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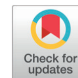
Original Article



Comparison of ADAM17 gene expression level in acute lymphoblastic leukemia patients

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Abstract

Background and aims: In acute lymphoblastic leukemia (ALL), large numbers of stem cells become lymphoblasts or lymphocytes. Among the genetic factors influencing cancer is the ADAM (a disintegrin and metalloprotease) gene family. Due to the important role of this family in cancer, this study aimed to compare the expression level of *ADAM17* gene in patients with ALL and healthy individuals.

Material and Methods: In this case-control study, 40 venous blood samples were taken from ALL patients referred to Omid hospital in Isfahan, Iran. Also, 40 venous blood samples were taken from healthy individuals in vitro. Lymphocyte isolation was performed using a ficol and cell RNA was isolated using an RNX-Plus kit. It was then converted to cDNA using the Yekta Tajhiz Azma kit. Finally, reverse transcription-polymerase chain reaction (RT-PCR) technique was used to evaluate the relative expression of ADAM17 gene in blood samples of healthy individuals and patients with leukemia, and the ratio was measured with the reference GAPDH gene. SPSS software version 22 and *t* test were used to analyze the data.

Results: The expression level of ADAM17 gene in patients with ALL compared to the control group showed a significant increase, which was statistically significant ($P = 0.043$).

Conclusion: It seems that increasing the expression of ADAM17 gene in people with ALL is a suitable biomarker to diagnose this disease.

Keywords: *ADAM17* gene, RT-PCR, Acute lymphoid leukemia, Biomarker

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Introduction

Cancer arises from the uncontrolled division of cells. The key genes involved in the development of cancer cells include tumor-blocking genes, oncogenes, DNA repair genes, and programmed death genes (1-3). Cancer is the second leading cause of death in the world and the third one in Iran (4-6). About 8% of all cancers are related to leukemia, and it is known as the fifth most common cancer in the world (7). Blood cancer is caused by the underdevelopment and proliferation of white blood cells and their precursors in the blood and bone marrow, and the bone marrow abnormally produces large amounts of blood cells. Following this process, the individual's ability to fight the diseases is lost (8).

Acute lymphoblastic leukemia (ALL) is the most common cancer in children that accounts for about 75% of blood cancers in them. In ALL leukemia, large numbers of stem cells become lymphoblasts or lymphocytes, and an increase in the number of lymphocytes in the bone marrow and blood creates less space for platelets, red blood cells, and white blood cells; so, the person bleeds easily (9). ALL is classified into three categories based on morphological criteria: ALL-L1, ALL-L2, and ALL-L3. This classification is based on criteria such as cancer cell size, nucleus chromatin status, nucleus shape, cytoplasm amount, basophilic intensity of the cytoplasm, condition and number of nuclei, and vacuolation of the cytoplasm (10). Different genetic factors are involved in cancer, including ADAM10, ADAM12, ADAM17, IKZF1, and BCL-2. ADAMs (a disintegrin and metalloprotease) are multi-domain proteins involved in various biological activities such as cell proliferation, cell migration, proteolysis, and cell adhesion, of which proteolysis plays the most important role. Many types of ADAM, including ADAM12, ADAM15, ADAM17, ADAM19, ADAM28, ADAMTS1, ADAMTS4, and ADAMTS5 are expressed in human malignant tumors. Many are involved in the regulation of growth factor activities and integral functions that lead to cell growth and invasion. Resultant cells have been reported from a variety of hematologic malignancies including leukemia, erythroleukemia, lymphoma, and myeloma. Although the expression of ADAM changes has been implicated in various diseases, their best role is in the formation and progression of cancer (11). For example, in their study of the existence of ADAMs in 2009, Turner et al found that expressing ADAM concentrations has been reported in a wide range of human cancers, and this group can affect the inhibition or progression of cancer and in

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most cases act as a positive regulator for cancer progression (12). ADAM17 (ADAM metallopeptidase domain 17) is a membrane protein that contains 824 amino acids and its gene is located on chromosome 2. ADAM17 is widely expressed in various tissues including brain, heart, kidney, and skeletal muscle and its expression changes during embryonic development and life in adulthood (13). ADAM17 protein can regulate cellular signaling and affect cellular behavior, but the outcome always depends on the cell substrate. Since both the substrate and the receiver are detachable, several scenarios are possible. The isolated substrate can attach to its receptor and then the activated receptor initiates downstream signaling events: ADAM17 can cleave HB-EGF, which in turn activates EGFR and initiates cell proliferation (14-16).

Given the high statistics of ALL and the role of ADAM17 gene as a key gene in cancer diagnosis and confirmation, for the first time, this study aimed to investigate the expression of ADAM17 gene in patients with ALL.

Materials and Methods

This case-control study was conducted in 2019. Due to the small number of children with ALL, 40 subjects referred to Omid hospital in Isfahan, Iran were included. Sampling was done before the start of chemotherapy through considering the same number of healthy individuals as the control group. An informed written consent was obtained from the parents of all subjects and an oncologist approved the diagnosis in laboratory conditions. To collect samples, 500 cc peripheral blood was taken from each person and poured into CBC tubes, and the samples were randomly classified into two groups: patient and healthy.

Inclusion criteria were being the 40 patient children, completing the informed consent form, having correct diagnosis of the disease, and not using chemotherapy drugs. The exclusion criteria were mortality and expression of ADAM17 gene in patients before chemotherapy. After collecting the samples, they were transferred to the laboratory complex of Islamic Azad University, Shahrekord Branch, Iran under standard conditions and on ice, and then other stages of the research were performed.

Isolation of lymphocytes

After blood sampling using a ficol, each sample lymphocyte was isolated separately under sterile conditions. Then, RNA was extracted from the lymphocyte of each sample using RNX Plus kit (CinnaGen Company, Iran). All steps were performed according to the kit instructions in standard conditions and on ice. To verify the extracted RNA, gel electrophoresis and nanodrop device were used; after ensuring the purity of the extracted RNA with RNX Plus kit, they were transferred to a -20 freezer for storage. To study the expression of ADAM17 gene, the primers of interest were designed by Gene Runner software and synthesized for primer sequence in kiagene Company based on ADAM17 and GAPDH gene sequences. The sequence of the genes of interest is given in Table 1.

Qualitative expression of genes by PCR

To qualitatively express the ADAM17 gene in a 0.2 microtubule, 6.5 μL of Master Mix was poured. Then, 1.5 μL of cDNA, 0.5 μL of Forward primer, 0.5 μL of Reverse Primer, and finally 4 μL of water were added to make a volume of 13 μL. Then, according to the temperature program, which included initial denaturation step for 5 minutes at 95°C, denaturation for 20 seconds at 95°C, and annealing for 20 seconds for both genes (temperature was 60°C for ADAM17 gene and 64°C for GAPDH gene), extension and final extension were done for 20 seconds at 72°C and for 5 minutes at 72°C, respectively. Then, polymerase chain reaction (PCR) was set up and samples were placed in the machine. PCR products were tested on 1% agarose gel and the results were recorded with a gel docking machine.

Reverse transcription-polymerase chain reaction (RT-PCR)

We performed the RT-PCR technique according to the Yekta Tajhiz Azma kit protocol and placed the samples in the device according to the temperature program given in Table 2. All steps were performed under the hood in sterile conditions.

Statistical analysis

Since the data had normal distribution, they were expressed in terms of mean ± SD. Data normality was

Table 2. RT-PCR reaction temperature program

Steps	Temperature	Time	Number of cycles
Initial denaturation step	95	5 minutes	1
Denaturation	95	20 seconds	44
Annealing *ADAM17* gene	60	20 seconds	
Annealing *GAPDH* gene	64	20 seconds	
Extension final	72	5 minutes	1

Table 1. Sequence of primers used in research

Primer	Sequence of primer	Temperature (°C)	Size of product (bp)
GAPDH F	5'-GCCAAAAGGGTCATCATCTCTGC-3'	64	183
GAPDH R	5'-GGTCACGAGTCCTTCCACGATAC-3'		
ADAM17 F	5'-CAGACCATCGCTTTTACAGACAC- 3'	60	128
*ADAM17*R	5'-TTTCTTCATTTGGATAACTTTTTG- 3'		

assessed using Kolmogorov-Smirnov test and after ensuring the normality of the data, the difference between the patients and control group was tested using t-test with SPSS software version 22. The *P* value of <0.05 was considered as significant.

Results

Results of PCR technique

To examine the cDNA prepared for all samples, RT-PCR was used for GAPDH gene and ADAM17 gene. Then, the 183 bp and 128 bp bands shown in Figures 1 and 2 for GAPDH and ADAM17, respectively, were observed. It should be noted that the absence of additional bands on the gel is a sign of specific binding and the correct choice of primers used.

Figure 1. The 183 bp band related to GAPDH with marker 100 bp and the 7th negatively controlled well.

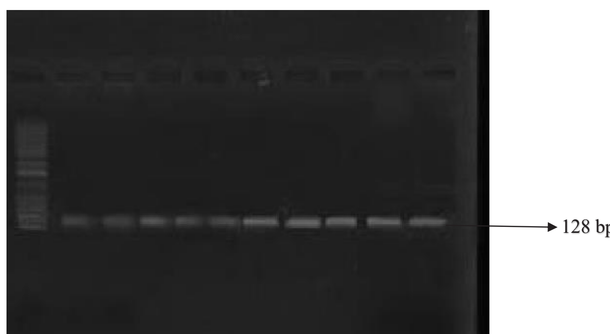

Figure 2. 128 bp band related to ADAM17 with 100 bp marker and the 12th negatively controlled well.

Results of RT-PCR

Proliferation charts

Figure 3A shows the proliferation curve for the healthy and patient samples for GAPDH and Figure 3B shows the proliferation curve for the healthy and patient samples for ADAM17.

Melting curve

To specifically investigate the RT-PCR response for target genes, the melting curve and peak were plotted by the device for each primer in each sample (Figure 3C for GAPDH and Figure 3D for ADAM17). Being single-peaked, aligned, and having a specific melting point indicated the correct proliferation of the target gene. Also, the lack of an extra peak indicated that the RNA is not contaminated and that the cDNA is synthesized correctly.

Comparison of gene expression levels

The obtained results indicated that the expression of ADAM17 gene increased in patients, which was statistically significant ($P = 0.043$). Thus, this gene can be used as a marker to identify and even treat cancer. The results are shown in Table 3 and Figure 4.

Discussion

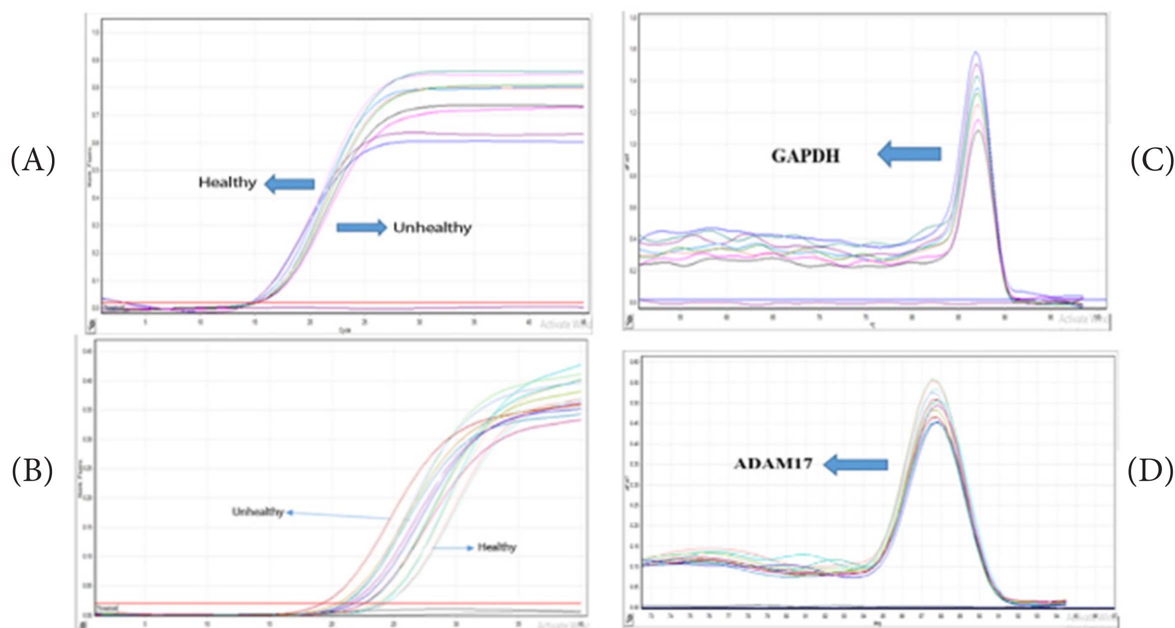

Figure 3. (A) GAPDH Proliferation Diagram. (**B)** ADAM17 Proliferation Diagram. (**C)** GAPDH Melting Curve. (**D)** ADAM17 Melting Curve

Various reports show that leukemia is on the rise in Iran. Identification of new diagnostic methods is one of the most important factors in the early diagnosis of this cancer. TNF-alpha is a converting enzyme (TACE) that is called ADAM17. It is a Zn-dependent type I transmembrane metalloproteinase that is involved in the extracellular domain of several transmembrane proteins such as cytokines, growth factors, receptors, and adhesion molecules. There is evidence that ADAM17 is involved in atherosclerosis, adipose tissue metabolism, insulin resistance, and diabetes. Many of the substrates broken down by ADAM17 make this enzyme an attractive candidate for investigating its role in inflammatory disorders and may be a treatment for inflammation or cancer. It has also been recognized that ADAM17 controls many vital functions in the body (17). ADAM17 is involved in cancer and reduces the growth factors enzyme for tumor progression and growth. In addition, by comparing the two studies it was revealed that *ADAM17* is an anti-tumor gene, in which the increase in expression reduces growth factors and prevents abnormal cell proliferation (18). The results of this study showed that the expression of *ADAM17* gene in the blood of people with ALL has a higher expression than that in the blood samples of healthy people and this increase in expression was statistically significant. For example, Shou et al found that ADAM17 was elevated in a variety of cancers, including gastric cancer, glioma, and kidney cancer, and was implicated in tumor growth and invasion (19). In 2012, Guo et al studied the expression of *ADAM17* in lung cancer tissue and found that the expression of ADAM17 protein in clinical samples was increased compared to normal tissues (20). In another study by Guo et al in 2013, it was found that ADAM17 is a key enzyme for activating the Notch signaling pathway and that inhibiting its activity effectively increases apoptosis and impairs the invasion of renal cell carcinoma (21). In 2019, Pavlenko et al examined the expression of the ADAM17 gene in colorectal cancer, and the results showed that the expression of this gene increased in cancer patients compared to healthy individuals (13). In 2019, Nayak et al examined the inhibition of *ADAM17* gene in uterine cancer, and their results showed that inhibiting this gene can significantly prevent the progression of uterine cancer (22). Li et al in 2019 examined the expression of *ADAM17* gene in gastric cancer and the results showed that this gene is significantly increased in gastric cancer and can be used in the future as a marker in gastric cancer (23). The results of the present study also showed that the expression of *ADAM17* gene in the blood of patients with ALL is higher than that in the blood samples of healthy individuals, and this increase in expression is statistically significant. These results are consistent with many other studies; and it seems that by increasing the expression of ADAM17 gene in people with acute lymphoid leukemia, it can be used as a suitable biomarker for the diagnosis of this disease.

Table 3. Adam17 gene expression

Samples	Expression of *ADAM17* gene Standard deviation ± mean
Healthy	0.3248 [a] ± 0.8929
Patient	0.5889 [b] ± 1.4226

Different letters indicate the existence of significant groups (*P* value <0.05).

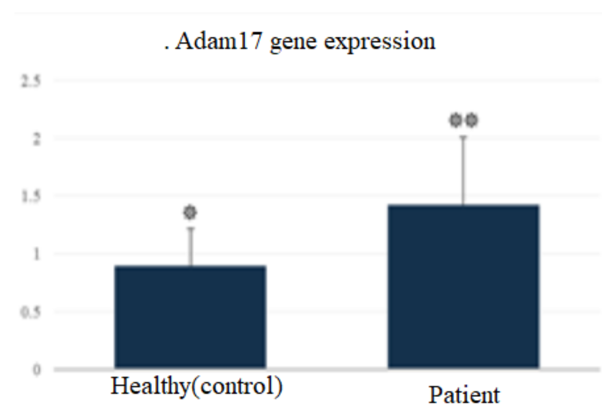

Figure 4. Comparison of *ADAM17* gene expression level ($P=0.043$). The different number of stars in each group indicates significance (*P* value <0.05).

Limitations and Suggestions

One of the limitations of the research was taking small samples of patients . For future studies, it is recommended to study the expression of *ADAM17* in other cancers, as well as the effect of chemotherapy drugs on the expression of the *ADAM17* gene.

Conclusion

This study showed that the expression of ADAM17 gene is significantly increased in people with ALL, indicating that the ADAM17 gene can be a suitable biomarker for the diagnosis of ALL.

Conflict of Interests

Authors declare no conflict of interests.

Ethical Approval

This research was approved by the Research Ethics Committee of Shahrekord Azad University, Iran (code: IR.IAU.SHK.REC.1398.039).

Authors' Contribution

SK and NZ: Study Design, Manuscript Preparation, and Literature Search. SK: Data Collection and Data Interpretation. SK and NZ: Statistical Analysis. All the authors approved the final version of the manuscript.

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