ABSTRACT

**Aim &Objectives:** Wilms’ tumor 1 (Wt1) gene is over expressed in majority of acute leukemia. The aim of this study is to evaluate (Wt1) gene expression in children with acute lymphoblastic leukemia and to find out the relationship between it and other morphological, immunophenotypic and prognostic factors.

**Subjects & Methods:** An case-control study was conducted in pediatric Hematology & oncology unit, zagazig university children's hospital zagazig university during the period of January 2011 to June 2013. we examined the level expression of (Wt1) gene in 44 newly diagnosed children with acute lymphoblastic leukemia and 20 controls patient with hematological problems other than hematological malignancies. A fresh peripheral blood samples were collected patients and controls and submitted to RNA extraction, reverse transcription of extracted RNA and real-time quantitative PCR.

**Results:** We detect a wide range of Wt1 gene expression level among 44 patients of acute lymphoblastic leukemia. Statistically, Wt1 gene expression level was significantly higher in T-cell acute lymphoblastic leukemia than in B-cell Precursor acute lymphoblastic leukemia (P<0.001) and also higher in those with expression of myeloid marker than those without expression of these markers. Analysis of relapsed cases (3/44) indicated that abnormally increase of wt1 gene expression level was associated with increased risk of relapse.

**Conclusion:** This study suggest that Wt1 expression in childhood acute lymphoblastic leukemia is very variable and much more expressed in t cell leukemia than BCP ALL and show higher levels in relapsed patients thus indicating a possible prognostic marker of childhood acute lymphoblastic leukemia.

**Keywords:** (Wt1) gene, childhood, acute lymphoblastic leukemia.

INTRODUCTION

Wilms’ tumor gene 1 (WT1) is an embryonic zinc-finger transcription factor, which was originally identified as a tumor suppressor gene inactivated in Wilms’ tumors [1]. WT1 is located on chromosome 11p13 and encodes a zinc finger transcription factor. Post-transcriptional mRNA modifications and the presence of possible several transcription initiation sites give rise to a number of different WT1 protein isoforms (at least 32) that are localized in specific subcellular and subnuclear regions and show different, partially overlapping but distinct functions [2-5].

In normal tissue, WT1 is expressed during embryogenesis where it plays a pivotal role in the development of the urogenital tract [6,7]. Alterations of WT1 expression (both under- or overexpression) have been described in a number of malignancies and premalignant syndromes. Remarkably, WT1 overexpression has been found in 80–90% of acute myeloid leukemia (AML) and 70–90% of acute lymphoblastic leukemia (ALL) patients, with an even higher frequency at relapse [8,9]. WT1 overexpression was also detected in the cerebrospinal fluid of patients with B-ALL where it showed a strong association with disease relapse [10].

The majority of acute leukemias express the normal, wild-type WT1 gene. In addition, point mutations and small deletions or insertions may also occur and have been identified in approximately 10–20% of acute leukemias, predominantly in biphenotypic and AML, but rarely in ALL. In AML, these alterations were shown to be associated with an unfavorable prognosis [2,3,11].

In this study, we focused on evaluation of WT1 expression and its clinical implications in prognosis of childhood ALL, where WT1 has been least studied and its impact remains most controversial.

MATERIALS AND METHODS

Samples

Patients samples: After ethical committee approval and informed consent, diagnostic PB samples from 44 patients with childhood ALL (from Hematology & oncology unit, zagazig university children hospital, zagazig university) were collected on a consecutive basis from December 2011 to June 2013 and analyzed. The male represent 52.3%, female represent 47.7%; the median age was 3.9 years (range 2-12 years).

They involved 36 B-cell precursor (BCP) ALL, 3 BCP ALL with aberrant expression of myeloid markers 5 T-cell ALL. The main risk
factors defined as age <1 or >10 years and WBC>50 x10^3 at diagnosis were present in 2, 3 and 7 patients, respectively. Patients were treated according to standard protocols (CCG protocol). The median follow-up is 9.5 months (range 1-28); during this period 3 of the 44 patients relapsed, and 5 patients died.

Controls samples:
To evaluate WT1 expression in normal hematopoietic tissues, peripheral blood samples obtained after informed consent from 20 patients with haematological problems other than haematological malignancies were examined. The control group was selected to match the patient group in sex and age.

Methods
Sample processing:
PB were collected into sterile tubes with anticoagulant (K3 ,EDTA) and immediately transported to the lab at room temperature. The initial processing of the samples was performed within 12 h after their collection, in most cases within the first 4 h. RNA was isolated from the samples using an RNeasy Mini Kit (Qiagen, Roche, Germany), and the concentration of extracted RNA was evaluated by spectrophotometry (Nanodrop 2000 thermoscientific). The cDNA synthesis was performed using the High Capacity cDNA Reverse Transcription Kits (Applied, Biosystems, USA.) starting from 1ug of total RNA.

Real time quantitative-PCR for WT1 gene
RQ-PCR for the detection and the quantification of WT1 was developed using (ipsogen WT1 ProfileQuant Kit, QIAGEN, GERMANY, cat.no.67692) and the TaqMan® Universal PCR Master Mix, NoAmpErase® UNG (Applied Biosystems, Forster City, CA). As a control gene (CG), ABL was detected in all samples without BCR-ABL translocation. The RQ-PCR reaction was performed Applied Biosystems 7500 Real-Time PCR System. For WT1 and CG the threshold was set at 0.1 to be in the exponential phase. The WT1 and CG copy number and WT1 normalized copy number (NCN, WT1/ABL copies) were calculated according to the standard curve method. Standard curves were created using plasmid DNA calibrators – ABL Control Gene Standards (Ipsogen).

Administrative design and Ethical issues:
An official permission from Zagazig University, Faculty of medicine was taken to pediatric hematology and oncology unit. The title and objectives of this study was explained to them to insure their cooperation. The head of the pediatric hematology and oncology unit was informed about the nature and steps of the study and written consent was taken. The study group was informed about the nature and the purpose of the study. The study group was not exposed to any harm or risk. Patient’s data was confidential.

Statistical analysis of the recorded data:
The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 16. For the statistical calculations, data coding was done, and Qualitative data were represented as frequencies and percentages, Chi-square test (\( \chi^2 \)), Mc Nemar test was carried out for testing the association between the qualitative data whenever possible. Quantitative data were compared using Analysis Of Variance (F test), Kruskall Wallis test and paired t test. The test results were considered significant when P-value <0.05.

RESULTS
WT1 expression in normal PB
WT1 expression was detected in all control samples (normal PB).

Table (1) WT1 gene expression in patients and controls:

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>controls</th>
<th>Mann test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT1 gene expression (NCN x 10)</td>
<td>median</td>
<td>0.121</td>
<td>M=650</td>
<td>0.00*</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>265±391.5</td>
<td>0.12±0.07</td>
<td>0.02±0.23</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.15-1827</td>
<td>0.02-0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table shows that WT1 gene expression is significantly higher in patients than controls.

WT1 expression in childhood ALL
WT1 overexpression was found in most of the childhood T-ALL cases, of median 895 and range 785-1827 and in BCP ALL, WT1 expressed of median 21.3 and range 0.15-785. In BCP ALL with aberrant expression of myeloid markers WT1 expressed of median 620 and range 553-750.

Table (2) WT1 gene expression in relation to immunophenotyping:

<table>
<thead>
<tr>
<th></th>
<th>T cell ALL</th>
<th>BCP ALL</th>
<th>BCP ALL with aberrant expression of myeloid markers</th>
<th>ANOVA test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT1 expression (NCN x 10) median</td>
<td>895</td>
<td>21.3</td>
<td>620</td>
<td>F=1.2</td>
<td>0.00*</td>
</tr>
</tbody>
</table>
This table shows that WT1 gene expression was significantly higher in patients with T cell ALL than that with BCP ALL and is also significantly higher in patients with BCP ALL with expression of myeloid marker than without expression of myeloid markers.

<table>
<thead>
<tr>
<th></th>
<th>107 ±4</th>
<th>121.6 ±20</th>
<th>641 ±10</th>
</tr>
</thead>
<tbody>
<tr>
<td>range</td>
<td>785</td>
<td>0.15 -785</td>
<td>553 -750</td>
</tr>
<tr>
<td>mean</td>
<td>25.6</td>
<td>3.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

With respect to the normal ranges of WT1 expression, we analyzed WT1 in a representative group of childhood ALL patients. In childhood BCP-ALL, we detected a very wide range of WT1 expression levels with an important difference of interest; ALL blasts with aberrant co-expression of myeloid markers, were associated with a significantly higher WT1 expression level than blasts without co-expression of myeloid markers.

Contrary to BCP-ALL, most of the childhood T-ALL patients showed WT1 overexpression, with the median being significantly higher than in BCP-ALL patients. Several studies matched with our results[26,27,28].

In previous studies, WT1 overexpression above the normal values was found in the majority or all of the ALL samples [14,15,16,17,19,22,24,29,30] and WT1 expression in acute leukemia below the ranges of normal controls was never detected. Most of the studies did not distinguish between B and T-ALL; in those analyzing these subtypes separately, controversial results were found, with higher WT1 overexpression detected in B-ALL in some of them[31] and in T-ALL in others.[29] However, these studies investigated WT1 mostly in adult ALL patients or in heterogeneous groups of children and adults, [14,17,19,22,32] in PB samples,[22,24] using potentially less sensitive PCR techniques of WT1 detection [1,16,17,29,30,32] and with a limited number or type of normal controls tested.[14,30,32].

Recent studies have identified WT1 overexpression as an independent adverse prognostic factor in acute leukemia associated with an increased risk of relapse.[23,17,18] Among our childhood ALL patients there was a trend toward higher WT1 expression in those who relapsed and this reached statistical significance. But insufficient number of relapsed cases may be the weakest point in these results.

CONCLUSION
The current management of childhood ALL relies on patient stratification and risk-directed therapy based on prognostic factors and initial
response evaluation, recently including minimal residual disease monitoring. Although the system of risk assessment as well as the techniques of MRD detection are well established and the treatment outcome is excellent, with 70–80% of patients achieving long-term remission, molecular aberrations that could act as new prognostic factors, MRD markers and therapeutic targets are investigated with the aim to improve further the prognosis of patients with childhood ALL. Among these, WT1 is one of the most promising. The role of WT1 in leukemogenesis is yet to be defined but in an increasing number of studies, WT1 overexpression has been identified as a potential independent adverse prognostic factor in acute leukemia, a suitable marker for MRD monitoring, mainly in AML, and a promising target for immunotherapy in leukemia and other malignancies.

Our study showed that WT1 expression in childhood ALL is very variable and, in general, much lower than in AML or adult ALL. Thus, WT1 will not be a useful marker for MRD detection in childhood ALL. But like in other types of acute leukemia, also, childhood ALL patients with WT1 overexpression have an increased risk of relapse, and WT1 overexpression represents a potential additional independent risk factor in childhood ALL. Interestingly, a proportion of childhood ALL patients express WT1 in levels below the normal physiological BM WT1 expression, and this WT1 under-expression also seems to be associated with an increased risk of relapse. These results only support the complex model of WT1 regulatory effects in normal and malignant hematopoiesis. Further studies, both functional and clinical, will be necessary to clarify the WT1 expression patterns and functions in acute leukemia.

REFERENCES
Wilms’ Tumor Gene 1 (WT1) Expression In......


