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# Expression of Homeobox Gene in Relation to Different Molecular Aberrations Associated with Adult Acute Myeloid Leukemia

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## ABSTRACT

***Background:*** Homeobox (*HOX*) gene expression is linked with definite cytogenetic and molecular abrasions in acute myeloid leukemia (AML) patients. This association may be a direct result of transcriptional process or may be related to the differentiation stage at which the leukemic cell is arrested.

***Objectives:*** Our aim was to detect the *HOX* genes expression pattern in leukemic patients is related primarily to the differentiation stage of myeloid cells or emerged during the process of malignant transformation.

***Patients and Methods:*** We examined the bone marrow (BM) of 38 adult patients with AML and BM of 20 healthy individuals. We sorted the various stages of myeloid differentiation and correlated them to their morphological counterpart of AML. The expression of mRNA of *HOX* genes (*HOXA* and *HOXB*) in BM was detected using Quantitative real time-PCR.

***Results:*** The AML cases were divided into five groups according to the presence of common molecular genetic abnormalities. The differentiation stage of the blasts was not statistically significant affecting *HOX* gene expression while it is more related to genetic and molecular abrasion associated with AML. *PML-RARa* fusion gene showed depressive effect on HOX gene expression, regardless the *FLT3/ITD* mutational status.

***Conclusions:*** We concluded that the *HOX* gene expression in AML patients is associated with molecular and genetic aberrations, rather than with the differentiation stage of leukemic cells.

***Key Words:*** *Homeobox genes – Acute myeloid leukemia – Myelopoiesis staging – Molecular abnormalities.*

## INTRODUCTION

The family of *homeobox* genes code for many transcription factors. There are many *HOX* genes clustered into four genomic regions (*HOXA, B, C* and *D* clusters) found on four chromosomes (chromosomes 7, 17, 12 and 2, respectively). Each cluster carries 9-11 genes **[1,2]**.

The expression of *HOX* genes occurs maximally in the stem and early hematopoietic cells during hematopoiesis. Then their expression progressively decreases with maturation till it reaches minimum expression in fully differentiated hematopoietic cells **[3,4]**. This process is firmly under control of regulators; the disruption of regulators leads to transformation to leukemic cell **[5,9]**.

The potential oncogenic effect of *HOX* genes was studied thoroughly for the last two decades. A number of *HOX* genes abnormal expression, either directly (e.g., *NUP98-HOX* fusion) or by their upstream regulators (e.g., *MLL* rearrangements), had been describedin Leukemia patients **[10,13]**. Also it was found that their over expression in leukemia patients is a poor prognostic factor where they induce myeloproliferation and with additional cytogenetic insult may cause leukemia **[14,16]**.

In the current study, we tried to answer the question of either the *HOX* genes expression in leukemic patients is related primarily to the differentiation stage of myeloid cells or emerged during the process of malignant transformation. The expression of *HOX* genes was studied in relation to the morphological stage, cytogenetic and molecular characteristics of leukemic cells in adult patients of acute myeloid Leukemia (AML).

## PATIENTS AND METHODS

*BM samples:*

We collected samples of BM from 38 patients newly diagnosed as AML from 2014 to 2017 at Dammam University Hospital, Kingdom of Saudi Arabia. After University Institutional Review Board (IRB) approval and patient's signature of informed consent, BM samples were processed to separate mononuclear cells using Ficoll-Paque Plus (GE Healthcare Life Sciences, Uppsala, Sweden) and stored at –80°C.

Twenty control BM samples were collected from BM transplantation donors.

*Methods:*

Different developmental stages of hematopoiesis were collected using Fluorescence Activated Cell Sorter (FACS) Aria (BD, San Jose, CA, USA). The different stages of myeloid lineage differentiation were identified by surface markers listed in Table (1).

From both patient and control groups, RNA was prepared with RNeasy Mini Kit (Qiagen, Hilden, Germany) and transcribed to cDNA by the iScript kit (Bio-Rad, Hercules, CA, USA).

Gene expression was measured by the iCycleriQ System (BioRad, Hercules, CA, USA); it was used to quantify the HOXA and HOXB gene expression in both groups. The PCR primer make up was settled for the detection of different *HOX* genes subtypes (*HOXA3, A4, A5, A6, A7, A9, A10, B2, B4, B5, B6,* and *B7*) and patient samples (*HOXA1, A3, A4, A5, A6, A7, A9, A10, A11, A13, B1, B2, B4, B5, B6, B7, B8,* and *B9*) **[17,18].** We quantified the ABL1 gene, which is known to be stably expressed during the developmental stages of myeloid lineage cells as internal control.

We selected 14 patients who were negative for the presence of the main molecular abnormalities to be examined for the presence of the gene mutation of the *NPMI, NRAS, KRAS, CEBPa, c-KIT* and *FLT3* genes (Table 2). The analysis was performed by Real Time PCR followed by sequencing **[19,22].**

According to previously published studies, the maturation stages were identified (Table 1) **[23,25].**

*Statistical analysis:*

The statistical significance among the subgroups was done by Mann-Whitney and Kruskal-Wallis tests with Dunn's multiple comparison post-test. Cluster analysis, was done to identify the subgroups with similar gene expression patterns using the Genesis software (Institute for Genomics and Bioinformatics, Graz University of Technology (IGB-TUG, Graz, Austria). All data is represented in tables, graphs and expressed in mean and median with calculation of statistical significance.

## RESULTS

For better assessment of the *HOX* gene expression throughout developmental stages, granulocytic and monocytic lineages were pooled together, so we classified the myeloid differentiation into four stages (stage 1=G1+M1, stage 2=G2+M2, stage 3=G3+M3 and stage 4=G4+M4). *HOXA* and particular *HOXB* (HOXB2 and HOXB4) genes expression gradually decreased during the process of maturation (Fig. 1).

According to French-American-British classification (FAB) AML subtypes certain *HOX* gene expression patterns was observed. M3 FAB was the subtype with lowest expression *HOXA* and *HOXB* genes when compared with other FAB subtypes. In contrast, AML M5 was the highest expression of *HOXA* genes. According to molecular genetics, significant differences were also found among subgroups where cases that carry *PML-RARa* fusion gene showed the lowest levels of *HOXA* and *HOXB* genes; on the other hand cases that carry *MLL* gene aberrations express the highest levels (Fig. 2).

According to FAB subtypes and genetic aberrations, *HOX* gene expression was significantly lower in AML M4 with *CBFb-MYH11+* rearrangement and AML M2 with the *RUNX1-RUNX1T1+* rearrangement patients when compared with patients lacking such rearrangement (Fig. 3).

In our study, patients who have mutated *FLT3/ITD* and *PML-RARa*, showed very low levels while those with *FLT3* mutation only,

showed higher expression levels of *HOX* genes (Fig. 4).

We sorted 20 normal BM subpopulations according to different myelopoiesis stages and compared the subpopulations with matched AML FAB subtypes (Table 1). When we compared promyelocytes (ID=G2) with AML M3, we found different *HOX* gene expression patterns. Also, a significant difference in the median of *HOX* genes expression was found when we compared between AML M4 and M5 subtypes with their matched normal subpopulations (Fig. 2).

Table (1): Subpopulations of healthy BM cells **[23,25]**.

| Normal sorted myeloid lineage populations | | | FAB Counterparts |
|---|---|---|---|
| *Granulocyte Lineage subpopulations:* | | | |
| G1 | Myeloid progenitor | CD117+ CD15- CD11b- | AML M1 AML M2 |
| G2 | Promyelocyte | CD117+ CD15+ CD11b- | AML M3 AML M2 |
| G3 | Promyelocyte-Myelocyte | CD117- CD13++ CD16- | |
| G4 | Myelocyte | CD117- CD13dim CD16- | |
| *Monocyte lineage subpopulations:* | | | |
| M1 | Monoblast | CD34+ SSc++ HLA-DR+ CD33- | AML M1-M0 |
| M2 | Myelo/monoblast | CD34+ SSc++ HLA-DR+ CD33+ | AML M2 |
| M3 | Promonocyte | CD34- FSc and Ssc corresponding to monocyte CD33+ Cd14- | AML M4-AML M5a |
| M4 | Monocyte | CD34- FSc and Ssc corresponding to monocyte CD33+ Cd14+ | AML M5b |

Table (2): Clinical, hematological and cytogenetic / molecular data of 38 acute myeloid leukemia patients.

| Parameter | N | % |
|---|---|---|
| Male/Female | 20/18 | 53/47 |
| Age: years - median (range) | 47 (28-54) | |
| *FAB Subtypes:* | | |
| M0 | 3 | 8 |
| M1 | 3 | 8 |
| M2 | 8 | 22 |
| M3 | 7 | 18 |
| M4 | 9 | 23 |
| M5 | 8 | 21 |
| *Cytogenetics/Molecular genetics:* | | |
| t(15;17) PML-RARa | 7 | 18 |
| t(8;21) RUNX1-RUNX1T1 | 6 | 16 |
| inv(16) CBFb-MYH11 | 3 | 8 |
| t(11q23) MLL rearrangements | 8 | 22 |
| Cytogenetically Normal **1** | 14 | 36 |
| NK-AML | 7 | 18 |
| FLT3/ITD | 5 | 13 |
| NPMI1 (mut) | 2 | 5 |

**1** Patients negative for the presence of four chromosomal translocations typical of AML.

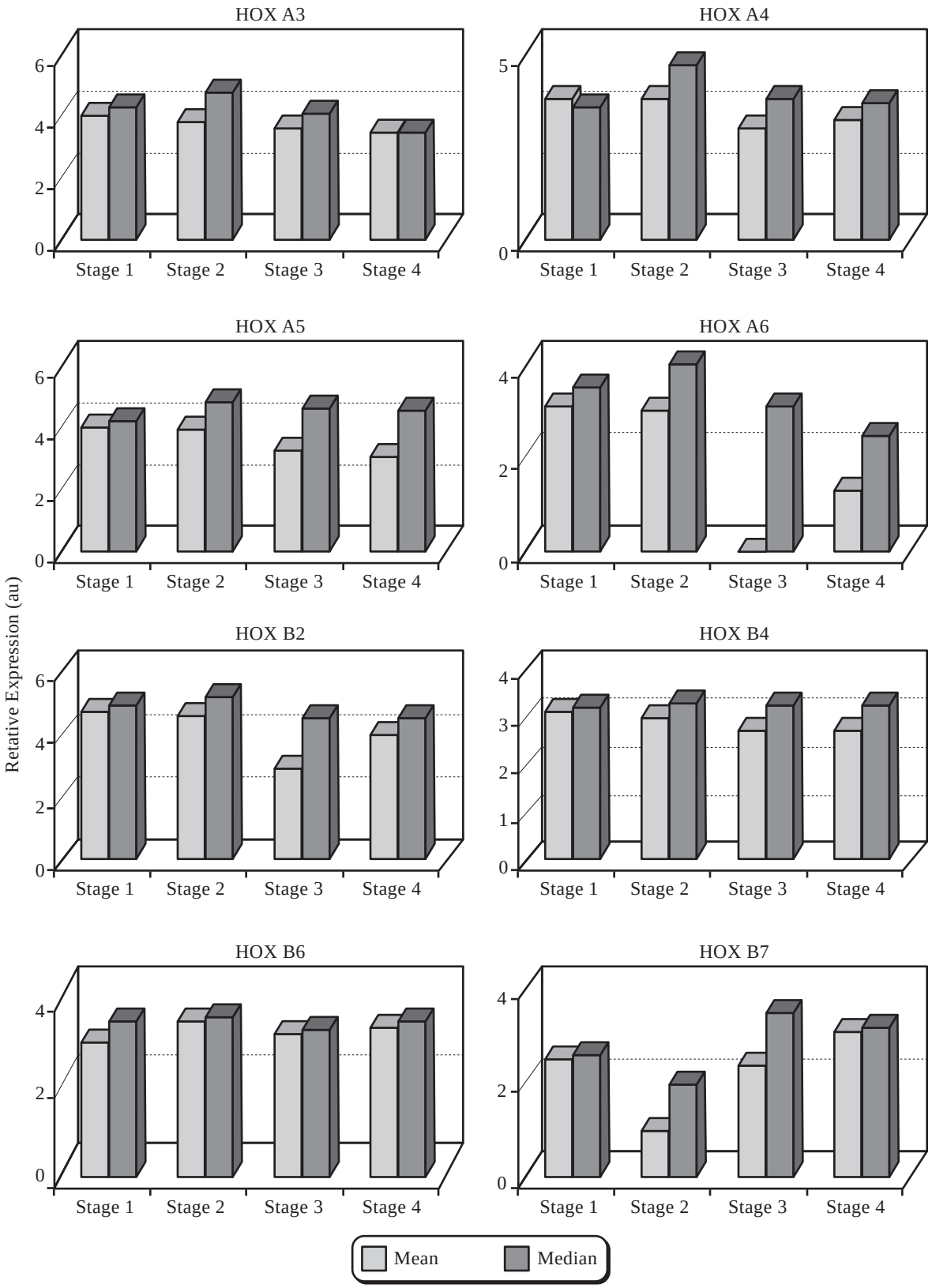


Fig. (1): Expression of different *HOXA* and *HOXB* genes in healthy BM. The myeloid differentiation was classified into four stages (stage 1=G1+M1, stage 2=G2+M2, stage 3=G3+M3 and stage 4=G4+M4).

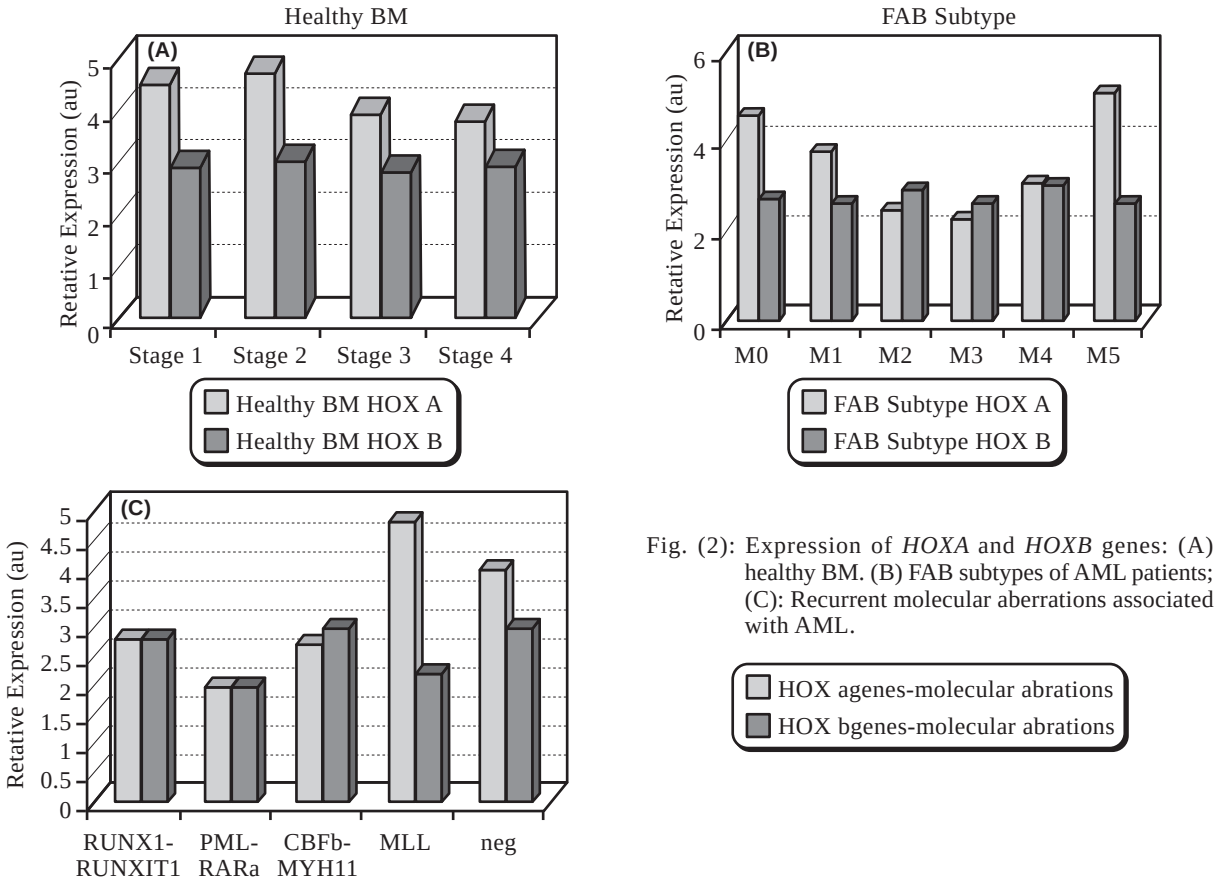


Fig. (2): Expression of *HOXA* and *HOXB* genes: (A) healthy BM. (B) FAB subtypes of AML patients; (C): Recurrent molecular aberrations associated with AML.

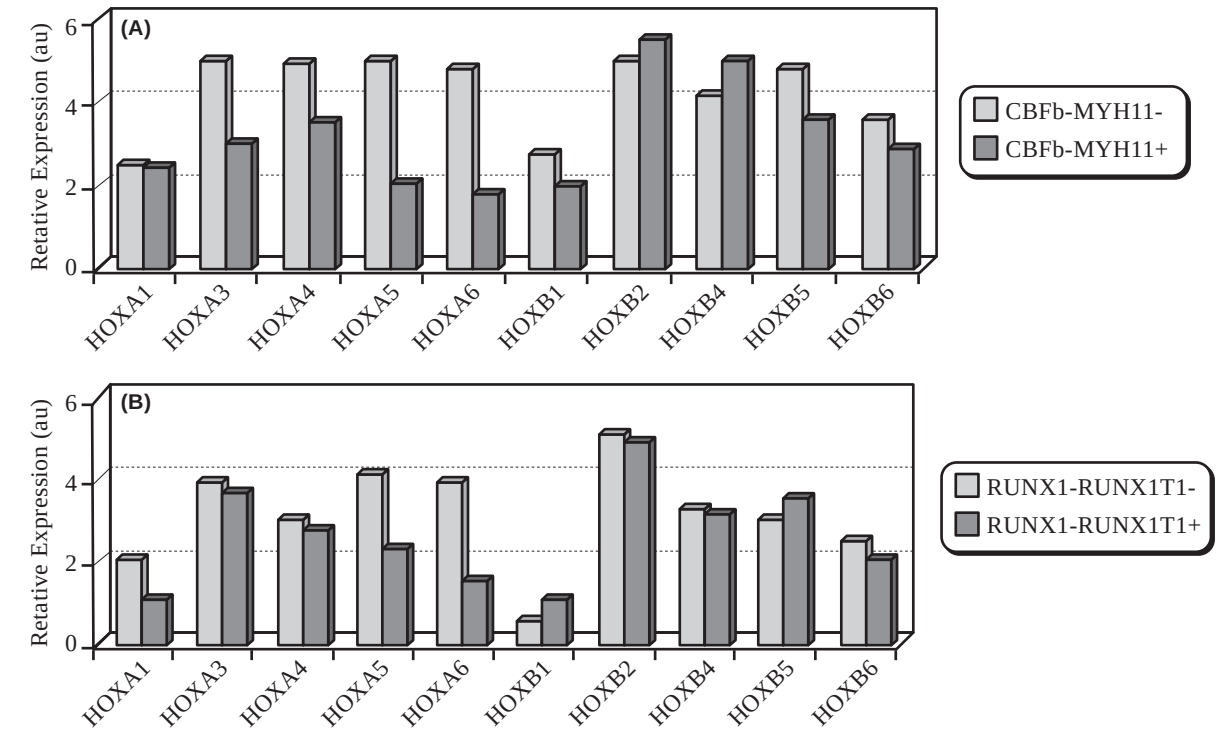


Fig. (3): Expression of different HOXA and HOXB genes with: (A) AML M4 patients with CBFb-MYH11 +/–. (B) AML M2 patients with RUNX1-RUNX1T1+/–. Statistical significance: *≤0.05, **≤0.01.

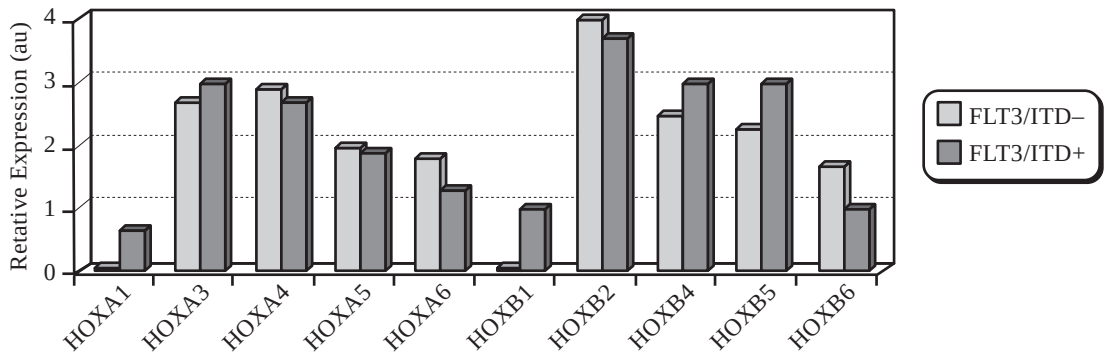


Fig. (4): Expression of different HOXA and HOXB genes in PML-RARa+ AML M3 patients with concurrent FLT3/ITD+ and FLT3/ITD–. Statistical significance: *≤0.05, **≤0.01.

## DISCUSSION

*HOX* genes are potent regulators of normal hematopoiesis, both during embryonic development and in adult life. However, the role of *HOX* genes in the process of leukemogenesis has not yet been proven **[26-28]**.

Abnormal expression of *HOX* genes was reported in most leukemia patients; however it is unclear whether it is a primary cause or a consequence of differentiation block **[29]**. Also the effect of the molecular abnormality present in leukemic cells and its relation to *HOX* genes expression was addressed **[30,31]**. In this study we sorted normal BM subpopulation according to the stage of differentiation and compared between the expression of *HOX* genes in the normal BM cells and their matched counterpart in AML patients*******. We found that at the early stages of hematopoiesis, the expression of *HOX* genes was higher and gradually decreased with maturation, which proves and matches with the claim that in the early stages of hematopoiesis, *HOX* genes play strong regulation roles **[27]**.

A different expression of *HOX* genes was found in leukemic cells other than that found in the normal matched counterpart at the same differentiation stage. This proves that the presence of abnormal of *HOX* gene expression is not enough to explain the developmental stage at which the cells have been arrested. Previous reports have documented that *HOX* gene expression in ALL patients' subgroups was different in malignant cells from their matched normal counterparts' **[15]**. Collectively, our results support the hypothesis that the abnormal control of *HOX* genes has a role in the pathogenesis of leukemia and malignant transformation.

In the current study, the molecular abnormality associated with the AML patient's subtypes found to affect the *HOX* genes expression. This was most clearly found in AML M3 patients with positive *PML-RARa* fusion gene where they expressed the lowest level of *HOX* genes. Also AML patients with other gene rearrangements such as *RUNX1-RUNX1T1+*, *CBFb-MYH11+* and *MLL* showed unique *HOX* gene expression patterns. This agrees with reports telling that MLL rearrangements have a prominent impact on HOX gene expression **[32-37]**.

Moreover, *FLT3/ITD+PML-RARa+* patients showed statistically significantly lower expression of *HOX* gene than those having *FLT3/ITD+* mutation without *PML-RARa* fusion protein **[23,38]**. Thus *PML-RARa* fusion gene has a more dominant role over *FLT3/ITD* in the process of neoplastic transformation.

In conclusion, particular differentiation stages are not the only factor affecting *HOX* genes expression patterns in AML malignant cells. The specific molecular aberration associated with AML is the main and primary factor affecting the pattern of expression of *HOX* genes.

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