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Detecting the Role of mi RNA Molecules and Methylation on Expression of BMAL1 Gene in Kids with Amyloid Leukemia in Mosul City

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Abstract

Leukaemia is the most common type of blood cancer among all ages marked by rapid growth of abnormal blood cells. Epigenetics entails the study of genomic modifications that do not change the core DNA nucleotide sequence. The study included 45 children aged 2 to 11years old referred to Al-Hadbaa Specialized Hospital between October and November 2024. The sample was divided into two groups: the first consisted of 35 children with leukaemia, and the second encompassed 10 samples as controls. Two millilitres of blood was obtained from the children, one ml was placed in an EDTA tube for DNA extraction and methylation assessmentand the second ml was placed in a Trizol tube for RNA extraction. The research comprises three phases. The initial task is to ascertain the gene expression level for the BAML1 genes. The second step is to measure the level of microRNA-155, which affects how genes are expressed. The third step is to determine the percentage of DNA methylation on the gene promoters using q-PCR. The study showed an elevation in gene expression levels of miRNA 155, recorded at 18.2 for leukaemia patients in compared to the control group. The study also showed a reduction in the expression level of the BMAL gene in leukaemia patients (measured at 0.46) compared to the control group.

Keywords*: BAML1 Gene, DNA Methylation, Gene Expression, Leukemia, miRNA.*

Introduction

Leukaemia is the most prevalent form of blood cancer across all age groups [1]. Blood cells multiplying too quickly and developing too quickly can cause this abnormal event, which can hurt red blood cells, bone marrow, and the immune system. Leukaemia is a form of haematological malignancy originating in the bone marrow, characterised by the proliferation of many atypical blood cells. These blood cells, commonly referred to as blasts or leukaemia cells, are not fully developed [2]. Epigenetics studies showed genomic modifications that do not change the core DNA nucleotide sequence. Three distinct epigenetic categories have been identified: methylation, histone modifications, and chromatin remodelling. Epigenetic factors alter gene expression. DNA methylation refers to the adding of a methyl group, a tiny chemical moiety, to a specific region of the DNA sequence known as CpG. This method is reliable and can be transmitted throughout generations [3].

CpG methylation is an essential mechanism in development, as it silences genes that are no longer necessary for expression. With advancing age, modifications in DNA methylation accumulate, correlating with genomic instability and many non-communicable diseases, such as cardiovascular disorders and cancer [4]. MicroRNA (miRNA) is a class of single-stranded, non-coding RNAs consisting of 19 to

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25 nucleotides. It is synthesised endogenously and regulates gene expression and post-transcriptional modifications of RNA by binding to the 3′ untranslated regions (3′ UTRs) of messenger RNA. This may result in the degradation of mRNA or the inhibiting its translation into protein [5]. Many bodily functions depend on microRNA, including cell division and proliferation, controlling the cell cycle, antibodies, cellular immunity, and apoptosis [6]. Research shows that changes in miRNA expression affect the tumour microenvironment and make treatments less effective [7].

The circadian clock is a regulatory system with an approximately 24-hour cycle that induces rhythmic alterations in several physiological processes. Growing evidence associates chronological disruption with anomalous functionality in clock gene expression, leading to various illnesses, including cancer. Tumour cells exhibit modified circadian rhythms relative to normal cells, disrupting their cell cycle, repair mechanisms, energy metabolism, and additional activities [8]. A small gene group controls the central circadian clock at the molecular level. When these genes are expressed, they create a feedback loop where transcription and translation happen at different times. The activating arm of the loop consists of circadian locomotor output cycle kaput (CLOCK)/neuronal PAS domain protein 2 (NPAS2) and brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1 (BMAL1), which form a heterodimer that interacts with its promoter at CACGTG E-box sequences. This heterodimer's binding to the E-box promotes the expression of clock-controlled genes (CCGs). This enables the transcription of various genes, such as the Cryptochrome (cry1 and cry2) and Period (per1, per2, and per3) genes [9, 10]. Circadian clock genes are crucial in regulating daily rhythms across cellular, tissue, and organismal levels. Clock genes form the molecular clock, consisting of interconnected feedback loops involving gene transcription and translation. In mammals, two critical clock genes, Clock and Bmal1, encode proteins that are part of the basic helix-loop-helix (bHLH)-PAS transcription factor family [11]. CLOCK and BMAL1 establish a heterodimeric complex in the cytoplasm through protein-protein interactions mediated by their PAS domains. Subsequently, they translocate to the nucleus, binding to E-box enhancer sequences, initiating the transcription of repressor clock genes [12]. The Period (Per1 and Per2) and Cryptochrome (Cry1 and Cry2) genes encode proteins that inhibit their own transcription by suppressing CLOCK: BMAL, thereby establishing an autoregulatory feedback loop. Multiple feedback loops have been recorded after identifying this "core" feedback loop. CLOCK: BMAL1 activates the transcription of the core clock gene Rev-erbα, which encodes an orphan nuclear receptor, through its interaction with E-box enhancers [13]. REV-ERBα protein inhibits Bmal1 production by binding to specific regions in the Bmal1 gene promoter, thereby contributing to the feedback mechanism [14]. BMAL1, an essential element of the circadian clock molecular machinery, is classified within the basic helix-loop-helix-PAS domain-containing transcription factor family [15]. The circuit operates with another primary component, CLOCK, which possesses intrinsic histone acetyltransferase activity [16]. BMAL1 regulates the expression of many genes by binding to E-boxes located in their respective promoters [17]. The everyday gene activity of mammals is controlled by a system that periodically activates and deactivates many genes. This system is governed by a complex comprising two proteins known as CLOCK and BMAL1. CRYPTOCHROME molecules inhibit CLOCK/BMAL1-dependent transactivation [18]. Most research suggests that the circadian clock operates as a tumour suppressor at the cellular, systemic, and

molecular levels. The circadian clock in peripheral tissue cells governs cell proliferation and apoptosis by influencing the expression of circadian-regulated genes [19].

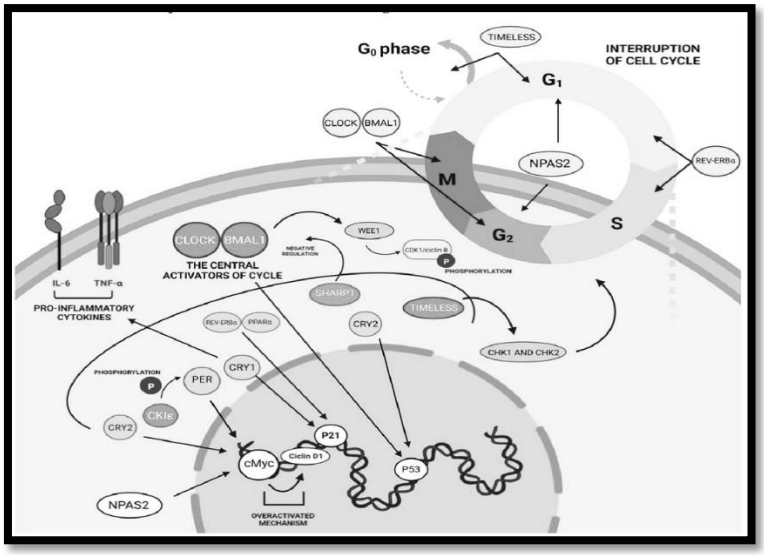

Figure 1. Role of the BMAL Gene of Circadian Rhythm 1 in Leukemic Transformation. (19)

Aim of Study

Identify of the association between miRNA and BMAL1 gene expression levels in leukaemia patients.

Materials and Methods

The study involved 45 children aged 2 to 11 years old, examined from October to November 2024, who were sent to Al-Hadbaa Specialised Hospital in Mosul. The children were classified into two categories. The initial group includes 35 children diagnosed with leukaemia, while the subsequent group contains 10 samples as controls. Two ml of blood was collected from the children and allocated it into two tubes. The initial tube contains EDTA for DNA extraction and methylation assessment. The second tube contains TRIzol for RNA extraction.

Detection of Gene Expression Level of the BMAL1 gene and miRNA 155 was Analyzed Depending on the q-PCR Technique

After collecting blood from the subjects, 750 µl of Trizol was mixed with 250 µl of blood, which included samples from both patients and controls. Subsequently, the RNA and miRNA 155 extraction was conducted using a kit provided by Transgenbiotech. The purity of the extracted RNA was then assessed using a Nanodrop device. For miRNA-155 to settle, E. coli poly(A) polymerase adds a poly(A) tail to the 3' end of RNA. This enzyme then adds ATP one at a time without needing a template. This enzyme does a great job of adding between 20 and 200 adenine bases to mRNA's tails, making translation more efficient. One active unit (U) of the enzyme equals to the amount needed to add 1 nmol of AMP to RNA in 10 minutes at 37°C. For miRNA processing, 20 µl is required which includes 2 µl of Master Mix Poly(A), 1 µl of ATP, 10 µl of RNA template, 1 µl of Poly(A) polymerase, and 6 µl of RNase-free water [20].

The extracted mRNA and microRNA-155 were converted into complementary DNA (cDNA) using the Transgenbiotech kit. The expression levels of the miRNA-155 gene were quantified using specific primers for the BMAL1 gene and a housekeeping gene, as detailed in Table 1. The qPCR reaction was performed in a final volume of 20 µl, using Ultra SYBR Green q-PCR master mix, forward and reverse primers, cDNA template,

and distilled water. The qPCR program included an initial pre-denaturation step at 95°C for 10 minutes, followed by denaturation at 95°C for 15 seconds, annealing/extension at 60°C for 1 minute, and a melting curve analysis at multiple temperature stages [21].

Table 1. Primers Used for Gene Expression Analysis

Primer	Sequence
microRNA-155-F	AAGCGACCTTAATGCTAATCGTGAT
microRNA-155-R	CAGTGCAGGGTCCGAGGT
U6-F	GTGCTCGCTTCGGCAGCA
U6-R	CAAAATATGGAACGCTTC
BMAL1-RT-F	GCCTACTATCAGGCCAGGCTCA
BMAL1-RT-R	AGCCATTGCTGCCTCATCATTAC
B-actin-F	TGACCCAGATCATGTTTGAG
B-actin-R	CGTACAGGGATAGCA CAG

Results and Discussion

The study, reliant on q-PCR, demonstrated an elevation in gene expression levels of miRNA 155, recorded at 18.2 for leukaemia patients in contrast to the control group 1, as illustrated in the subsequent table 2.

Table 2. Displays the CT Values and Gene Expression Levels of microRNA 155 and the Housekeeping Gene in leukaemia Patients Relative to the Control Group

	CT. target	CT. H.K	ΔCT target	ΔCT control	ΔΔCT	Folding
Control	28.25	19.75	8.5	8.5	0	1
Patients	24.16	19.85	4.31	8.5	-4.13	18.2

The study results indicated a reduction in the expression level of the BMAL gene in leukaemia patients (measured at 0.46) in comparison to the control group, as illustrated in Table 3.

Table 3. Displays the Values of CT and the Gene Expression Levels of the BMAL Gene and the Housekeeping Gene for Leukaemia Patients in Comparison to the Control Group

	CT. target	CT. H.K	ΔCT target	ΔCT control	ΔΔCT	Folding
Patients	28.43	22.78	5.65	5.65	0	1
Control	31.33	24.59	6.74	5.65	1.09	0.46

MicroRNA 155 is crucial in regulating gene expression by targeting mRNA for specific genes. This highlights the significant role of miRNA 155 in regulating and inhibiting the expression level of the BMAL gene in children with leukaemia. This results in a defect in cell cycle regulation. The circadian clock works at the cellular level through feedback loops that involve positive and negative actions. These loops control the regular patterns, cell activity, metabolism, and body functions. The circadian clock is regulated positively by the CLOCK gene transcription and LOCK/BMAL1 will increase the levels of key clock genes, including PER1, PER2, and PER3, as well as CRY1 and CRY2. Higher levels of PER and CRY help them attach to CLOCK-BMAL1, which reduces their expression and creates a system that controls their activity negatively.

The insufficient production of PER proteins results in decreased levels within the cell nucleus. When CLOCK-BMAL1 doesn't bind to something [22]. It allows these complexes to start transcription, making it easier to manage their activity. In the early evening, the elevated expression of CG throughout the day leads to a subsequent rise in PER levels. This increase helps PER enter the nucleus [23],

where it connecting with casein kinase 1 epsilon (CK1) to create the PER-CRY-CK1 complex. This complex subsequently inhibits the positive transcription of CLOCK-BMAL1, thereby initiating a new cycle [24, 25].

Hypermethylation in the Promoter of the BMAL Gene

The result of DNA methylation in the promoter of BMAL shows hypermethylation, which prevents binding of RNA polymerase to the promoter of genes. This leads to inhibition of the process of gene expiration, which decreases the level of gene expression.

Conclusions

The study's result show that miRNA 155 and hypermethylation play vital roles in the expression of the BMAL gene in leukemia patients.

Conflict of Interests

The authors declare no conflict of interest regarding the publication of this research.

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