Prevalence and Transcriptional Study of Bcl2 Gene Among Leukemia Patients in Arbil City

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ABSTRACT

Cancer is a significant human affliction, and hematological health is a major issue. Leukemia cannot be effectively cured, even advancements in treatment options. The B-cell lymphoma (BCL2) gene is crucial for the diagnosis and treatment of leukemia, particularly in understanding the BCL2 protein's significant regulatory role in apoptosis. This study was conducted at Nanakaly Cancer Hospital in Arbil, Iraq, from January 1, 2022, to June 30, 2022, utilizing cancer patient data spanning from 2010 to 2022. Five milliliters of blood samples were obtained from cancer patients and healthy individuals for molecular detection. RNA was extracted from blood, followed by the synthesis of cDNA. The quantification of cDNA was performed using a Nanodrop spectrophotometer, and subsequently, PCR was employed to analyze the BCL2 gene. BCL2 protein levels were significantly diminished in the regulatory molecular tissues of a hematological malignancy cell. There is no observable difference in expression level. Our data indicated that reduced BCL2 expression correlates with significant clinical characteristics, including tumor grade, stage, and type of blood cancer. We found that BCL2 is linked to leukemia and may be a biomarker for hematologic malignancies.

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1. Introduction

Blood cancer comprises several malignancies. Cancers of the bone marrow, blood, and lymphatic systems are included in this group, which includes lymph nodes, lymphatic vessels, tonsils, thymus, spleen, and lymphoid tissue of the digestive tract. Leukemia and myeloma, which originate from the bone marrow, and lymphoma, which develop in the lymphatic system, are the most common types of blood cancer [1]. Although the exact cause of blood cancer is unclear despite several factors that have been linked to its development. Blood cancer is more happen and frequently seen in elderly people. Moreover, genetic factors play an important role in developing leukemia through families. Infections, autoimmune diseases, and compromised immune systems enhance the risk of several blood malignancies [2]. Leukemia is one kind of blood cancer when a patient has leukemia, the DNA in the cells mutates, leading the body to produce an excess number of immature white blood cells, and blasts [3 [4]. Leukemia may affect a variety of blood cells, and the disease is classified into four types based on the cells affected [5]. When overproduced cells conduct the function of the bone marrow, the disease is developed [6]. Bcl2 is the apoptosis-regulatory gene(mainly inhibitor), it promotes cell longevity and leads to tumor formation by inhibiting programmed cell death. The Bcl2 gene's abnormal expression is closely linked to resistance to chemotherapy and radiation [7].Bcl-2 gene that encodes a 21-kDa protein termed BAX-alpha, which scientists believe is vital in controlling intrinsic apoptosis [8].In this investigation, the expression of the Bcl2 genes in blood cancer will be identified using PCR.

2. Material and methods

2.1 Study area and sampling

The study was carried out in Nanakaly Cancer Hospital Arbil City, Kurdistan Region/ Iraq after issuing the Ethical approval: Ref. of ethical approval: HMU [64-7/3/2022]. The duration of this study took 6 months from January 2022 to June 2022 and the cancer data was collected from 2010 and 2022. Only five ml of blood samples were collected from cancer patients and normal people as controls for gene expression. Primers were designed for exon regions of the *Bcl2* gene by using the NCBI Primer Blast database. Primer sequences for the exons of the *Bcl2* gene, as well as their length, annealing temperature, GC content, and PCR product length.

2.2 Molecular detection

RNA isolation kit (QIAGEN, Cat. No. / ID: 56304, Germany) was used to isolate RNA from the blood and then measured in its quantity by Nanodrop. The isolated RNA was converted into cDNA by using a random primer. The polymerase chain reaction is used for the detection of the Bcl2 gene by using PCR (Table 1&2).

2.3 Statistical analysis

The One-way ANOVA test was used and, all data was statistically measured by the ECXEL program and analyzed by using GraphPad Prisma.

Table 1: PCR components and their amounts for the Bcl2 expression

PCR components	**amounts(µl)**
Master mix	10
10 pm Forward primer	0.5
10 pm reverse primer	0.5
Distilled water	7.5
cDNA	1.5
Total volume	20

Table 2: PCR conditions of Bcl2 detection

Step	Temperature (°C)	Duration
1st denaturation	95	5 min
2nd denaturation	95	40 sec
Primer annealing	55	45 sec
Extention	72	40 sec
Last extension	72	7 min
	4	4 min

3. Results and discussion

The current analysis summarized the patterns in cancer incidence between 2010 and 2022 and predicted an upward tendency for the following ten years. Between 2016 and 2022, the total cancer incidence rate significantly increased. This study provides estimates of leukemia incidence from 2010 to 2022 it shows that the number of leukemia increased in the last ten years in Kurdistan region/north Iraq, in 2010 the estimated number of leukemia cases was 35 cases only, while during 2011-2012 the cases were increased to 48-50 cases. In 2014 the leukemia cases reached 100 cases, and in 2016 was estimated to be over 150 cases, however during 2018-2020 higher rate was recorded at 180-220 cases, and in 2020 the total cases reached over 250 cases (Figure 1). Moreover, the number of new cases worldwide was about 1,529,560 cases [10, 11]. The leukemia cases increased rapidly and this could be because of several risk factors including heavily processed meals, sweet drinks, Type 2 diabetes, obesity, sedentary lifestyles, and alcohol use [2]. In Iran, the total number of leukemia cases was estimated at 3.7-4.9 cases per 100000 population [17]. However, in Turkey, the higher prevalence of leukemia was recorded at 4.87-5.5 cases per 100000 population [18].

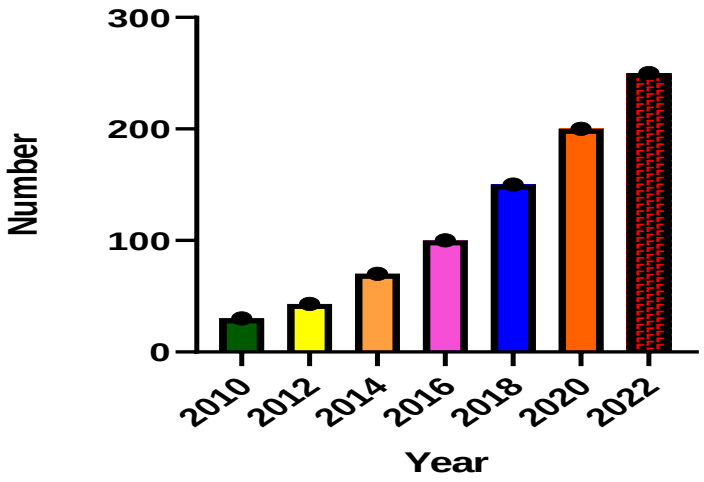

Figure 1: Cancer data from 2010 to 2022 in the Kurdistan region/Iraq

According to our data collected in the Kurdistan region age affects the chance of developing cancer, which increases with age, 20 years old was 3%, 6% at 30 years old, 8% at 40 years old, 9% at 50 years old, 21% at 60 years old, and 53% at 70 years old respectively. This could be because our cells are experiencing senescence. This damage can accumulate as we age, eventually leading to cancer (Figure 2).

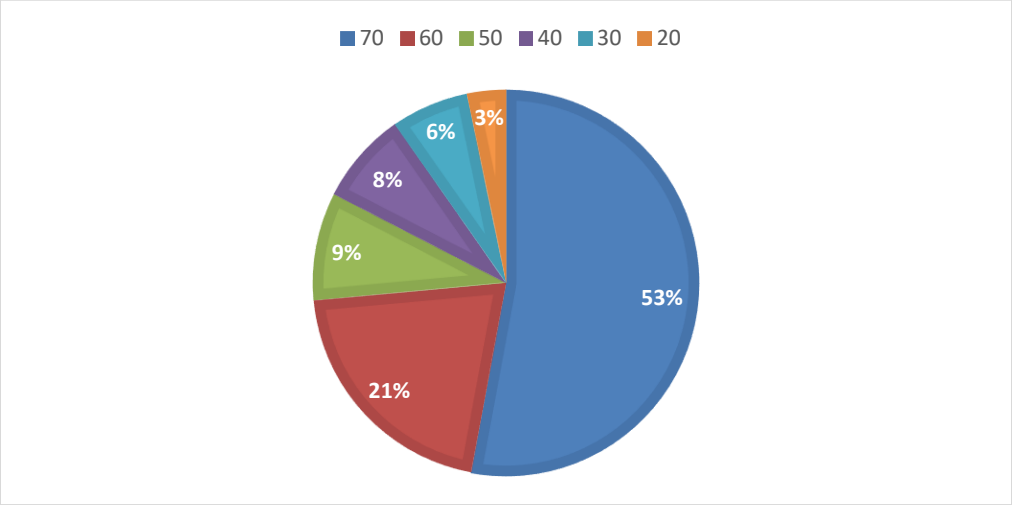

Figure 2: Number of cancer cases in 2022 according to age

Among the samples tested for the presence of the Bcl2 gene via PCR, 15 samples originated from normal individuals or those with other diseases, exhibiting Bcl2 gene levels within the normal range (control). In contrast, one sample from a leukemia patient demonstrated an absence of the Bcl2 gene due to a mutation affecting the gene.

The present study evaluates the Bcl2 gene in blood leukemia. For leukemia patients, the result of PCR revealed that normal and DNA bands appeared, and one of them shown at the last lane (right-hand side of the photo) had leukemia and the band was absent due to mutation (Figure 3).

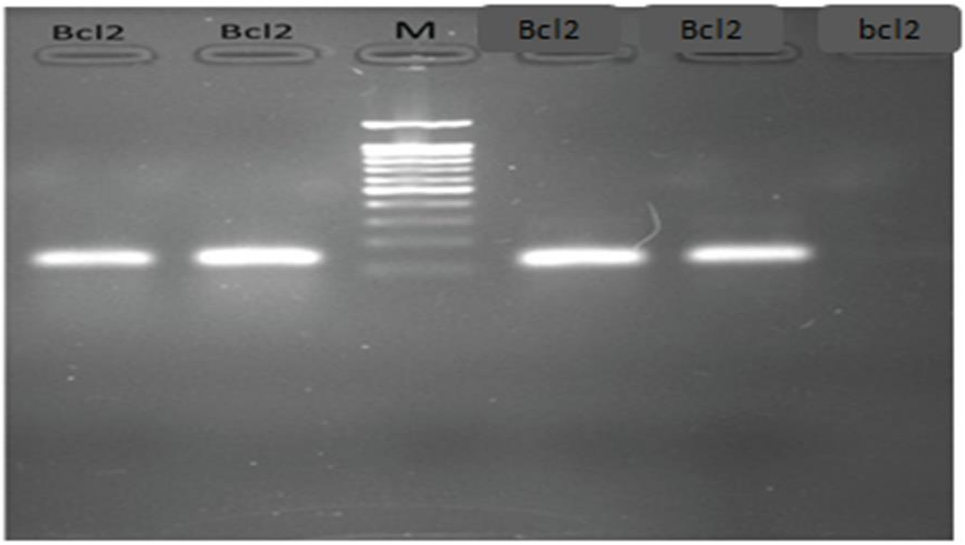

Figure 3: Results from samples tested by PCR, lines 1,2,4, and 5 are a patient group and line 3 are a control group (marker).

Figure 4 illustrates the difference between normal and cancer groups regarding the Bcl2 gene. The cancer group Bcl2 gene defect caused down-regulate and statistically appeared a significant difference between the control group and leukemia patients.

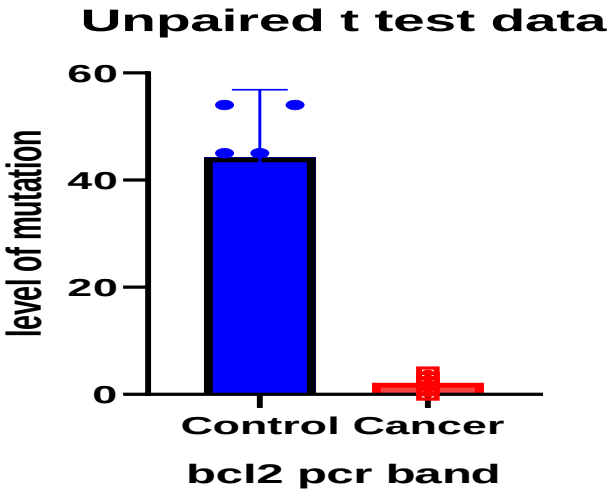

Figure 4: Relation between mutation and Bcl2 gene

Data we collected from the patients who had leukemia before and after treatment showed that the number of lymphocytes decreased during the disease and increased after treatment but did not reach the normal range. However, all WBCs disappeared during the disease, and reached above the normal range after treatment, and the number of PLT decreased during the disease, and increased after treatment (half of the normal range). Finally, HBG: HBGs decreased slightly and increased above the normal range after treatment. One-way ANOVA results compared the means of two or more independent groups to see if there is statistical evidence that the means of the linked population differ significantly [15]. WBC, lymph, PLT, and HBG, and the number of lymphocytes decreased during the disease because if a high number of cancerous blood cells begin to accumulate in lymph nodes or the spleen, creating swellings, blood cancer might affect the lymphatic system. This cell accumulation can cause the lymphatic system to malfunction, increasing the likelihood of infection (Figure 5). Because bone marrow produces blood cells, the number of PLT, HBG, and WBCs decreases. The body produces abnormal blood cells that proliferate and divide in leukemia. The aberrant cells gradually outweigh healthy ones, including HBG and PLT, which are white blood cells. As a result, your body has lower-than-normal levels of them [16].

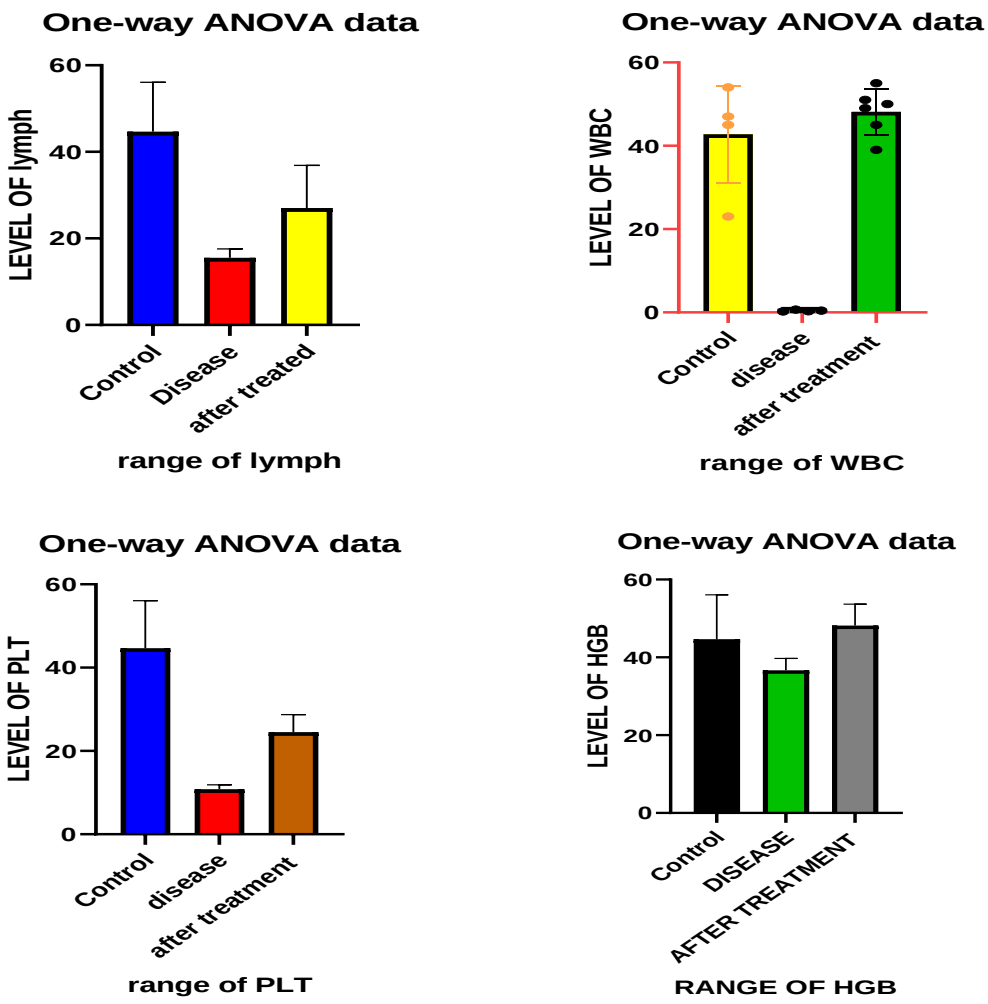

Figure 5: Blood cell leukemia after treatment

Conclusions

The current study's findings concluded that the prevalence of leukemia is low in Kurdistan, the Bcl2 gene is lacking in leukemia, and the overall cancer rate is rising. Leukemia is the world's tenth most frequent cancer. The quantity of blood cells decreases in cancer and WBC disappears, but increases following therapy.

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دراسة و أنتشار ونسخ جين Bcl2 بين مرضى سرطان الدم في مدينة أربيل

أيشان رفعت ياسين

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الخلاصة

السرطان هو أحد أخطر الأمراض التي تصيب الإنسان، والدم مشكلة صحية كبيرة. لا يمكن علاج سرطان الدم بشكل كاف، على الرغم من التقدم في الأساليب العلاجية. ومع ذلك، فإن جين سرطان الغدد الليمفاوية في الخلايا البائية (BCL2)يلعب دورًا رئيسيًا في تشخيص وعلاج سرطان الدم، ومعرفة دور بروتين BCL2 بشكل مكثف كمنظم رئيسي أثناء موت الخلايا المبرمج. تم إنجاز هذه الدراسة في مستشفى نانكالي للسرطان أربيل / العراق في الفترة من 1 كانون الثاني (يناير) 2022 إلى حزيران (يونيو) الماضي 2022 وكانت بيانات مرضى السرطان من عام 2010 إلى عام 2022. تم جمع خمسة مل من عينات الدم من مرضى السرطان والأفراد الطبيعيين للكشف الجزيئي. تم الحصول على الحمض النووي الريبي (RNA) من الدم وتم إنتاج [كدنا]، ثم تم إجراء قياس الحمض النووي وأخيرا، تم استخدام PCR لتحديد الجين .BCL2. تم تخفيض مستويات البروتين BCL2 بشكل كبير في الأنسجة الجزيئية المنظمة لخلية سرطان الدم. وعلى النقيض من بروتين باكس، لا يوجد اختلاف ملحوظ في مستوى التعبير. كشفت النتائج التي توصلنا إليها أيضًا أن انخفاض تعبير BCL2 يرتبط بمعلمات سريرية مهمة للمريض، مثل درجة الورم والمرحلة ونوع سرطان الدم. تشير النتائج التي توصلنا إليها إلى أن BCL2 يلعب دورًا في سرطان الدم وقد يكون علامة حيوية محتملة للكشف عن سرطان الدم وعلاجه.