Summary and Conclusions

Childhood precursor B cell ALLs frequently carry specific chromosomal translocations. More than 50 distinct rearrangements have been described, four, i.e., t(12;21), t(1;19), t(9;22), and t(4;11), constitute the most frequently observed abnormalities.

These recurrent chromosomal translocations lead to the formation of fusion genes and their proteins expression as chimeric transcripts, are of relevance in leukemogenesis because they influence the expression of key regulatory genes in the hematopoietic system. These four chromosomal translocations have therapeutic importance for risk assessment in current multi-agent chemotherapy protocols because they are predictors of prognosis.

The identification of these four translocations is thus important not only for understanding the biology of ALL but also as a guide for patient management.

The purpose of this work is to develop a multiplex reverse transcription-PCR assay for detection and quantification of leukemia-specific chimeric transcripts that identify the genetic subgroups of acute lymphoblastic leukemias (ALLs) proposed by the WHO classification.

The study included 40 newly diagnosed ALL patients (all were children). They were diagnosed at NCI and Tanta University Hospital. Eight healthy volunteers of matched age and sex were included in the study.

All the patients were subjected to the following:

1. Full history taking and clinical examination:

Full clinical examination was done with a special stress on the presence of fever lymphadenopahy organomegaly purpura gum hypertrophy and manifestations of CNS infiltration.

Diagnosis was based on peripheral blood and bone marrow morphological, cytochemical examination and immunophenotyping.

2. Peripheral blood examination

Ten ml PB were collected under complete aseptic conditions. These were aliquoted as follows

a. 2 ml on K3 EDTA for complete blood count (CBC) and morphological studies

b. 4 ml on K3 EDTA for RT-PCR

c. 4 ml on Na heparin for cytogenetic studies

CBCs and morphological studies

CBCs were performed onto automated cell counter.

The diagnosis of acute lymphoblastic leukemia was primarily based on the morphological and cytochemical characteristics of the blast cells. PB and BM films were stained with Leishman stain and were used for the morphological identification of various cell types.

3. Bone marrow examination

Bone marrow aspirate were collected under strict aseptic conditions.

The sample was used to prepare bone marrow films and the remaining was divided to be used for immunophenotyping and cytogenetics studies.

4- Immunocytochemistry

Myeloperoxidase (MPO) reaction was performed on PB and BM films.

5- Immunophenotyping

Immunophenotyping were done on Becton Dickinson FACS caliber flow cytometer equipped with cell quest software.

6- Cytogenetic analysis:

FISH for the following translocations was performed using LSI probes:

- t(4;11)
- t(1;19)
- t(12;21)
- t(9;22)

7- Multiplex RT-PCR analysis

RT-PCR analysis was done for all cases to detect the following fusion gene transcripts at the time of diagnosis and repeated after complete remission:

- t(4;11)
- t(1;19)
- t(12;21)
- t(9;22)

Screening for the presence of the frequently occurring translocations, t(9;22) with BCR-ABL, t(12;21) with TEL-AML1, t(1;19) with ETO-PBX and t(4;11) with MLL-AF4 was performed by simultaneous detection of all of them in one step (multiplex RT-PCR), followed by confirmatory individual detection of each of them by FISH technique.

Statistical analysis of this work reveled that, there was a complete concordance between the results obtained with the FISH and the multiplex RT-PCR in both the negative and positive cases.

The t(12; 21) proved to be the most common 8/40 (20%). On the other hand the t(4; 11) is the least common of the translocations as it constitute 2/40 (5%) of the ALL cases. The frequency of t(1; 19) and

t(9; 22) lie in between comprising 3/40 (7.5%) and 10% (4/40) respectively.

The relationship between the immunophenotypes and the different translocations shows no correlation between the fusion genes and immunophenotyping. The t(12; 21) was restricted to CALL positive and pre-B subtypes, while the t(1; 19) was distributed among the 3 precursor B subtypes, the 4 cases with t(9; 22) were CALL positive, and the 2 cases with t(4; 11) were one pro-B and one pre-B phenotype.

As regard the prognostic value, treatment response, and outcome, we estimated the disease free survival (DFS) after 2 years from the beginning of induction chemotherapy using Kaplan-Meier method.

The 2 cases with t(4; 11), one case died after 28 days from induction chemotherapy, and the other case died after 360 days with estimated DFS of 51% .

The 8 cases with t(12; 21), one case died after 360 days, while the remaining 7 cases live till day 720 with estimated DFS of 72%.

The 3 cases with t(1; 19), 2 of them died one at day 20 from beginning of induction chemotherapy, the other case died after day 360, and the third case live till day 720, with estimated DFS of 58%.

The 4 cases with t(9 ; 22), 2 of them died after 360 days from the start of induction chemotherapy, the other 2 cases live till day 720 with estimated DFS of 69%.

The one step detection of these translocations by multiplex RT-PCR is time saving, less costly, decrease the number of PCR reaction involved and so decreases the risk of contamination and the occurrence of false positive results and thereby, adds precision onto diagnostic and treatment strategy of acute leukemias. Therefore, the patients would

receive the best opportunity for curative outcome using these fusion transcripts for risk stratification.

The recognition of this has allowed precursor B ALL to be subdivided into molecular entities in the recently proposed WHO classification. The presence of t(12;21) is associated with good response and detected in 20–25% of pediatric ALL . The t(1;19) is observed in 5-6% of all ALLs but constitutes 25% of the pre-B subgroup and predicts intermediate response. The other two translocations, t(9;22) and t(4;11), are each observed in 5–6%, and both associate with poor response but the latter occurs mostly in infant patients.

Fluorescence *in situ* hybridization, Southern blot, or RT-PCR strategies can successfully detect these translocations. However, all these methods are sample/time consuming and are not accurate for quantification purposes Except for RT-PCR.

Conclusion

In the present study we concluded the following:

1. The t(12; 21)(TEL/AML1) was reported in 8/40 cases (20 %) and was found to be associated with good prognosis with DFS of 72 %.
2. The t(9; 22)(PCR/ABL) was reported in 4/40 cases (10%), and was found to be associated with bad prognosis with DFS of 69 %
3. The t(1; 19)(E2A/PBX) was recorded in 3/40 cases (7.5%). And was found to be associated with bad prognosis as one case dying during induction chemotherapy, the other case died after one year from relapse .The third case live till 720 day with estimated DFS of 58%.

4. The t(4; 11)(MLL/AF4) was recorded in 2/40 cases (5%) and was found to be associated with bad prognosis and resistance to aggressive induction chemotherapy, with estimated DFS of 51 % .
5. The presence of translocations in ALL not only influences the biology of leukemic clone but also predicts clinical outcome, therefore multiplex RT-PCR assay to detect the four most common rearrangements in childhood precursor B ALL can be used for rapid, simple, and reliable classification of pediatric ALL. It also facilitate studies on minimal residual disease detection.
6. The primers were designed to amplify the two known variants of TEL/AML1, mBCR/ABL, and E2A/PBX1.
7. The MLL/AF4 has been reported with at least 10 different rearrangements, and the primers used here amplify 6 of them representing more than 85 % of all t(4; 11). We attempted to use set of primers that will amplify all known (4; 11) variants
8. The quality and integrity of cDNAs were tested by initially amplifying the housekeeping gene GAPDH, and all samples was tested and analyzed by standard RT-PCR reactions for each of the four rearrangements.