Our findings revealed that lncRNA *RP11-68118.10* was downregulated in ALL samples compared to the control samples (P<0.0001) (Figure 6). Additionally, in our study, the association between the expression variation of this lncRNA and immune-phenotype in ALL patients was not significant (Table 1).

4. Discussion

The current study evaluated the expression levels of lncRNAs *RP11-137H2.4, RP11-203E8, RP11-446E9, 4RP11-624C23,* and *RP11-68I18.10* in patients who were positively diagnosed with Acute Lymphoblastic Leukemia (ALL) and their possible alterations compared to the normal cases.

ALL is one of the most prevalent cancers among children with an average age of <10 years[26] and is defined by the aggressive multiplication of white blood cells and their mal-developed stem cells.[27] ALL is categorized into several groups (mainly based on their CD cell surface markers) with respect to the immune-markers and clinical data, namely: T-cell, Pro B-Cell, Pre B-Cell, B-Cell, and Mixed. This categorization is done mostly based on blood lymphocyte markers (CD markers).[28]

Considering high mortality rate associated with this disease, early diagnosis and appropriate treatment strategies can be very helpful.

One of the most effective strategies for early diagnosis is the use of molecular biomarkers. Investigating the changes in the expression levels of the biomarkers indicates the importance of early diagnosis and treatment.[29] LncRNAs are regarded as one of the most reliable and effective molecular biomarkers. These non-coding molecules, which play significant roles in modifying gene expression and epigenetic alterations, are expressed in different levels in different cancers such as breast tumor,[30] lung cancer,[31] prostate malignancy,[32] and ALL.[33,34]Therefore, evaluation of the expression levels of these molecules at different stages of cancer may present new biomarkers for cancer detection.

Changes in the expression of lncRNAs can subsequently lead to changes in the expression of target genes, in addition to stimulation of changes in the dependent signaling pathways. Thus, the study of lncRNAs can be of considerable importance while investigating the transcription level and subsequent translation. Furthermore, expression of lncRNAs can lead to changes in the induction of signal pathways such as RAS / MAP kinase, NFKB, AKT, b-catenin, and so on.[35]

In addition to the abovementioned, various lncRNAs have shown different expression levels in various cancers, including breast, prostate, and gastric, as well as acute lymphoid carcinoma in children, and the association with cancer phenotypes such as migration, metastasis, and apoptosis has also been observed. A study examined the changes in the expression of lncRNAs in ALL cancer and proved it.

Fernando et al. displayed that CASC15 lncRNA could regulate the SOX4 gene in acute myeloid leukemia (AML).[36] This gene confers a critical role in the development and evolution of B-cells. Furthermore, Sox4 plays an important role in the B-catenin signaling pathway, and consequently, altering the expression level of the lncRNA CASC15 can induce changes in this signaling pathway. It displays the importance of evaluating the investigated lncRNAs.[37,38]

In a study by Wallaert et al. on important subclasses of lncRNAs for each of the T-ALL genetic subclasses, linked pattern of lncRNA expression in T-ALL subclasses with diverse stages of healthy T cell evolution in the thymus was evaluated.[39] Similarly, Casero et al., in their study, concluded that the co-expression of protein-coding genes near lncRNA genes demonstrated development for oncologies associated with the lymphoid variation.[40]

We chose lncRNAs *RP11-68I18.10, RP11-137H2.4, RP11-446E9, RP11-624C23.1*, and *RP11-203E8* and investigated the expression profile of these five lncRNAs in ALL patients. Recent studies have shown that these five lncRNAs significantly affect different cellular processes in pre B-ALL. The relationship between the immune-phenotype of ALL samples and the expression level of each lncRNA was also evaluated.

In the present study, we identified and analyzed the medical and pathological indication of immune-phenotype in patients with ALL, and assessed their association with the expression levels of the abovementioned lncRNAs one by one. Analysis of samples by classifying ALL in each case and comparing the expression level of each lncRNA showed no significant relationship between immune-phenotype of ALL samples and expression level of each lncRNA.

To confirm the findings of the current study and the expression changes of lncRNAs in ALL and healthy samples, Fong et al. evaluated the expression levels of different lncRNAs in MLL-r cancer, and revealed changes in the expression levels of many of these lncRNAs. These changes in expression levels were compared between the control and unhealthy samples. The results of this study are consistent with those of our study in that they indicated a change in the expression of lncRNAs in both leukemia samples and control samples. The results of our study displayed a significant decrease in the expression of LncRNAs *RP11-68I18.10, RP11-446E9, RP11-624C23.1, RP11-203E8,* and *RP11-137H2.4* in ALL

samples compared to the healthy control samples. These expression changes can specifically reflect expression changes in a particular type of cancer.

Consistent with the present study, a study was conducted by Gioia et al. on 56 pre B-ALL cancer samples to investigate the expression changes and roles of lncRNAs *RP11-203E8, RP11-624C23.1,* and *RP11-446E9*. These lncRNAs, which play a role in regulating metastasis and relocation of blood leukemic cells, were down-regulated in this cancer. Fernando et al., illustrated that the gene expression levels of lncRNAs *RP11-446E9* and *RP11-624C23.1* decreased in ALL. Our findings showed that the lncRNA *RP11-624c23.1* expression was significantly decreased in ALL patients compared to the control subjects (P<0.0001). Additionally, we studied the association between lncRNA *RP11-624c23.1* expression variations and immune-phenotype in these ALL patients. Our findings revealed that the alteration in the expression level of this lncRNA was not significantly associated with the immune-phenotype in ALL patients (P=0.963).

Our results revealed that lncRNA *RP11-203E8* was downregulated in ALL patients compared to the control samples (P=0.0007), and the association between the expression level of this lncRNA and immune-phenotype in ALL patients was not significant (P=0.161). Our results also showed that the lncRNA *RP11-446E9* was downregulated in ALL patients (P =0.616). The results of our study about lncRNAs *RP11-203E8* and *RP11-624c23.1*corroborate the results of Fernando et al and is consistent with those of Gioia et al. Furthermore, the results of these two articles are in line with those of our study in terms of lncRNA *RP11-446E9* expression in ALL cases.

Increased expression of lncRNAs *RP11-203E8* and *RP11-624C23.1* increases apoptosis and decreases phosphorylation of H2A.X, which is involved in response to DNA damage. Interestingly, the increased expression of both lncRNAs produced similar phenotypic effects, which indicate that the two lncRNAs contribute to the same molecular pathway.[37,38]

Moreno et al. reported that the increased expression of lncRNA *RP11-446E9* reduced the rate of migration and proliferation of leukemia cells, indicating the important role of this lncRNA in signaling pathways, which are dependent on the migration and proliferation. Another study demonstrated that an increased expression of lncRNAs *RP11-624C23.1, RP11-446E9,* and *RP11-203E8* results in augmented apoptosis while facing genotoxic stress, which displays the contribution of these lncRNAs in apoptosis-dependent signaling pathways. Moreover, it was shown that *RP11-203E8* and *RP11-624C23.1* play important roles in regulating DNA damage response (DDR). LncRNA *RP11-446E9* plays a role in activating cell death initiated by DNA damage, however it does not play a role in response to DNA

damage. In addition, the increased expression of this lncRNA results in a decreased proliferation and cell migration.[41]

The studies of Ouimet et al. demonstrated that lncRNAs *RP11-137H2* and *RP11-68I18.10* were downregulated in pre B-ALL. Our study reflected a decrease in the expression level of lncRNA *RP11-137H2.4* in ALL samples, which agrees with previously done research.[10-24] In addition, our study indicated the relationship between the LncRNA *RP11-137H2.4* expression changes and the immune-phenotype in ALL patients was not significant (*P*=0.55) (Table 1).The current study further revealed that lncRNA *RP11-68118.10* was downregulated in ALL patients compared to the control samples; this is consistent with a recent article. The association between this lncRNA expression variations and immune-phenotype in ALL patients also was not significant (Table 1). LncRNA *RP11-68I18.10* is overexpressed in cardiac and skeletal muscles and this result is not in agreement with our study results.

Previous studies have displayed that overexpression of lncRNA *RP11-137H2.4* in pre B-ALL cells can encourage malignant performances, for example, improved resistance to apoptosis, cell proliferation, and cell migration. In addition, genes of the MAPK signaling pathway are downregulated, resulting in *RP11-137H2.4* silencing. The study of Ouimet et al. revealed that lncRNAs *RP11-137H2* and *RP11-68I18.10* were downregulated in pre B-ALL, and describing the precise roles of *RP11-137H2.4* in cell cycle pathways and NRAS/BRAF/NF-κB MAPK cascade are significant enough to expand new therapeutic methods to overcome GC opposition in children treated for ALL. Furthermore, *RP11-137H2.4* knockdown significantly increases apoptosis in the cells treated with camptothecin, prednisolone, and doxorubicin. Although suppressing *RP11-68I18.10* was obligatory, it had no influence on apoptosis. Additionally, the levels of unusual effects on apoptosis are comparatively different, and they cause the deregulation of lncRNA in pre B-ALL. This effect determines specific lncRNAs despite having great effects on leukemia types. In this study, the expressions of aforementioned lncRNAs were reported to be reduced. Further studies are needed on the mentioned lncRNAs, and for defining the association of specificity and difference in lncRNA expression levels with ALL classes.

Conclusion

Results displayed a significant decrease in the expression levels of LncRNAs *RP11-203E8, RP11-624c23.1, RP11-446E9, RP11-137H2.4,* and *RP11-68118.10* in ALL patients compared to the control cases. Given the obtained results, an important prospective prognostic assessment is required to be done on these lncRNAs. They can also be used as a new diagnostic kit, as well as therapeutic tolerance in future. Moreover, studies on the mentioned lncRNAs and other lncRNAs are needed to identify the signaling pathways and the target genes of these lncRNA and their role in the tumor progression.

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Ethical considerations

The ethical approval was obtained from the Ethics Committee of Tabriz University of Medical Sciences (Code of Ethics: IR.TBZMED.REC.1398.732). The written informed consent form for participation in the study was also signed by the parents or legal guardians of the children.

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