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Research Article

# Upregulation of HOTAIR Predicts Poor Outcome in Acute Myeloid Leukemia

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ABSTRACT

**Background:** Acute myeloid leukemia (AML) is a clonal hematopoietic malignancy, in spite of the marked improvement in the treatment of AML; Molecular biomarkers open the door to improve disease outcome. Accumulating evidence suggested that the long non-coding RNA "HOTAIR" has an oncogenic role in hemopoietic malignancies. Recently, it has been evident that knockdown of HOTAIR inhibits cell proliferation and induces apoptosis by modulating c-Kit expression via acting as competing for endogenous RNAs (ceRNAs) to sponge miR-193a at the post-transcriptional level.

**Objectives:** we aimed to evaluate the diagnostic and prognostic value of HOTAIR in AML, to investigate its association with and c-Kit and miR-193a.

**Subjects &Methods:** we examined the expression levels of HOTAIR, miR-193a, and c-Kit in 100 de-novo AML patients using quantitative, the association of genes expressions with risk factors and patient's outcome were statistically analyzed.

**Results:** the expression of HOTAIR was significantly upregulated by four folds in AML compared to healthy controls; higher expression levels were associated with high-risk factors, poorer overall survival (OS) and shorter leukemia-free survival (LFS). In addition; a negative correlation was detected between Lnc-HOTAIR and miR-193a, although significance didn't reach.

**Conclusion:** The obtained results suggested that HOTAIR expression was upregulated in peripheral blood samples of de-novo AML patients and was associated with leukemic burden and disease outcome. Therefore, it may represent an effective diagnostic and poor prognostic tool for AML.



## Introduction

Acute Myeloid Leukemia is considered as one of the most aggressive types of cancer, its rank is the 5$^{th}$ between the lethal types of cancers in the male population [1]. AML is intrinsically heterogeneous, destructive disorder and hematological malignancy with a spectrum of morphologic, cytogenetic, immune-phenotypic and molecular characteristics [2]. AML quickly becomes fatal, and historically unless it wasn't treated, it has always been associated with a poor prognosis, on the other hand, at the few last decades, it was probably treated [3]. It is a result of clonal expansion of the myeloid progenitor in both peripheral blood (PB) and bone marrow (BM) with a spread which is possible for spleen and liver [1]**.** When the BM cells were changed into leukemic cells a sudden division take place to give billions of up-normal cells [4]. The classification of AML includes a variety of several groups, for instance, AML with a translocation between chromosomes 8 and 21, AML with changes in chromosome number 11, therapy-related AML & AML with myelodysplasia-related changes [1]. Lately, it has been shown that long noncoding RNAs (lncRNAs) play a crucial role in hematopoietic differentiation and hematological malignancies, including AML [5].

Long non-coding RNA (Lnc-RNA) is a hetero-generous class of RNAs that are commonly described as non-protein-coding transcripts longer than 2 hundred nucleotides. LncRNA which become considered as best transcriptional "noise" in the beyond a long time can participate in

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numerous essential organic techniques, along with chromatin remodeling, gene transcription [6]. These days, increasingly researches have proven that lncRNAs are deregulated in a huge form of cancers. There is a usually developing listing of lncRNAs which are associated with gene expression regulation and illnesses. However, little or no is known approximately their unique function. LncRNAs that can alter gene expression thru unique molecular mechanisms.so far, the wide variety of human lncRNA genes is close to 9,000. However, just a few of them had been assigned a function in myelopoiesis [7, 8].

Hox transcript antisense intergenic RNA (HOTAIR) is a 2,158 bp lncRNA this is transcribed from the antisense strand of the homeobox C gene locus of chromosome 12 [5]. HOTAIR coordinates with chromatin modifying enzymes and regulates gene silencing. Several latest research has recognized the aberrant expression of HOTAIR in a number of most cancers types, including breast, gastric, pancreatic, cervical, colorectal and lung cancer, and a higher expression level of HOTAIR has been correlated with high tumor burden and cancer progression, Thereby, knockdown of HOTAIR is able to inhibit the malignant invasion and proliferation and inducing cells apoptosis, consequently indicating that HOTAIR might also characteristic inside the modulation of cancer development [8-16]. Presents proof in their look at for the primary time that HOTAIR can also act as an oncogenic gene in AML, and that it could constitute a capability biomarker of bad prognosis and an ability therapeutic goal for AML intervention [5, 15]. However, the right molecular mechanisms in the back of the involvement of HOTAIR in AML require similarly investigation [16].

Micro RNAs (miRNA) are small non-coding RNA molecules, it's far composed approximately of (20-25nucleotides), they are corresponding for suppression gene expression via binding to complementary segments of messenger RNA and interfering with the formation of proteins by way of translation [13, 15-18]. According to Xing et al, miR-193a was downregulated in AML blasts because of hypermethylation in its promoter area. Ectopic expression of miR-193a inhibited cell proliferation, facilitated differentiation, and triggered apoptosis in AML blasts via immediately focused on mobile cycle control genes (c-package, DNMT3a, CCND1, and MDM2) [19]. However, it's far doubtful whether or not HOTAIR acts as a sponge to modulate miR-193a in AML cells. miR-193a has been reported overexpression of miR-193a inhibit cell proliferation and induce apoptosis in AML, THP1, and HL-60 cells line [19]. Therefore, this study was designed to assess the diagnostic and prognostic value of Lnc_HOTAIR in AML, moreover, to find out the association between HOTAIR expression and expression of miRNA-193a and c-kit gene as targeting genes for HOTAIR.

## Results

### I Demographic and clinicopathological characteristics of AML patients

The mean age of AML enrolled patients was 50 years; the majority were males. Referred to risk factors for AML; the studied patients were categorized into low and high-risk groups. Patients at low risk predominate in our study regarding age, gender, TLC, hemoglobin concentration, platelets count, BM blasts and MRD. The most frequent phenotype was M2 (42%), however, M3 represented the least frequent phenotype (8%). Few patients (34%) were relapsed (Table 1).

**Table 1:** Demographic and Clinical characteristics of the studied subjects

| **Variable** | **AML group N=100** | **Control group N=50** |
|---|---|---|
| **Age (years)** | Mean ±SD:50.3±14.4<br>range 28 - 77 | 44.2±13.8<br>27 - 72 |
| **Age subgroups** | | |
| ≤40 | 42(42) | |
| >40 | 58(58) | |
| **Gender** | | |
| Male | 76(76) | 35(70) |
| Female | 24(24) | 15 (30) |
| **AML phenotype** | | |
| M0-M1 | 28(28) | |
| M2 | 42(42) | |
| M3 | 8 (8) | |
| M4-M5 | 22(22) | |
| **Cytogenetic abnormalities** | | |
| No | 46(46) | |
| Yes | 54(54) | |
| **TLC ($x10^6$/L)** | | |
| ≤50 | 72(72) | |
| >50 | 28(28) | |
| **Hemoglobin (gram/dl)** | | |
| ≤10 | 82 (82) | |
| >10 | 18 (18) | |
| **Platelet (($x10^{12}$/L)** | | |

| | | |
|---|---|---|
| ≤100 | 26(26) | |
| >100 | 74(74) | |
| **BM blasts (%)** | | |
| ≤70 | 70(70) | |
| >70 | 30(30) | |
| **MRD (%)** | | |
| ≤0.01 | 62(62) | |
| >0.01 | 38(38) | |
| **Clinical Response** | | |
| Remission | 66(66) | |
| Relapse | 34(34) | |

M0: Undifferentiated myeloblastic leukemia; M1: Acute Myeloblastic leukemia with minimal maturation; M2: Acute myeloblastic leukemia with maturation; M3: Acute promyelocytic leukemia; M4: Acute myelomonocytic leukemia; M5: Acute monoblastic leukemia; TLC: total leucocytes count; BM: bone marrow; MRD: minimal residual disease

**Table 2:** Expression levels of Lnc-HOTAIR, miR-193a and c-Kit in studied subjects

| **Variable** | **Lnc-HOTAIR** | | **miR-193a** | | **c-Kit** | |
|---|---|---|---|---|---|---|
| | **Median(range)** | **Statistics** | **Median(range)** | **Statistics** | **Median(range)** | **Statistics** |
| **Subjects** | | | | | | |
| AML | 38(11 -133) | U=39 | 6(1.2-12) | U=19 | 33(17-88) | U=114 |
| Control | 9.5(3.4-18) | p=0.001 | 16 (8.5-31) | p=0.001 | 11(11-37) | p=0.001 |
| **Age subgroups** | | U=272 | | U=261 | | U=291 |
| ≤40 | 21(6.5-89) | P=0.5 | 2(0.2-8.5) | P=0.4 | 33(17-79) | P=0.8 |
| >40 | 22(11-133) | | 3(0.2-8.2) | | 32(18-88) | |
| **Gender** | | U=197 | | U=204 | | U=180 |
| Male | 21(6.5-133) | p=0.5 | 2.6(0.2-8.2) | p=0.6 | 32(18-80) | p=0.3 |
| Female | 34(12-93) | | 1.8(0.2-8.5) | | 45(17-88) | |
| **AML phenotype** | | F=0.4 | | F=0.09 | | F=0.3 |
| M0-M1 | 19(11-133) | p=0.8 | 3(0.2-6.3) | p=0.9 | 33(18-78) | p=0.8 |
| M2 | 23(7-93) | | 3(0.2-8.5) | | 39(18-88) | |
| M3 | 49(13-134) | | 3(0.2-5.8) | | 32(22-61) | |
| M4-M5 | 33(9 – 78) | | 2(1.2-7.4 | | 34(17-70) | |
| **Cytogenetic abnormalities** | | U=304 p=0.9 | | U=283 p=0.6 | | U=256 p=0.3 |
| No | 21(11-113) | | 2(0.2-7.4) | | 32(17-80) | |
| Yes | 21(6.5-133) | | 3(0.2-8.5) | | 33(19-88) | |
| **TLC(x10$^6$/L)** | | U=0.1 | | U=231 | | U=193 |
| ≤50 | 18(11.2-97) | p=0.001 | 3(0.2-8.5) | p=0.8 | 32(17-88) | p=0.3 |
| >50 | 112(103-132) | | 3(0.6-8.2) | | 44(18-78) | |
| **Hemoglobin (gram/dl)** | | U=103 p=0.05 | | U=165 p=0.6 | | U=137 p=0.2 |
| ≤6 | 71(14-133) | | 3(0.2-8.5) | | 45(18=78 | |
| >6 | 21(7-113) | | 3(0.6-8.2) | | 32(17-88) | |
| **Platelet count** ((x10$^{12}$/L) | | U=192 p=0.3 | | U=195 p=0.3 | | U=151 p=0.05 |
| ≤100 | 42(6.5-133) | | 2(0.2-8.2) | | 45(19-78) | |
| >100 | 21(11-93) | | 3(0.2-8.5) | | 32(17-88) | |
| **BM blasts (%)** | | U=0.1 | | U=250 | | U=176 |
| ≤70 | 18(11-97) | p=0.001 | 3(0.2-8.5) | p=0.6 | 31(17-79) | p=0.05 |
| >70 | 112(92-132) | | 2(0.6-8.2) | | 45(18-88) | |
| **MRD (%)** | | U=246 | | U=257 | | U=211 |
| ≤0.01 | 21(11-92) | p=0.3 | 3(0.2-74) | p=0.5 | 32(17-80) | p=0.09 |
| >0.01 | 29(6.5-133) | | 2(0.2-8.5) | | 41(18-88) | |
| **Clinical Response** | | U=24 | | U=225 | | U=132 |
| Remission | 18(11-97) | p=0.001 | 2(0.2-7.4) | p=0.3 | 31(17-80) | p=0.002 |
| Relapse | 111(20-132) | | 3(0.6-8.5) | | 48(19-88) | |

Test is significant at level≤0.01, Acute Myeloid Leukemia; Lnc: long non coding RNA. Mann-Whitney U M0: Undifferentiated myeloblastic leukemia; M1: Acute Myeloblastic leukemia with minimal maturation; M2: Acute myeloblastic leukemia with maturation; M3: Acute promyelocytic leukemia; M4: Acute myelomonocytic leukemia; M5: Acute monoblastic leukemia; TLC: total leucocytes count; BM: bone marrow; MRD: minimal residual disease

**Table 3:** Impact of high/low expression levels of lnc-HOTAIR, miR-193a and c-Kit on OS and LFS free survival AML

| Variable | Overall survival (OS) | | | | Leukemia free survival | | | |
|---|---|---|---|---|---|---|---|---|
| | MST | 95% CI | $X^2$ | P value | MST | 95% CI | $X^2$ | P value |
| **Lnc-HOTAIR ($log^{10}$)** | | | 17 | 0.001 | | | 5.5 | |
| ≤38 | 22.0 | 21-24 | | | 18.0 | 15.9-20 | | 0.02 |
| >38 | 13.0 | 8.8-17 | | | 12.4 | 7.4-17.5 | | |
| **miR-193a ($log^{10}$)** | | | 2.8 | 0.09 | | | | 0.9 |
| ≤6 | 18.6 | 16.4-21 | | | 17.0 | 14-19 | 0.02 | |
| >6 | 21.6 | 19-24 | | | 16.3 | 12-20 | | |
| **c-Kit ($log^{10}$)** | | | 4.9 | 0.03 | | | 9.0 | 0.003 |
| ≤33 | 21.6 | 19.8-23 | | | 19.3 | 17-21 | | |
| >33 | 16.4 | 12.7-20 | | | 12.6 | 9.2-16.5 | | |

Log Rank (Mantel-Cox) X: Chi-square value, LFS: Leukemia free survival, HOTAIR: Home box transcript antisense intergenic RNA, Lnc: long non coding; OS: overall survival; LFS: leukaemia free survival

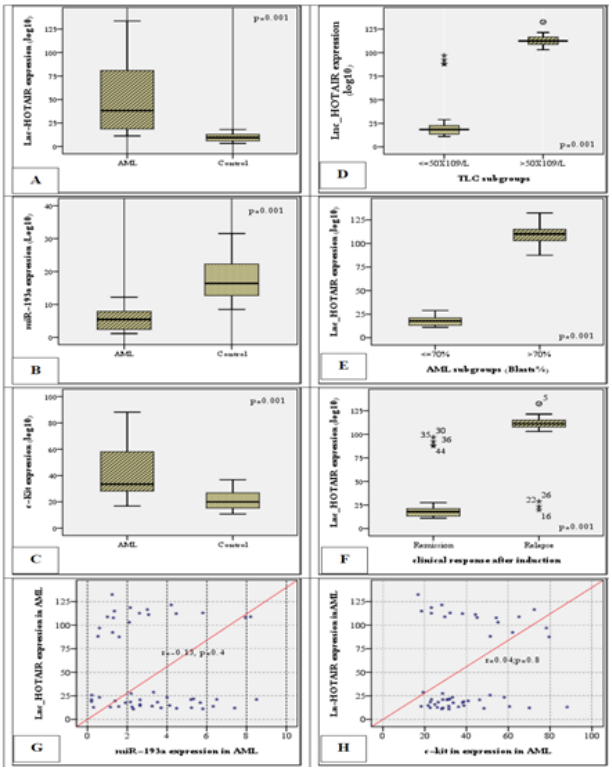


**Figure 1:** Boxplot graphs comparing the expression levels of Lnc-HOTAIR, miR-193 and c-kit in peripheral blood sample (A-C); respectively between AML and healthy controls; also, between high risk and low risk AML (D-F). Moreover; a negative correlation was observed between expression levels of Lnc-HOTAIR and miR-193a in AML samples (G) (r=0.13); whereas; a positive correlation was detected between Lnc-HOTAIR and c-kit (H) (r=0.4); although both correlations didn't showed significance (p>0.05)

**II Expression of Lnc-HOTAIR, miR-193a, and c-Kit**

A significant difference between AML patients and healthy controls was detected for the expression of HOTAIR, miR-193a, and c-Kit (p=0.001) (Table 2), (Figure 1A, 1B, 1C). Meanwhile; the expression of HOTAIR and c-kit were upregulated by approximately 4 and 3 folds; respectively; miR-193a expression was decreased. Higher expression of HOTAIR was significantly associated with AML at high risk who had Hb concentration ≤6 gm/dl, TLC>50,000/ul, BM blasts>70% in addition to AML patients who relapsed after induction chemotherapy. On the other hand; no significant difference was detected between HOTAIR expression and the other risk factors (p>0.05) (Table 2), (Figure 1D, 1E, 1F).

Comparing between gene expression of HOTAIR with miR-193a and c-Kit; higher expression levels of HOTAIR was associated with high expression of c-kit ($r$=-0.13) and lower expression of miR-193a ($r$=0.4) (Figure 1G, 1H) although no significant difference was reached (p>0.05).

**III The influence of Lnc-HOTAIR expression on the overall survival (OS) and leukemia-free survival (LFS)**

To investigate the impact of the expression of HOTAIR and its target gene on the overall survival (OS) and LFS; a Kaplan –Meier survival curves were plotted and the cut-off value was adjusted to 38, 6 and 33 for Log expression of HOTAIR, miR-193a and c-Kit; respectively, a significant difference was obtained for the expression of HOTAIR and c-Kit (p≤0.05), higher expression value was associated with shorter OS and LFS (Table 3), (Figure 2A,2C,2D,2F). Meanwhile, the expression of miR-193a did not significantly influence the AML patients outcome (p>0.05) (Table 2), (Figure 2B, 2E).

**Methods**

**I Patient enrollment**

Peripheral blood samples (PB) were collected at diagnosis from 100

patients with AML; diagnosis was confirmed based on bone marrow morphological, cytochemical, cytogenetics, immunophenotyping, and molecular basis. According to the definitive risk factors; patients were classified into low and high-risk groups. The study was approved by the ethical committee of the faculty of Medicine, Ain Shams University. Written consent was signed from all participants in whom it was assured that all information generated in this study remained confidential. The study was conducted according to the World Medical Association Declaration of Helsinki. All patients received a standard regimen of induction chemotherapy consists of Vincristine, Corticosteroids, L-asparaginase, an anthracycline. Patients were followed up after four weeks after induction for assessment of clinical response.

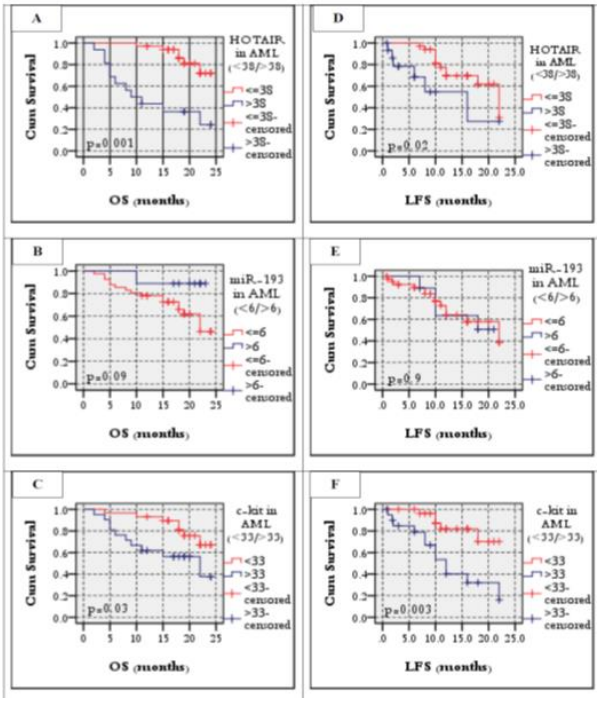


**Figure 2:** Kaplan-Meier survival curves for the OS (A-C) and LFS (D-F) for low and high expression of agency Hatia me 198a and c-kit; respectively in AML patients, the p-value is calculated by log-rank (Mantel-Cox) test.

### II RNA isolation & purification and cDNA synthesis

The collected peripheral blood samples were lysed with Trizol and miRNAs was extracted using the miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The extracted RNA concentration and purity were evaluated spectrophotometrically at 260 and 280nm; cDNA was synthesized using the miScript II RT kit (Qiagen, Hilden, Germany).

### III Gene expression analysis using quantitative PCR (qPCR)

### Lnc_HOTAIR and c-kit expression analysis

The expression of Lnc-HOTAIR, miR-193a and c-Kit were measured by quantitative PCR technique, RT2 lncRNAs qPCR primer assays and RT2 lncRNA qPCR kit were used for the amplification of human HOTAIR (lncRNA HOTAIR; cat no: 330701) and c-Kit (Hs_c-kit quantities primer assay, cat no: 2499900), the GABDH gene expression was amplified as a housekeeper gene for normalization of the expressed genes. The 25 ul reaction mixture/reaction consists of RT2 cyber green PCR master mix, *10 µM* primer assay and 50pg- 3ng cDNA. Both targets were amplified in duplicates for each sample. The thermal protocol consists of 10 min for HotStarTaq DNA Polymerase activation at 95ºc followed by 40 cycles of primer annealing at 55ºc for 40 seconds, and extension at 70ºc for 30 sec). The $2^{\Delta\Delta Ct}$ method was conducted for the analysis of gene expression levels, using GABDH as an endogenous reference control for normalization purposes.

### miR-193a expression analysis

The quantification of miR-193a levels was performed using the SYBR-Green fluorescent-based primer assay (Hs_miR-193a_1 miScript Primer assay; cat no: Ms00008932), the small nucleolar RNA, C/D box 48 (SNORD48), (NCBI RefSeq: NR_002745.1) was used as a reference gene. Expression assays were purchased from Qiagen, Hilden, Germany and conducted on the5-plex Rotor-Gene PCR System (Qiagen, Hilden, Germany). The qPCR was performed in the 5-plex Rotor-Gene PCR System (Qiagen, Hilden, Germany). The 20ul reaction mixture/reaction consist of 2x QuantiTect cyber green PCR master mix, 10x script universal primer, 2 ul primer assay and 50pg- 3ng cDNA. Both targets were amplified in duplicates for each sample. The thermal protocol consists 15 min for HotStarTaq DNA Polymerase activation at 95ºc followed by 40 cycles of denaturation at 94ºc for 15 minutes, primer annealing for 30 seconds at 55ºc and extension at 70ºc for 30 sec). The $2^{\Delta\Delta Ct}$ method was conducted for the analysis of miR-193a expression levels, using RUN6 as an endogenous reference control for normalization purposes.

### IV Statistical Analysis

Statistical analysis was performed using SPSS v.23 (Chicago, IL, USA). The comparative analysis for gene expression between different studied groups was conducted using non-parametric Mann–Whitney U test. Spearman's correlation analysis was used to investigate the association between HOTAIR/miR-193a and c-Kit in AML patients. To assess the impact of gene expression levels on disease outcome; the cut-off value was determined for each gene expression using the median values and survival analysis was conducted. Significance was set at level$\leqslant$0.05.

### Discussion

It has been evident that more than 10,000 discovered lncRNAs have been contributed to human solid and hematopoietic cancers [20].Adjacent with the high needs for new non-invasive diagnostic and prognostic biomarkers that target human cancer; the lncRNAs occupy the forefront of use in leukemia [19]. Recent studies provide evidence that the long non-coding RNA –HOTAIR "HOX transcript antisense RNA" has an oncogenic role in AML[2,18]; however; the precise underlying molecular mechanism still under investigations [21]. Few studies tried to determine the different pathways by which HOTAIR influence myelopoiesis; they reported that knockdown of HOTAIR blunted the expression of HOX1 and HOX4 during myeloid differentiation [19]. In the present study, by using quantitative Real-time PCR (qPCR), it was

confirmed that HOTAIR expression is markedly upregulated in peripheral blood samples of de-novo AML compared to normal healthy controls and higher expression is associated with high-risk groups as well as poor prognosis. Moreover; we demonstrated a significant association between high expression of HOTAIR with downregulation of miR-193a and higher levels of the c-Kit gene.

HOTAIR upregulation has believed to be contributed to a variety of hematological malignancies and cancers. Inconsistent with our results; evidence supports the idea that HOTAIR acts as an oncogene and mediates tumor invasion and metastases. It has been found that HOTAIR is upregulated in AML [5, 6]. Wu S. et al demonstrated that the expression of HOTAIR was significantly upregulated by three folds in de-novo AML compared to healthy control; moreover; higher expression levels were associated with high BM blasts, total leucocytes counts, and low hemoglobin concentration and platelets counts [23].On the contrary, it was reported by a study conducted on Iranian patients that the expression level of HOTAIR could not be considered as a definitive diagnostic or prognostic biomarker for AML as it's expression levels insignificantly differed in AML patients and healthy controls [16]. This odd result could rely on several limitations that include a few small sample sizes and restricted to Iranian's patients. The oncogenic behavior of HOTAIR has been demonstrated in different types of solid tumors as colorectal cancer, gastric, hepatocellular carcinoma, cervical and pancreatic cancer [12-14, 21, 24]. In fact, the line-HOTAIR is located on the 12q13.13 region, it regulates multiple genes in cooperation with Polycomb Repressive Complex 2 (PRC2) that is involved in the polycomb-dependent chromatin modification. Accordingly, the upregulation of HOTAIR increases undifferentiated cancer and contributes to cancer development and progression [12].

The mechanism by which HOTAIR contributes to carcinogenesis have been investigated by in vivo studies, it was found that knock-down of HOTAIR suppressed myeloid blasts proliferation and tumor growth via sponging miR-193a and miR-613 that regulates c-Kit and notch-3 in AML and pancreatic cancer; respectively [19, 25]. Furthermore; it was recently demonstrated by Li et al that knockdown of HOTAIR inhibits cervical cancer cell's proliferation and invasion in vivo and in vitro, he suggested that this action is achieved via competitive binding of HOTAIR to miR-23b which further modulates the expression of MAPK1 in cervical cancer cells [21].

It was evident that miR-193a was downregulated in AML blasts; the dysregulation of miR-193a inhibited cell proliferation, induced apoptosis, and enhanced differentiation through targeting c-Kit, DNMT3 and MDM[2, 4, 15, 16]. Moreover, the interlink between HOTAIR and miR-193a has been demonstrated by Xing et al, he suggested that HOTAIR may modulate c-Kit expression through endogenously competing with miR-193a in AML cell lines [19]. In addition, he recommended applying the HOTAIR/miR-193a/c-Kit axis as therapeutic targets in AML. In the current study, for the first time, we investigate the association between the HOTAIR, miR-193a, and c-kit in clinical samples of AML, our results revealed a significant association between the three biomarkers and that which could reflect their regulatory interlink that was proved experimentally on leukemic cell lines [2, 12].

In the present study, the HOTAIR expression level was assessed in high risk-AML patients and compared to the low-risk group; higher expression levels of HOTAIR was significantly associated with AML patients with total leucocyte count >50,000, bone marrow blasts percentage >70% and hemoglobin concentration≤6 gm/dl, which represented aggressive clinic-pathological features. Moreover; HOTAIR expression level was significantly correlated to disease outcome in AML, patients with higher HOTAIR expression tended to have a poorer overall survival (OS) and Leukemia-free survival (LFS) when compared to those with lower expression. Similar results were obtained by Wu et al who concluded that HOTAIR overexpression is an independent poor prognostic biomarker in AML patients [23]. Additionally, the prognostic value of HOTAIR has been investigated in different types of cancers in which their results were in agreement with our findings. The data suggested that upregulation of HOTAIR is associated with shorter OS and progression-free survival (PFS) in cancer cervix, non-small cell lung cancer (NSCLC), breast cancer and gastric adenocarcinoma[4, 9, 16-19, 21, 27].

Referred to the mentioned findings; we can conclude that the expression of HOTAIR has a significant diagnostic and prognostic value in AML and higher expression levels are associated with poor prognosis. Besides; we confirmed the results which have previously proved in vivo experimental analysis that suggested the mechanistic pathway of HOTAIR/miR-193a/c-Kit on blasts proliferation in AML, which finally supports the oncogenic role of HOTAIR in development of AML. Furthermore; we suggest that the HOTAIR-miR-193a-c-kit axis may additionally represent a novel therapeutic application in AML within the coming days.

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**Conflict of Interest**

The Authors declare no conflict of interest.

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