The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2023

Volume 12, Pages 13-17

ICMeHeS 2023: International Conference on Medical and Health Sciences

Determination of Some miRNA Expression Levels in Chronic Lymphoid Leukemia Patients

Ayse Dalyan
Gaziantep University

Sibel Bayil-Oguzkan
Gaziantep University

Mehmet Ozaslan
Gaziantep University

Abstract: Chronic lymphoid leukemia (CLL) is a type of cancer that occurs as a result of the accumulation of morphologically small, mature-looking lymphocytes. CLL is the most common type of leukemia in Western countries, where it accounts for 30% of all leukemias. Accounts for only 10% of all leukemias in Asian populations. MicroRNAs (miRNAs) are non-protein-coding single-stranded RNA molecules approximately 18-25 nucleotides long, forming a class of endogenous small RNAs. Research has shown that microRNAs can function as oncogenes or tumor suppressors in CLL. Although the expression levels of microRNA 133a and microRNA 452 have been determined in many cancers, including lung, prostate and colon cancer, expression levels in CLL patients have not been determined. Therefore, in our study, the expression levels of miRNA 133a and miRNA 452 in CLL patients will be calculated quantitatively using the Real-Time PCR method. As for the method steps, in the first stage, whole blood samples will be taken. miRNA will be isolated from the whole blood samples taken, cDNA will be synthesized from the miRNA samples, and finally, expression levels will be determined with the Real-Time PCR method using miRNA 133a, miRNA452 specific primers and U6 primer as the reference gene. The data obtained will be analyzed and interpreted with the SPSS package program. This study will be conducted to determine whether these two miRNAs can guide early diagnosis and diagnosis in CLL patients and to provide preliminary information to clinicians and contribute to the literature on this subject.

Keywords: CLL, miRNA, Real-time PCR, Expression

Introduction

Cancer is a disease at the cellular level. Clinically cancer; It is expressed as a condition that includes nearly a hundred complex diseases that exhibit different behaviors depending on the cell type from which they originate. Cancers; They vary according to their age of onset, growth rate, spread, stage, and treatment response. On the other hand, all cancer types have common characteristics at the molecular level that bring them together under a single title (Klug et al., 2011).

Cancer is one of the deaths with known causes both in the world and in our country; It is an important public health problem as it is the second cause of death after heart and circulatory system diseases. At the same time it is a chronic disease that is increasing worldwide and causing significant material, spiritual, social and economic losses in societies. Cancer causes the death of 8.2 million people and infects 14 million people every year in the world; It affects all people (Ergin et al., 2019). Leukemia, one of these cancer types, is a type of cancer caused by the abnormal proliferation of developing leukocytes. It is divided into two as acute or chronic according to

the rate of proliferation, and myeloid or lymphoid according to the cell of origin. While acute leukemias are seen in children, chronic leukemias are seen more in adults (Chennamadhavuni et al., 2023).

Chronic lymphoid leukemia (CLL) is a disease characterized by clonal proliferation and accumulation of neoplastic B lymphocytes in the peripheral blood, spleen, bone marrow and lymph nodes (Rozman & Montserrat, 1995). CLL is the most common type of leukemia in Western countries, accounting for approximately 25-30% of all leukemias. The majority of patients are asymptomatic, so it is difficult to determine its actual frequency. The incidence rate is approximately 2-6 new cases per 100,000 people and is stated to increase with age, reaching 12.8/100,000/year at the age of 65, which is the average age of diagnosis. Although the average age of diagnosis is 65, in recent years, it has been reported that one-third of new cases are also seen in young individuals under the age of 55. It has been stated that chronic lymphoid leukemia disease is more common in men than in women and the gender ratio is approximately 1.5-2:1 (Ghia et al., 2007).

Although the etiology of CLL is not fully known, there has been no apparent relationship between its occurrence and any known environmental factors such as ionizing radiation, chemicals and drugs. Epidemiological evidence indicates that genetic factors and familial predisposition are important in the pathogenesis of the disease (Kalil & Cheson, 1999).

CLL can be found in two forms: aggressive and slowly progressing disease. While some CLL patients do not need treatment for many years, patients with aggressive form CLL need urgent treatment. Furthermore slow CLL can progress to an aggressive form, so it is important to find new markers for specific and early diagnosis of CLL prognosis and staging (Balatti et al., 2016). Two different staging systems used in CLL are Rai and Binet staging systems. When both staging systems are examined, it can be seen that both have some deficiency. Many parameters that determine prognosis in CLL have been defined. These parameters; The doubling time of the leukocyte count, mutation status in the variable gene of the immunoglobulin heavy chain(IgVH), CLL cells CD38 and (ZAP70) expressions, Lactate Dehydrogenase(LDH) level, serum ß2-microglobulin level, serum thymidine kinase activity can be counted(Soysal, 2013).

CLL is a type of leukemia that occurs as a result of malignant transformation of mature B-lymphocytes. As 90% of CLL cells don't have proliferation capacity, they are in G0/G1 cell transformation. For this reason, it is suggested that CLL disease is caused by the disruption of the apoptosis mechanism, but not by excessive proliferation of B-cells. Studies have shown that the proto-oncogene bcl-2(B-cell lymphoma-2) is overexpressed in B-cell CLL. The Bcl-2 family, which includes pro-apoptotic (bax, bok, and bak) and anti-apoptotic (bcl-2, bcl-w, bcl-xl, and mcl-1) proteins, has been implicated as important regulators of apoptosis. Among the 11 proteins involved in the control of apoptosis, proliferation and differentiation, bcl-2 is stated to be one of the most important proteins that determine the pathogenesis of CLL and ensures the longevity of the relevant cells (Alkan, 2006; Del Principe et al., 2016).

CLL is divided into two subgroups depending on the presence or absence of somatic mutations in the variable genes of the immunoglobulin heavy chain locus (IgVH). IgVH mutation status has different biological and clinical significance: Compared to cases with unmutated IgVH, cases with mutated IgVH show stable disease and longer survival. Mutation of TP53, a tumor suppressor, is associated with chromosome 17p13 deletion, and they generally have poor prognosis and resistance to treatment (Mraz et al., 2009). Like the TP53 mutation, mutations in many genes including SF3B1, NOTCH1, MYD88, ATM, SAMHD1, NRAS and BIRC3 have been stated to be associated with CLL prognosis (Kaur et al., 2020).

Cytogenetic anomalies seen in CLL are important in determining prognosis and treatment options during diagnosis. There are no single specific cytogenetic abnormalities unique to CLL. Detection of chromosome abnormalities has increased thanks to new methods such as Fluorescent in situ hybridization (FISH). The most common cytogenetic abnormalities are 13q14 deletion (-51%), 11q22-23 deletion (17-20%), trisomy 12 (15%) and 17p13 deletion. While cases with 13q14 deletion show good prognosis, cases with trisomy 12 constitute the intermediate risk group. The presence of deletions 11q and 17p has been associated with non-response to chemotherapy, short survival and poor prognosis(Soysal, 2013; Kalil & Cheson, 1999)

What is miRNA? and miRNA CLL Relationship

miRNAs were first identified as genes that play a regulatory role in developmental timing events in a model organism, C. elegans (Kato & Slack, 2008). miRNAs are small non-protein-coding RNA molecules approximately 18-25 nucleotides long. These molecules influence many biological processes, including cellular

proliferation, differentiation, and apoptosis, and play important roles in normal development, physiology, and disease (Çelik et al., 2013; Chandrasekaran et al., 2019). Apart from these features, more than half of miRNA molecules have been found to be located in cancer-related gene regions or fragile regions in the human genome. In cancer development, miRNAs may act as oncogenes or tumor suppressors in relation to the mRNAs they target, therefore miRNAs appear to be regulators of tumor progression, metastasis and invasion. (Çelik et al., 2013; Saydam et al., 2011). Revealing new mechanisms and relationships will also contribute to the development of diagnostic and treatment methods.

The first study revealing a miRNA relationship with cancer was the study conducted by Calin et al. They found that this region, located on chromosome 13q14, a region that is deleted in approximately half of B-cell chronic lymphoid leukemias (B-CLL), contains miR15 and miR16 genes. İt was determined that miR15 and miR16 are located within a 30-kb region of loss in CLL. They then studied miR15 and miR16 expression in blood samples from CLL patients. Looking at the results, it was stated that the expression of these miRNAs is reduced or absent in approximately 68% of CLL patients. The emergence of these expression differences has further strengthened the roles of miRNAs in cancer pathogenesis. (Calin et al., 2002; Sassen et al., 2008). After this study, studies on cancer and miRNA have accelerated.

In their study to create miRNA expression profiles in 94 CLL patients, Calin et al. reported 13 miRNAs that correlated with CLL. (Calin et al., 2005) In the study conducted by Farzadfard et al. to investigate the expression of a group of miRNAs (miRNA 30d, 25-3p, 19a-3p, 133b, 451a, 145 and 144) in CLL patients; They used the RT-PCR method on serum samples of CLL patients. The results showed that these miRNAs were dysregulated in CLL samples compared to healthy controls. They also concluded that miR-19a-3p and miR-25-3p were significantly increased in CLL patients and that they may play important roles in the pathogenicity of CLL (Farzadfard et al., 2020). In their study by Khalifa et al. to evaluate the prognostic value of members of the miRNA 17-92 gene cluster in Egyptian CLL patients, expression levels of miR17-92 gene cluster members, including miR17, miR18a, miR19a, miR19b-1, miR20a and miR92a-1, were evaluated by qRT-PCR. Among other research, they used serum LDH, serum β2 microglobulin (β2M), CD38 and ZAP70 expression by flow cytometry, fluorescence in situ hybridization (FISH) and imaging studies for 17p deletion. Overexpression of the entire cluster was detected in Egyptian CLL patients, and significant associations were found between the miR17-92 gene cluster and various parameters. They concluded that although miR18a, miR19b-1 and miR92a-1 showed negative prognostic value, miR17 may be a good prognostic marker and those with high miR19a expression showed good overall survival compared to those with low expression(Khalifa et al., 2021) Rahimi et al evaluated the expression of miR-32-5p, miR-98-5p, and miR-374b-5p using the Real-Time PCR method in samples from Kermanshah province, Iran and also investigated the signaling pathways regulated by the examined miRs. It showed that there was a significant decrease in the expression of miR-32-5p, miR-98-5p and miR-374-5p in CLL patients at the time of diagnosis and before receiving any treatment. The decreased expression of these miRNAs suggested that they have a tumor suppressor role in CLL. They concluded that these significant changes occurring in the early stages of the disease may also make them candidates as potential biomarkers for early diagnosis of CLL(Rahimi et al., 2021).

Many studies have been put forward for the early diagnosis and treatment of CLL and studies are still ongoing, we have only mentioned a few of them above. As a result of these studies, new information is revealed that will shed light on this disease. More detailed studies aimed at understanding the roles of miRNAs in the development and progression of the disease in CLL patients and identifying new miRNA genes may lead to more accurate diagnoses, prognoses and therapeutic applications in the treatment of CLL disease. Although miRNA133a and miRNA452 have been investigated in other types of cancer, there is no study on their expression levels in CLL patients. For this purpose, we aim to determine miRNA133a and miRNA 452 expression levels in CLL patients by Real-Time PCR method.

Considering the relationship of miRNA133a and miRNA452 with other types of cancer; miRNA 133 has been described as one of the most studied and best characterized miRNAs to date. miRNA 133 has 3 known genes in the human genome; miRNA-133a-1, miRNA-133a-2 and miRNA-133b. miRNA 133a has been associated with cancer and has been implicated in breast cancer, colorectal cancer, lung carcinoma, bladder cancer, prostate cancer, etc. It has been identified as a key factor in the development of cancer, including (Yu et al., 2014; Wang, 2020) miRNA 452 is a recently identified cancer-associated miRNA and is divided into two subtypes: miR-452-5p and miR-452-3p. Abnormal expression of MiR-452 has been identified in many types of cancer, including kidney cancer, prostate cancer, non-small cell lung cancer, osteosarcoma, breast cancer and colorectal cancer, and it has been stated that it plays two different roles as a potential tumor suppressor gene and oncogene (Karimi Dermani et al., 2023).

Conclusion

This study will be conducted to determine whether these two miRNAs can guide early diagnosis and diagnosis in CLL patients and to provide preliminary information to clinicians and contribute to the literature on this subject.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

*This study was supported by Gaziantep University (Scientific Research Project Unit). Project number; FEF.DT.22.14

* This article was presented as an oral presentation at the International Conference on Medical and Health Sciences (www.icmehes.net) held in Antalya/Turkey on November 16-19, 2023.

References

Alkan, S. (2006). *Kronik lenfositik losemi (KLL): Patogenezi ve biyolojisi*. Retrieved from http://www.thd.org.tr/thdData/userfiles/file/kroniklenfositik.pdf

Çelik, D. A., Koşar, P. A., & Özçelik, N. (2013). MikroRNA lar ve kanser ile iliþkisi. *SDÜ Tıp Fakültesi Dergisi*, *20*(3), 121-127.

Balatti, V., Acunzo, M., Pekarky, Y., & Croce, C. M. (2016). Novel mechanisms of regulation of miRNAs in CLL. *Trends in cancer*, *2*(3), 134–143.

Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., Rassenti, L., Kipps, T., Negrini, M., Bullrich, F., & Croce, C. M. (2002). Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(24), 15524–15529.

Calin, G. A., Ferracin, M., Cimmino, A., Di Leva, G., Shimizu, M., Wojcik, S. E., Iorio, M. V., Visone, R., Sever, N. I., Fabbri, M., Iuliano, R., Palumbo, T., Pichiorri, F., Roldo, C., Garzon, R., Sevignani, C., Rassenti, L., Alder, H., Volinia, S., Liu, C. G., … Croce, C. M. (2005). A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *The New England journal of medicine*, *353*(17), 1793–1801.

Chandrasekaran, A. R., MacIsaac, M., Dey, P., Levchenko, O., Zhou, L., Andres, M., Dey, B. K., & Halvorsen, K. (2019). Cellular microRNA detection with miRacles: microRNA- activated conditional looping of engineered switches. *Science advances*, *5*(3), eaau9443.

Chennamadhavuni, A., Lyengar, V., Mukkamalla, S. K. R., & Shimanovsky, A. (2023). *Leukemia*. StatPearls Publishing.

Del Principe, M. I., Dal Bo, M., Bittolo, T., Buccisano, F., Rossi, F. M., Zucchetto, A., Rossi, D., Bomben, R., Maurillo, L., Cefalo, M., De Santis, G., Venditti, A., Gaidano, G., Amadori, S., de Fabritiis, P., Gattei, V., & Del Poeta, G. (2016). Clinical significance of bax/bcl-2 ratio in chronic lymphocytic leukemia. *Haematologica*, *101*(1), 77-85.

Ergin, A., Özdilek, R., & Dutucu, N. (2019). 2012-2017 yılları arasında kadınlarda görülen kanser türleri ve dağılımları: bir üniversite hastanesi örneği. *Kadın Sağlığı Hemşireliği Dergisi*, *5*(1), 1-21.

Farzadfard, E., Kalantari, T., & Tamaddon, G. (2020). Serum expression of seven MicroRNAs in chronic lymphocytic leukemia patients. *Journal of Blood Medicine*, *11*, 97–102.

Ghia, P., Ferreri, A. M., & Caligaris-Cappio, F. (2007). Chronic lymphocytic leukemia. *Critical reviews in oncology/hematology*, *64*(3), 234–246.

Kalil, N., & Cheson, B. D. (1999). Chronic lymphocytic leukemia. *The Oncologist*, *4*(5), 352–369.

Karimi Dermani, F., Datta, I., & Gholamzadeh Khoei, S. (2023). MicroRNA-452: A double-edged sword in multiple human cancers. *Clinical & translational oncology: Official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico*, *25*(5), 1189–1206.

Kaur, G., Ruhela, V., Rani, L., Gupta, A., Sriram, K., Gogia, A., Sharma, A., Kumar, L., & Gupta, R. (2020). RNA-Seq profiling of deregulated miRs in CLL and their impact on clinical outcome. *Blood cancer journal*, *10*(1), 6.

Kato, M., & Slack, F. J. (2008). microRNAs: small molecules with big roles - C. elegans to human cancer. *Biology of the cell*, *100*(2), 71–81.

Khalifa, M. M., Zaki, N. E., Nazier, A. A., Moussa, M. A., Haleem, R. A., Rabie, M. A., & Mansour, A. R. (2021). Prognostic significance of microRNA 17-92 cluster expression in Egyptian chronic lymphocytic leukemia patients. *Journal of the Egyptian National Cancer Institute*, *33*(1), 37.

Klug, W. S., Cummings, M. R., & Spencer, C.A. (2011). *Genetik kavramlar*. Ankara: Palme Yayıncılık.

Mraz, M., Malinova, K., Kotaskova, J., Pavlova, S., Tichy, B., Malcikova, J., Stano Kozubik, K., Smardova, J., Brychtova, Y., Doubek, M., Trbusek, M., Mayer, J., & Pospisilova, S. (2009). miR-34a, miR-29c and miR-17-5p are downregulated in CLL patients with TP53 abnormalities. *Leukemia*, *23*(6), 1159–1163.

Rahimi, Z., Ghorbani, Z., Motamed, H., & Jalilian, N. (2021). Aberrant expression profile of miR-32, miR-98 and miR-374 in chronic lymphocytic leukemia. *Leukemia research*, *111*, 106691.

Rozman, C., & Montserrat, E. (1995). Chronic lymphocytic leukemia. *The New England journal of medicine*, *333*(16), 1052–1057.

Sassen, S., Miska, E. A., & Caldas, C. (2008). MicroRNA: implications for cancer. *Virchows Archiv : an İnternational Journal of Pathology*, *452*(1), 1–10.

Saydam, F., Değirmeci, İ., Güneş, H. V. (2011). MikroRNA' lar ve Kanser. *Dicle Tıp Dergisi, 38*(1), 113-120.

Soysal, T. (2013). Kronik Lenfositik Lösemi-Küçük Lenfositik Lenfoma. Türk Hematoloji Derneği. HematoLog, 3: 253-270.

Wang, Z. (2020). Diagnostic performance for declined microRNA-133a in pancreatic cancer. *Journal of cellular biochemistry*, *121*(8-9), 3882–3886.

Yu, H., Lu, Y., Li, Z., & Wang, Q. (2014). MicroRNA-133: Expression, function and therapeutic potential in muscle diseases and cancer. *Current drug targets*, *15*(9), 817–828.

Author Information

Ayşe Dalyan
University of Gaziantep Department of Biology, 27310 Gaziantep, Turkiye
Contact e-mail: *agurlekdalyan@gmail.com*

Sibel Bayıl-Oguzkan
Gaziantep University, Vocational School of Health, Department of Medical Services and Techniques, 27310, Gaziantep, Turkiye

Mehmet Ozaslan
University of Gaziantep Department of Biology, 27310 Gaziantep, Turkiye

To cite this article:

Dalyan, A., Bayil-Oguzkan, S., & Ozaslan, M.. (2023). Determination of some miRNA expression levels in chronic lymphoid leukemia patients. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 12*, 13-17.