for poor prognosis and suggests that blood vessel-AML interactions may contribute to refractory disease.

In most patients with myelofibrosis with myeloid metaplasia, Mesa et al., 2000 **[12]** demonstrated an increase MVD compared with patients with either polycythemia vera or essential thrombocythemia, they also detected that increased angiogenesis, along with other factors, was a highly significant risk factor for survival.

In patients with multiple myeloma (MM), increased BM angiogenesis and prognosis are well related, and MVD is considered as an independent prognostic factor for OS together with beta2-microglobulin and C-reactive protein **[13,14]**.

Conclusion:

We found that there was no relation between increased angiogenesis expressed by MVD and

response to induction therapy in newly diagnosed Egyptian pediatric ALL patients. However, further large prospective studies are recommended to prove or deny this finding.

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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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