BCL-2 GENE EXPRESSION EFFECT ON HAEMATOLOGICAL PROFILES AMONG CML PATIENTS IN ILE –IFE. OSUN STATE. NIGERIA

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Abstract

Background: B-cell lymphoma 2 (BCL-2) was the first gene identified to inhibit programmed cell death and function as an anti-apoptotic regulator. Its anti-apoptotic activity represents a major oncogenic mechanism in haematological malignancies, largely driven by the aberrant upregulation of BCL-2. This overexpression arises from multiple underlying mechanisms. BCL-2 gene is located on chromosome 18q21.33. It was first discovered by cloning the breakpoint region of the t(14;18) translocation, a chromosomal abnormality commonly found in follicular lymphomas (FL). Also established is BCL-2 MicroRNA (miRNA) deregulation, which leads to increased BCL-2 expression in the incidence of chronic lymphocytic leukemia (CLL) and CML is associated with increased Bcl-2 expression at the protein and mRNA levels. The intrinsic (mitochondrial) pathway of apoptosis is tightly regulated by the balance of pro-apoptotic and anti-apoptotic BCL-2 family proteins. Dysregulation of this system shift the balance toward cell survival, an important step in many hematologic malignancies. The mechanisms of BCL-2 alteration in Hematologic malignancies include genetic alterations, aberrant signaling pathways, infectious agents and environmental exposure etc. The aim of this study is to assess BCL-2 gene alterations through its expression levels and to evaluate their effects on haematological profiles among Chronic Myelogenous Leukemia (CML) patients in Ile-Ife. This investigation is particularly important given the scarcity of research on the role of the BCL-2 gene in the development of CML, especially among Nigerian cancer patients.
Objectives: To evaluate the relationship between BCL-2 expression levels and haematological parameters for their potential diagnostic relevance in CML patients. To compare the expression of the BCL-2 gene and haematological parameters in patients with CML and apparently healthy individuals and to a**ssess** the potential of BCL-2 gene expression as a molecular marker and possible driver in the pathogenesis of haematological malignancies, particularly CML.

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Methods: This study was cross-sectional and a total of 100 consenting participants were recruited: 50 known CML patients and 50 control subjects. Among the CML participants, the largest proportion (42%) were between 41 and 60 years old, while only a small fraction were within the 81–90-year age range. Females accounted for 58% of this group. Most participants (86%) had attained tertiary education, whereas 6% had no formal education. In the control group, the predominant age category was 21–40 years, and males constituted 60% of the participants. Nearly half of the controls (48%) had secondary-level education. All CML participants were on CML-specific treatment. Among them, 16% commenced therapy in 2008, 14% in 2013, 18% in 2017, and 26% began treatment in 2021, with all continuing their therapy up to the study period in 2024. Full blood count was done using 3-part haematology auto analyzer (Mindray), while RNA extraction and qPCR Bcl-2 quantification were done using real time PCR equipment.

Results:

In this study, participants' age, sex, level of education, and CML-specific treatment did not have a significant impact on the study outcomes. The results demonstrated that BCL-2 gene expression was significantly higher in CML samples compared to controls ($p < 0.05$), suggesting that dysregulated BCL-2 may play a role in the pathogenesis of CML. Haematological parameters were also significantly affected by BCL-2 expression ($p < 0.05$). Specifically, 30% of CML patients with BCL-2 overexpression exhibited leukocytosis, compared to normal WBC counts in 43 (86%) of the controls and 29 (58%) of CML patients with no gene expression. Additionally, more than half (24%) of CML patients with BCL-2 overexpression had anaemia, whereas the majority of CML patients without expression (44%) and controls (70%) had normal PCV values. Thrombocytosis of 14% was observed in CML patients with gene expression, which was compared to 2% in those without gene expression and 4% in the control group, indicating a prognostic significance.

Conclusion: The overexpression of BCL-2 observed in this study is characteristic of CML and its association with anaemia, leukocytosis and neutrophilia proves that Bcl-2 expression level is a marker for the role of BCL-2 gene in the incidence of CML disease and can be used in the stratification and evaluation of hematologic malignant (CML) disease. Targeting a driver of haematologic malignancies (H.M.) is an effective approach to identify possible prognosis for these diseases and aid in the BCL-2 therapy.

Keywords: *B-Cell Lymphoma 2 gene, chronic myelogenous leukemic, leucocytosis, anaemia, neutrophilia*

Introduction

B Cell Lymphoma-2 (BCL-2) is one of the Bcl-2 protein family encoded by the BCL-2 gene.[1] It was the first anti-death gene, anti-apoptotic modulator associated with cancer.[2] BCL-2 was also the first gene demonstrated to prolong cell survival without promoting increased proliferation[3-4]. The suppression of apoptosis mediated by BCL-2 represents a critical step in tumorigenesis [3-4]. BCL-2gene is located on chromosome 18q21.33. It was first discovered by cloning the breakpoint region of the t (14;18) translocation, a chromosomal abnormality commonly found in follicular lymphomas (FL).[5] Bcl-2 resides within the mitochondria, the cell's powerhouse. The BCL-2 protein is the founding member of the BCL-2 family of apoptosis regulators. It functions by neutralizing pro-apoptotic proteins like Bax and Bak, preventing them from triggering the release of cytochrome c, a crucial step in the intrinsic apoptotic pathway. Mimetics (BH3-only proteins) circumvent BCL-2 and BCL-XL, sequester and inhibit it, however, freeing BAX and BAK to initiate the cascade caspase leading to cell death.[6] this delicate balance between pro- and anti-apoptotic proteins maintains cellular homeostasis. However, when Bcl-2 expression surpasses a certain threshold, it disrupts this balance, enabling cell survival and hindering apoptosis[7-8]. Mechanisms underlying BCL-2 dysregulation in

hematologic malignancies include gene mutations, aberrant signaling pathways, infectious agents, and environmental exposures. For example, elevated BCL-2 expression is observed in Multiple Myeloma (MM), particularly in patients carrying the t(11;14) translocation[9]. In Chronic Lymphocytic Leukemia (CLL), the loss or downregulation of miR-15a and miR-16-1, which normally suppress BCL-2 mRNA, results in increased BCL-2 expression[10-11]. Studies also indicate that BCL-2 overexpression is more common in Acute Myeloblastic Leukemia (AML) patients with specific cytogenetic abnormalities, such as t(8;21)[12-13]. Additionally, certain oncogenic viruses can directly or indirectly induce BCL-2 upregulation in hematologic contexts[14-15]. Benzene exposure has been shown to induce BCL-2 upregulation contributing to hematotoxicity and luekemogenic transformation[16]. Bcl-2 is highly expressed in erythroid precursors which promotes their survival, which might disrupt their apoptosis, influencing iron recycling, impacting overall iron homeostasis and eventual haemoglobin synthesis disruption[17],which leads to an increase in the production of macrocytosis and slight increase in mean cell volume (MCV) and possibly mean cell haemoglobin (MCH)[18] Bcl-2 overexpression has been associated with the development of lymphoid malignancies, such as chronic lymphocytic leukemia (CLL)[19] as it promotes B-lymphocyte survival, contributing to an abnormal increase in circulating lymphocytes, a hallmark of the disease. CML is associated with increased BCL-2 expression at the protein and mRNA levels, leading to uncontrolled proliferation of leukemic cells[20]. The BCR-ABL1 oncogene activates various signaling pathways, including NF-κB and PI3K/Akt, which upregulate Bcl-2 transcription and protein stability [21]. This elevated Bcl-2 confers resistance to apoptosis in CML blasts, promoting their survival and disease progression (Wang *et al.,* 2017)[22]. Studies suggest that Bcl-2 dysregulation could promote megakaryocyte survival and differentiation, potentially leading to increased platelet production[23]. So this finding has established the direct proportion between leukocytosis, neutrophilia, lymphopenia, anaemia and BCL-2 overexpression in the diagnosis of CML. The aim of this study is to assess BCL-2 gene alterations through its expression levels and to evaluate their effects on haematological profiles among Chronic Myelogenous Leukemia (CML) patients in Ile-Ife. The objectives are to evaluate the relationship between BCL-2 expression levels and haematological parameters for their potential diagnostic relevance in CML patients, to compare the expression of the BCL-2 gene and haematological parameters in patients with CML and apparently healthy individuals and to **assess** the potential of BCL-2 gene expression as a molecular marker and possible driver in the pathogenesis of haematological malignancies, particularly CML. This investigation is particularly important given the scarcity of research on the role of the BCL-2 gene in the development of CML, especially among Nigerian cancer patients.

Materials and Methods

Study Area

The study was carried out in Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, and Seventh Day Adventist Hospital (SDAH), Ile-Ife, Osun State, South West, Nigeria. Ile-Ife is about 218 kms northeast of Lagos, 40km from Osogbo. Ife is Latitude 7^0 28'N and 7^0 45'N and longitudes 4^0 30'E and 4^0 34'E., east of the city of Ibadan. It has a population of 501,000 people[24]. OAUTHC is along Ilesa road while SDA is at Idiomo in between Mayfair and Lagere, Ile-Ife. (Ife central local government).

Study Design

The study is a cross-sectional study representing a random sampling of the haematological malignant patients in Ife metropolis. The research was designed to assess the BCL2 expression effect on haematologic profiles among CML patients in Ile-Ife. This study was carried out within three months.

Study Population and Subjects

The participants were known Chronic myelogenous leukemic (CML) patients who attend the referral haematology clinic. 50 CML patients were recruited for the study. Among the CML patients, 42% were aged between 41 and 60 years, with 6% participants in the 6 – 20 age range and 2% participants in the 81–90 age range. Females accounted for more than half of the CML group (58%). Most participants 86% had attained tertiary education, while only 6% had no formal education. The control group were prospective healthy subjects with no history of haematologic malignancy. 50 control subjects were recruited. They were screened before being recruited. Among the control group, the predominant age range was 21–40 years, with males representing the larger proportion 60%, and approximately half 48% have completed secondary education.

Inclusion and Exclusion criteria

Inclusion: Known/diagnosed CML patients who attended haematology clinic were allowed for the study
Newly diagnosed CML patients
All CML patients on treatment with CML drugs
CML patients irrespective of age were recruited
Exclusion: Other haematological malignancy excluded
CML patients on Mimetics drug or any other drug that repress BCL-2
No other associated ailment was considered

Sample size determination

Using formula validated by Adebola *et al.*[25] the sample size was determined as follows:
$n = Z \times 2 \times p \times q/d^2$
(P = prevalence, Q=1-P, d – degree of accuracy (0.05), Z = (1.96), P=0.22, d=0.05, confidence level: 95%).
According to Otu and Ejike, (2021), the prevalence of Haematologic Malignancies in Abuja was 6.66%
P= prevalence of Haematologic malignancies - 6.66% [26]
Then the formular will be considered: Z= 1.96 (for 95% confidence level) d= 0.05 (confidence interval or tolerance error)
N= Sample size $N = \frac{Z \times 2 \times P(1-P)}{d^2}$

$$= \frac{1.96 \times 2 \times 0.067(1-0.067)}{0.05^2}$$

$$= \frac{3.92 \times 0.067 \times 0.933}{0.0025} = 98.02$$

Therefore, the minimum sample size-N is pegged at 100.

Ethical considerations

Ethical clearance was obtained from the review board of Seventh Day Adventist Hospital (SDAH), Ile-Ife, Osun state (SERC-2024-3-0035). All research procedures were conducted in accordance with the ethical standard of the responsible committees of the two hospitals and in line with the principles outlined in the WMA Declaration of Helsinki.
Informed Consent**:** All participants were provided with detailed written information outlining the purpose of the study, the procedures involved and anticipated benefits.
Questionnaire was administered to obtain written informed consent from each participant before their enrollment. Participants were informed of their right to withdraw from the study at any point without penalty.

Confidentiality: To ensure confidentiality, all participant data were anonymized using unique identifiers. Only authorized members of the research team had access to the data. Electronic data were stored on password-protected computer laptop, while physical documents were securely stored in locked filing cabinets.

Participant Well-being: Participants retained the right to withdraw from the study at any time without consequence. Any adverse events associated with blood sample collection, such as bruising or hematoma, were addressed promptly by qualified medical personnel in charge. Participants were also provided with the contact information of the principal investigator and the Institutional Review Board (IRB) to address any concerns or inquiries related to the study. The whole research is self-funded, no conflict of interest and no risk involved.

Blood samples collection, storage and transportation

About 4 mL venous blood samples were collected, 2 mL were dispensed into two anticoagulated (EDTA- Ethylene–diamine tetraacetic acid) sample bottles each, i.e., 2 sample bottles containing 2ml of blood each for each participant (test and control). One blood sample was meant for full blood count while the second was for RNA extraction and qPCR analysis. Samples were well mixed after collection and labelled appropriately. 200 blood samples were collected in total. 50 blood samples from CML patients and 50 blood samples from the control were used for full Blood Count, blood samples were analysed within 8 hours of collection using "auto analyzer machine (3 parts)". Another blood samples from CML patients and the control respectively meant for RNA extraction and qPCR analysis were kept at -4^{0}C to prevent haemolysis until sample size 100(50 CML+ 50 CONTROL) were realized. The blood sample size-100(50 CML + 50 CONTROL) were transported in cold chain (2 to 8^{0}C) to Biorepository and Clinical Virology Laboratory, College of Medicine, University of Ibadan for RNA extraction and eventual RT-PCR analysis for BCL-2 gene expression.

Full Blood Count (FBC) Using (Mindray) Haematology Auto Analyzer

Procedure: Blood samples were placed on blood mixer machine. One after the other, the blood sample was put under the probe of the autoanalyzer. Auto analysis was done for the following parameters: PCV, Total and Absolute WBCs count, Platelet count, and Red Cell indices.

RNA Extraction Protocols (Qiagen Kit)

Arranged in a plastic rack were 2 mL Eppendorf tube according to the number of the expected samples to be processed. 560 µl of the prepared AVL buffer containing 5.6 µl of carrier RNA (probe) was dispensed into the arranged and labelled Eppendorf tube. Into each of the Eppendorf tube, 140 µl of the fresh or frozen blood samples were dispensed into corresponding labelled tube. Each sample was vortex for 10 secs and spun down briefly using the microcentrifuge and incubated at room temperature for 10 mins. 560 µl of absolute ethanol 96% -100% was added into each labelled tube containing lysed samples (lysis buffer + sample + carrier RNA) (inactivation). Each sample was vortexed for 10secs and spun down briefly using the microcentrifuge placed in the biosafety cabinet. 630 µl of each lysate (sample + lysis buffer + ethanol) was aspirated into corresponding labelled spin column, and centrifuged for 1 min at 12000 rpm. Collection tubes were discarded and each spin column was placed into a new collection tube. The remaining 630 µl of each lysate (sample + lysis buffer + ethanol) was aspirated into corresponding labelled spin column and centrifuged for 1 min at 12000 rpm. Collection tubes were discarded and each spin column was placed into a new collection tube. 500 µl of WASH buffer AW2 was dispensed into each spin column containing the collection tube and centrifuged for 1 min at 12000 rpm (9000 x g). Collection tubes containing waste was discarded and each spin column was placed into a new collection tube and centrifuged at 14000 rpm (10500 x g) for 3 mins. The spin column was placed into a new microcentrifuge tube (1.5 mL) and 60 µl of RNase-free elution buffer was dispensed into each tube containing the spin

column. It was then incubated at room temperature for 1 min and centrifuged for 1 min at 12000 rpm. The spin column were discarded

Real Time Polymerase Chain Reaction (Rt-PCR)

Procedure of One Step Rt-PCR

Primer design

In order to design specific qPCR primers specific to the quantification of Homo sapien BCL-2 genes, Homo sapien BCL-2 were downloaded from the NCBI website (National Center for Biotechnology Information) and multiple cluster analysis was performed to reveal the conserved regions. Homo sapien BCL-2 genes of accession number NM_000633.3 was used as a DNA reference sequence and used in the primer designing. https://www.idtdna.com/PrimerQuest/Home/Index site was accessed and sequence pasted in the sequence entry box and multiple intercalating dye PCR primers were generated. It is very necessary to ensure that the primers will have a perfect match, this will enhance primer annealing during PCR. To do this, primers must anneal to regions where the sequences are conserved. Each primer pair was then checked for specificity to be sensitive to only the genes of interest to which it was designed to detect and also ability to cut across all aligned genes then the best primer was selected and synthesized at Inqaba in South Africa.

RNA treatment

20 ng total RNA was then treated with NEB DNase 1 (M0303) to totally eliminate extracted DNA briefly, a mixture of 2µl of 10ng/ µl RNA, 10 µl DNase I Reaction Buffer (10X), 1 µl DNAse I (RNase-free) and up to 100 µl with Nuclease-free H_2O. The mixture was then incubated at 37°C for 10 minutes followed by adding 1 µl of 0.5 M EDTA (to a final concentration of 5 mM). Then Heat inactivated at 75°C for 10 minutes and stored in the -20°C till use

Gene quantification

A volume of 20 µl reactions following manufacturer's instructions using Luna® Universal qPCR Master Mix Protocol (M3003) was used to detect the presence of miRNA genes in the extracted RNA. Expression of Actin gene was used as an internal control. Briefly, a mix of 10 µl Luna Universal qPCR Master Mix, 0.5 µl Forward primer (10 µM) 0.5 µl Reverse primer (10 µM) and 0.06 Reverse Transcriptase (Promega) made up to 18 µl with Nuclease-free Water to which 2 µl of the treated RNA Template was added. This was then ran with the profile Initial Denaturation 95°C for 60 seconds followed by 40-45 of Denaturation 95°C 15 seconds Extension and plate reading at 60°C for 30 seconds followed by a termination at 72°C for 10 minutes. Amplification was conducted using the CFX96TM REAL TIME SYSTEM FROM BIO-RAD following manufacturer manual. One-Step RT-PCR: It is a type of RT – PCR where the reverse transcription and the amplification reactions occur in a single tube. All the required components are added in a single tube. First, reverse transcription occurs, forming cDNA, which is then amplified in a PCR process. Luna Universal qPCR Master Mix and other reaction components was thawed at room temperature, then placed on ice. After thawing completely, each component was mixed briefly by inversion, pipetting or gentle vortexing. The total volume for the appropriate number of reactions was determined, plus 10% overage and assay mix of all components was prepared except DNA template accordingly. Then it was mixed thoroughly but gently by vortexing. Liquid collected to the bottom of the tube by brief centrifugation. Aliquot Assay mix was aliquoted into qPCR tubes or plate. For best results, accurate and consistent pipetting, precise volume measurements, and the minimization of bubbles were ensured. DNA templates was added to qPCR tubes or plate. Tubes were sealed with flat optically transparent caps, and plates sealed with optically transparent film. To prevent artifacts caused by evaporation, care was taken to have plate edges and corners sealed properly. Tubes or plates were spun briefly to remove bubbles and collect liquid (1 minute at 2500 – 3000 rpm). Real – time instrument was programmed with indicated thermocycling protocol. It was ensured

that a "plate read" is included at the end of the extension step. The STBR or SYBR/FAM scan mode setting on the real-time instrument was used, along with the "Fast" cycling profile. During the RT phase, conducted at 42 °C for 30 minutes, the Promega reverse transcriptase (RT) enzyme converted the extracted RNA into cDNA. Data was analyzed according to real – time instrument manufacturer instructions.

Statistical analysis

The collected data was cleaned, coded, summarized, and checked for accuracy, consistency and completeness. The data is carefully entered into the Statistical Package for social science (SPSS IBM version 22) statistical software and analyzed using descriptive statistics such as mean, median, mode, standard deviation and bivariant analysis with T-test and cross tabulation/ correlation analysis with bar chat and histograms.

Results

In Figure 1, a Mann-Whitney U test was performed to compare the quantified expression of the BCL-2 gene between CML patients and healthy control individuals for prognosis. There was a statically significant difference in BCL gene expression across the two groups($U = 1677.50; Z = 2.947; p < 0.05$). The lower the Ct values, the more the gene PCR product, the more the gene expression. The CML patients shows higher Bcl-2 expression from -0.7 to + 5.0 at the frequency of 5 to 10 compared to control subjects with peak gene expression from 0.00 to 10.0 at the frequency 5 to 9.

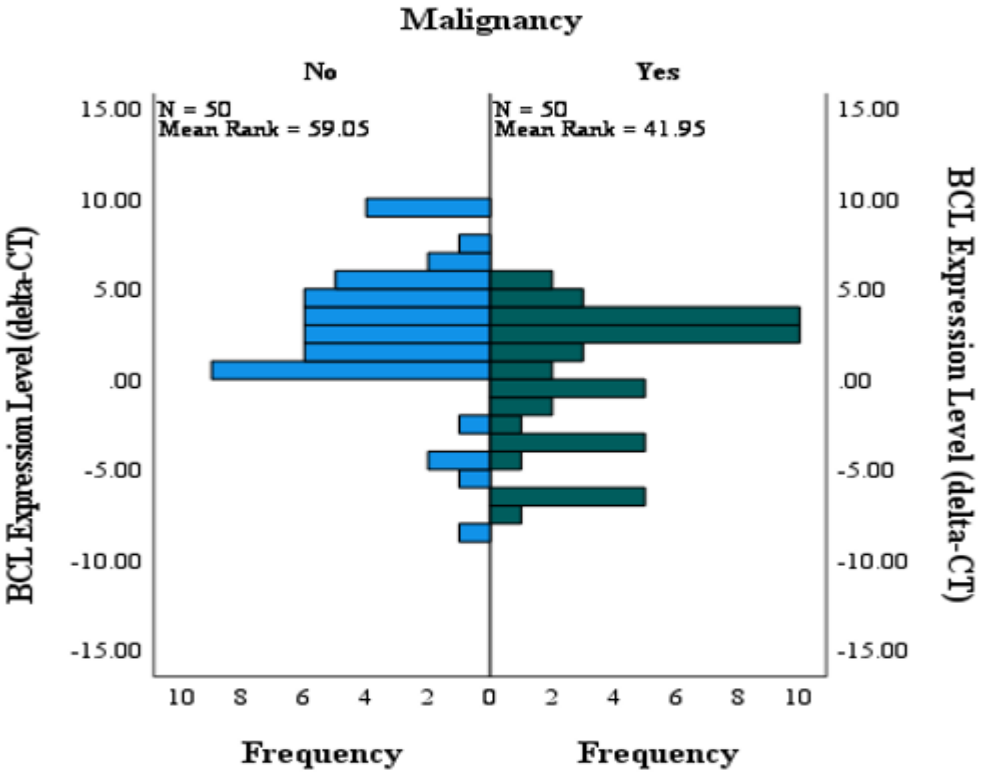


Figure 1: Comparing the quantified expression of the BCL-2 gene between CML patients and healthy control individuals for prognosis.

KEY: Threshold cycle (deltaCT): negative (-ve) values means Bcl-2 gene overexpression positive(+ve) value means under expression.

Figure 2 demonstrates the association between BCL-2 gene expression and WBC counts in CML patients. Among the 40% of patients showing BCL-2 overexpression, most—15 patients

(30%)—presented with leukocytosis (>12.0–450.0 × 10⁹/L), while 5 patients (10%) maintained WBC values within the normal range (3.0–12.0 × 10⁹/L). In contrast, among the 60% of patients without detectable BCL-2 expression, the majority—29 patients (58%)—had normal WBC levels, and only 1 patient (2%) exhibited leukopenia (<3.0 × 10⁹/L). This pattern suggests that BCL-2 overexpression is more frequently associated with elevated WBC counts in CML.

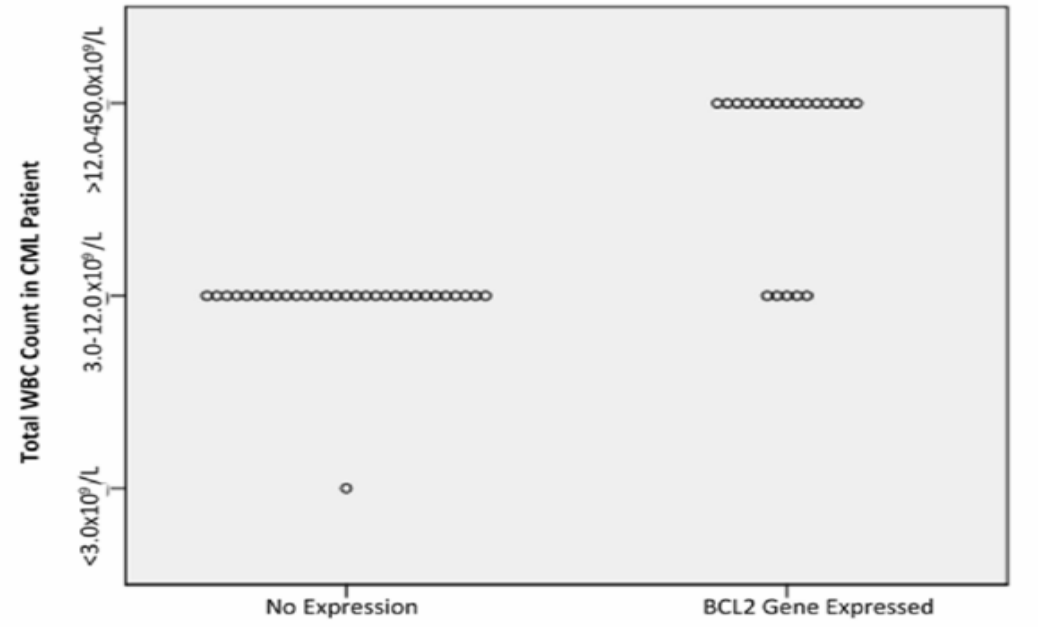
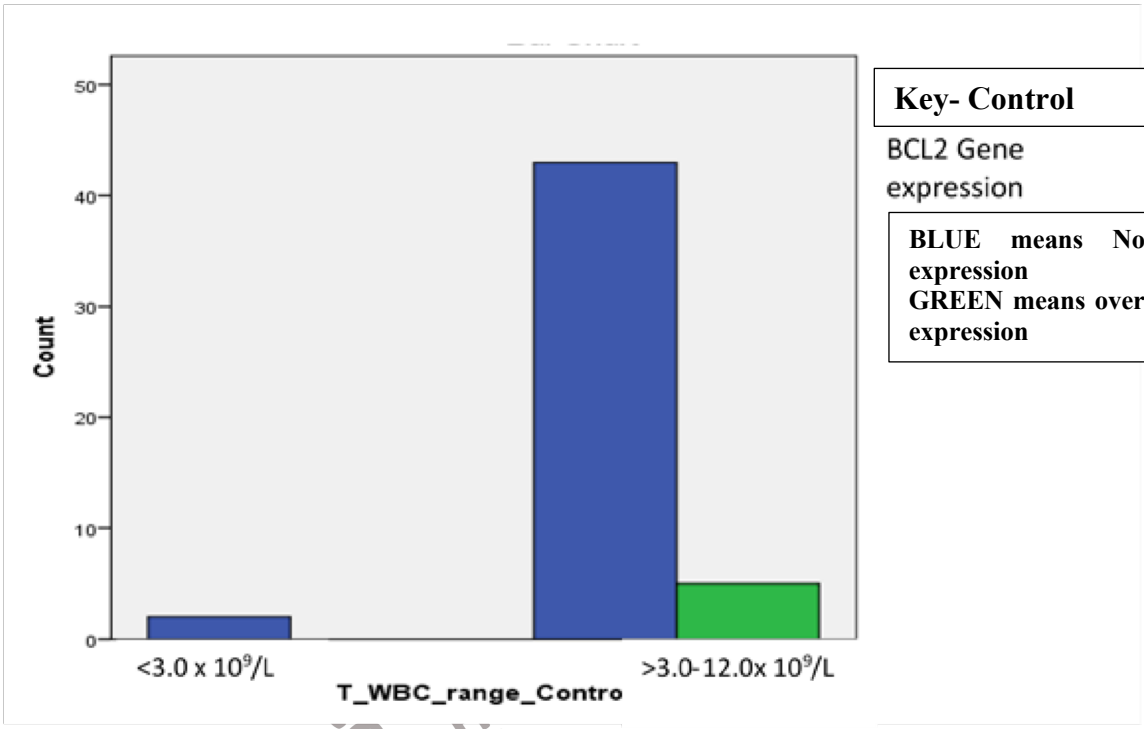


Figure 2: Bcl-2 gene expression in CML patients in relation to WBCs count (P=0.00)

In Figure 3, the relationship between BCL-2 gene expression and WBC count among the control subjects is presented. In this study, 43 (86%) of the 50 control subjects with no detectable BCL-2 expression had WBC counts within the normal reference range (3.0–12.0 × 10⁹/L). Additionally, 5 (10%) of the controls with BCL-2 overexpression also had WBC counts within the normal range, while 2 (4%) of those with no BCL-2 expression exhibited leukopenia (<3.0 × 10⁹/L).



Control Twbcs range (Total White blood cells range)

Figure 3: The relationship between BCL2 gene expression and WBC in control subjects.

Table 1 highlights the haematological characteristics of participants in relation to BCL-2 expression. A notable association was identified between BCL-2 expression and packed cell volume (PCV) among CML patients. Among those with BCL-2 overexpression (40%), over half—12 patients (24%)—had reduced PCV values (<35%). Among the 60% of CML patients with no BCL-2 expression, 22 (44%), over half fell within the normal PCV range. In comparison, among the control participants, the 5 participants (10%) exhibiting BCL-2 overexpression included 4 (8%) with a normal PCV. Among controls without expression (90%), 30 (60%), over half maintained normal PCV levels. Regarding platelet profiles in CML patients, BCL-2 overexpression was associated with a broader distribution of platelet abnormalities. Of the 40% with overexpression, 11 (22%) had normal counts. Among the 60% without BCL-2 expression, over half-19 (38%) had normal counts. In the control group, the majority of those without expression (90%) had normal platelet levels (80%). Notably, all 5 controls with BCL-2 overexpression had platelet counts within the normal reference range. Other haematological parameters in relation to BCL-2 expression: Neutrophils: Among BCL-2–expressing participants, 20% out of 40% CML patients showed neutrophilia. All control participants with BCL-2 expression (10%) had normal neutrophil counts. Among those without BCL-2 expression, 38% out of 60% CML patients exhibited normal counts whereas controls had 78% normal counts. Lymphocytes: In BCL-2–expressing participants, CML patients exhibited 24% lymphopenia. All controls with BCL-2 expression (10%) had lymphocytosis. Among participants without BCL-2 expression, CML patients had 52% lymphocytosis while the control group had 54% lymphocytosis. Red cell indices in relation to BCL-2 expression: Among 40% CML patients with BCL-2 expression: MCV: 28% normal. MCH: 16% normal, 16% low. MCHC: 30% normal. Among 60% CML patients without BCL-2 expression: MCV: 52% normal. MCH: 40% normal. MCHC: 56% normal. Among control participants: MCV: 44% normal, 46% low. MCH: 38% normal, 50% low. MCHC: 80% normal.

Table 1: Haematological profiles among the Bcl-2 overexpressed and Non- expressed participants

Profiles	Expression				No Expression				P-Value
	CML n=20(40%)	mean	Control n=5(10%)	mean	CML n=30(60%)	mean	Control n=45(90%)	mean	
Neutrophil Low Normal High	 1(2.0%) 9(18.0%) 10(20.0%)	 39.2 59.54±12.5 80.12±4.1	 - 5(10.0%) -	 - 46.06±1.91 -	 11(22.0%) 19(38.0%) -	 35.0±5.4 52.4±6.3 -	 6(12.0%) 39(78.0%) -	 36.0±4.1 51.3±8.0 -	0.047
Lymphocyte Low Normal High	 12(24.0%) 3(6.0%) 5(10.0%)	 11.61±3.84 26.36±2.80 44.0±4.66	 - - 5(10.0%)	 - - 46.38±2.72	 - 9(18.0%) 21(42.0%)	 - 34.02±3.77 51.33±7.74	 1(2.0%) 17(38.0%) 27(54.0%)	 19.6 34.72±4.25 47.27±4.63	0.000
Mixed Cells Low Normal High	 - 14(28.0%) 6(12.0%)	 - 7.70±1.89 12.88±1.84	 - 5(10.0%) -	 - 8.22±1.43 -	 - 26(52.0%) 4(8.0%)	 - 7.24±1.20 11.32±0.72	 - 34(68.0%) 11(22.0%)	 - 7.18±1.53 12.36±2.31	0.001
MCV Microcytic Normocytic Macrocytic	 6(12.0%) 14(28.0%) -	 72.13±7.72 86.62±4.92 -	 3(6.0%) 2(4.0%) -	 64.86±12.32 84.50±3.25 -	 2(4.0%) 26(52.0%) 2(4.0%)	 48.50±19.65 88.75±4.59 102.75±2.47	 23(46.0%) 22(44.0%) -	 72.17±6.68 86.72±4.01 -	0.000
MCH Hypochromic Normochromic Hyperchromic	 8(16.0%) 8(16.0%) 4(8.0%)	 24.07±3.22 29.47±1.32 41.10±10.18	 3(6.0%) 2(4.0%) -	 20.30±5.07 29.30±0.98 -	 6(12.0%) 20(40.0%) 4(8.0%)	 24.55±1.72 29.76±1.11 33.17±1.21	 25(50.0%) 19(38.0%) 1(2.0%)	 23.68±2.81 29.21±0.96 33.2	0.012
MCHC Hypochromasia Normochromasia Hyperchromasia	 1(2.0%) 15(30.0%) 4(8.0%)	 29.0 32.98±0.53 43.92±8.19	 1(2.0%) 4(8.0%) -	 28.9 33.40±1.67 -	 2(4.0%) 28(56.0%) -	 30.65±0.07 33.49±0.75 -	 5(10.0%) 40(80.0%) -	 30.06±0.64 33.22±0.87 -	0.001
PCV <35% 35-54% >54%	 12(24.0%) 7(14.0%) 1(2.0%)	 30.35±3.74 40.75±5.85 66.30	 2(4.0%) 3(6.0%) -	 32.90±0.42 41.13±3.55 -	 8(16.0%) 22(44.0%) -	 29.36±5.16 41.57±3.65 -	 14(28.0%) 30(60.0%) 1(2.0%)	 32.78±2.18 41.31±4.37 54.50	0.011
PLATELET Thrombocytopenia Normal Thrombocytosis	 2(4.0%) 11(22.0%) 7(14.0%)	 69.50±13.43 248.91±77.2 644.0±129.5	 - 5(10.0%) -	 - 234.8±86.3 -	 10(20.0%) 19(38.0%) 1(2.0%)	 119.2±22.57 214.6±54.70 490	 3(6.0%) 40(80.0%) 2(4.0%)	 132.0±13.45 224.9±49.66 453.0±7.07	0.000

Key: haematology parameters normal reference range: Neutrophil: 40-75%, Lymphocyte: 21-40%, Platelets: 150,000- 400,000/ml, Mixed cells: 2-10%. Red cell Indices: MCV=80-98fl, MCH=27-32pg, MCHC=31.5-36.0g/dl

Discussion: The socio-demographic profile of the study participants indicated that the majority of CML patients 42% were aged between 41 and 60 years, with very few participants in the 81–90 age range. Females accounted for more than half of the CML group 58%. Most participants 86% had attained tertiary education, while only 6% had no formal education. In the control group, the predominant age range was 21–40 years, with males representing the larger proportion 60%, and approximately half 48% having completed secondary education.

In this study, participant age was not included as a variable. However, previous work by Liu *et al*., (2019) reported that BCL-2 expression exhibits a complex age-related pattern, decreasing in certain tissues while increasing or remaining unchanged in others [27]. Gender was likewise not considered, as BCL-2 plays a fundamental role in hematopoiesis by supporting the survival of hematopoietic cells[4]. Although chronic myeloid leukemia (CML) may occur at any age, it is rare in children[28].

All CML participants in this study were receiving CML-specific therapy. Among them, 16% initiated treatment in 2008, 14% in 2013, 18% in 2017, and 26% began therapy in 2021, with all continuing treatment through the study period in 2024. Treatment history was not analyzed as a variable because findings by Lee et al. (2021) indicate that the gene of interest significantly upregulates BCL-2 expression in CML patients, independent of disease duration [29]. Furthermore, BCL-2 overexpression is known to block the intrinsic apoptotic pathway activated by tyrosine kinase inhibitors (TKIs) by suppressing pro-apoptotic proteins[30]. This mechanism contributes to TKI resistance and the prolonged survival of CML stem cells[31].

Among the 40% of CML patients who demonstrated BCL-2 overexpression, 14% had normal packed cell volume (PCV), 24% had reduced PCV, and 2% had elevated PCV. Thus, nearly one-quarter of the overexpression group presented with anemia. In contrast, among the 60% of patients without BCL-2 expression, 16% had low PCV while 44% had normal PCV. This indicates that more than half of the patients with BCL-2 overexpression were anemic, whereas over half of those without BCL-2 expression maintained normal PCV values, a difference that was statistically significant ($P < 0.05$).

Similarly, within the subgroup of CML patients exhibiting BCL-2 overexpression, 30% presented with leukocytosis and 10% had normal white blood cell (WBC) counts. Among the 60% of patients without BCL-2 expression, 58% had normal WBC counts, while 2% showed leukopenia.

Conclusion

The overexpression of BCL-2 observed in this study is characteristic of CML and its association with anaemia, leukocytosis and neutrophilia proves that Bcl-2 expression level is a marker for the role of BCL-2 gene in the incidence of CML disease and can be used in the stratification and evaluation of hematologic malignant (CML) disease. Targeting a driver of haematologic malignancies (H.M.) is an effective approach to identify possible prognosis for these diseases and aid in the BCL-2 therapy.

References

1. Jannah Patty, D. N. ., Ayu Safitri, T. and Saraswati, H. (2020): Specific Primer for Human Bcl-2-Protein Expression Analysis", *KnE Life Sciences*, 5(2). doi: 10.18502/kls.v5i2.6438.

2. Reed John, C. (2008). Bcl-2–family proteins and hematologic malignancies: history and future prospects. *Blood*. ; 111:3322-3330.

3. Vaux, D.L., Cory, S., Adams, J.M. (1988). Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature*. 335(6189):440–2.

4. Cory, S. and Adams, J.M. (2002).The BCL2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer*; 2(9):647-656.
5. Tsujimoto, Y., Gorham, J., Cossman, J., Jaffe, E. and Croce CM. (1985). The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. *Science*. 229(4720):1390–1393.
6. Moia, R., Diop, F., Favini, C., Kodipad, A.A. and Gaidano, G. (2018). Potential of BCL2 as a target for chronic lymphocytic leukemia treatment. *Expert Rev Hematol*; 11(5):391-402.
7. Huang, D. C. and Strasser, (2000). A. BH3-only proteins - Gatekeepers of apoptosis. *Cell Death and Differentiation*, 14(7), 1515-1523.
8. Huang,Y., Wu, S., Zhu, X., Wu, J. and Zhong, S. (2018). The BCL-2 family contributes to neuronal development and survival by regulating apoptotic and non-apoptotic functions. *Cell Death and Differentiation,* 2018; 25(1), 127-142.
9. Roberts, A.W. and Huang, D. (2017). Targeting BCL2 with BH3 mimetics: basic science and clinical application of Venetoclax in chronic lymphocytic leukemia and related B cell malignancies. *Clinical Pharmacology Therapeutics*. 101(1):89–98.
10. Fresa, A., Innocenti, I., Tomasso, A., Stirparo, L., Mosca, A., Iadevaia, F., Autore, F., Ghia, P. and Laurenti, L. (2024). Treatment Sequencing in Chronic Lymphocytic Leukemia in 2024: Where We Are and Where We Are Headed. *Cancers*, *16*(11), 2011. https://doi.org/10.3390/cancers16112011
11. Perini, G. F., Ribeiro, G. N., Pinto Neto, J. V., Campos, L. T. and Hamerschlak, N. (2018). BCL-2 as a therapeutic target for hematological malignancies. *Journal of Hematology & Oncology, 11*(1), 65. https://doi.org/10.1186/s13045-018-0615-4
12. Xu, J., Zhai, L., Liu, Y., Zhou, Z. and Li, C. (2022). Bcl-2 family proteins; Guardians of cardiomyocyte survival in physiological and pathological conditions: *Frontiers in cell and Development Biology,* 10, 820205.
13. Miettinen, J. J., Pulkki, K., Mustonen, J., Kokko, A. and Elenius, K. (2021). BCL-2 protein expression in acute myeloid leukemia with t(8;21) translocation and its prognostic significance. *Leukemia Research,* 108, 104595.
14. Ross, A.M., Leahy, C.I., Neylon, F., Steigerova, J., Flodr, P., Navratilova, M., …Murray, P.G. (2023). Epstein-Barr Virus and the Pathogenesis of Diffusse Large B-Cell Lymphoma. Life, 13(2), 521. https://doi.org/10.3390/life13020521
15. Zhang, W., Du, F., Wang, L., Bai, T., Zhou, X. and Mei, H. (2023). Hepatitis Virus-associated Non-Hodgkin Lymphoma: Pathogenesis and Treatment Strategies. Journal of Clinical and Translational Hepatology, 11(5), 1256-1266. https://doi.org/10.14218/JCTH.2022.000795
16. Obazelu, P.A. and Anyafulu, F.C. (2025). Expression of BCL-2 and BAD Genes in Benzene-induced Haematotoxicity Bearing Wistar Rats Treated with Bi-Herbal Formular of Picralima Nitida and Cymbopogon Citratus Extract. *African Journal of Laboratory Haematology and Transfusion Science*, 4(2), 172-186. Retrieved from htpps://www.ajol.info/index.php/ajlhts/article/view/300333
17. Liu, Y., Zou, Z., Ou, Q., Sun, L., Liu, J., Sun, Y. and Wang, Y. (2018). FoxO3a controls erythrocyte differentiation and iron metabolism through FBXW7-mediated degradation of Bach1. *Nature Communications*; 9(1), 1-13.
18. Ku, C. H., Liu, Y., Cheng, Y. H., Chen, S. W. and Chen, K. Y. (2018). Bcl-2 overexpression extends erythrocyte lifespan but reduces deformability in mice. *Blood Cells, Molecules, and Diseases*,; 71(1), 74-80.
19. Yip, K. W., Wong, K. S. and Au, F. T. K. (2020). Bcl-2 family proteins in chronic lymphocytic leukemia: Therapeutic targets. *Blood;* 136(1), 49-60.

20. Melo, J.V., Rocha, V., Martins, L.R., Jerónimo, C., Lobo, C., Gärtner, F. and Medeiros, R. (2010). Bcl-2 overexpression in chronic myeloid leukemia: A survival mechanism. *Leukemia Research*; 34(5): 607-613.
21. Hu, Y., Liu, Y., Zhuang, L., Xu, Y., Guo, Y., Li, Q. and Zhao, K. (2012). BCR-ABL1 upregulates Bcl-2 expression through JAK2/STAT3 and PI3K/Akt pathways in human chronic myeloid leukemia cells. *Biochemical and Biophysical Research Communications*, 429(1-2), 120-125.
22. Wang, C., Li, M., Li, S., Liu, X. and Peng, Y. (2017). The role of Bcl-2 family proteins in chronic myeloid leukemia. *Oncotarget*, 8(10), 16224-16234.
23. Sina Ojuade (1992). The issue of "Oduduwa" in Yoruba Genesis: The Myths and Realities," in TransAfrican *Journal of History*, Vol. 21, p 143
24. Huang, D., Wang, X., Li, L., Zhang, Y., Zhang, H., Li, Z. and Li, J. (2019). Bcl-2 deficiency impairs megakaryocyte maturation and platelet production through BIM activation. *Cell Death & Differentiation*; 26(11), 2576-2590.
25. Adebola, F.B., Sanusi, A.R. and Adegoke, N.A. (2015). Consequences of road Traffic accident in Nigeria: Time series approach dept of statistics, FUTA Akure. *International journal of computer applications Technology and Research*; 4:262-273.
26. Out, T.I. and Ejike, U.G. (2021). The frequency and Pattern of Haematological Malignancies in A Tertiary Hospital in Abuja, Federal Capital Territory, North Central Nigeria: A sixteen Year Review. *IOSR journal of Dental and Medical Sciences (IOSR-Jdms);* 20(5 Ser.11), 38-44.
27. Liu, X., Zou, H., Liu, Y. and Zhu, Y. (2019). Regulation of Bcl-2 family proteins in aging. *Aging (Albany NY)*, 11(12), 4005-4022.
28. Giona, F. and Bianchi, S. (2022). Update in Childhood Chronic Myeloid Leukemia. Hematology, 3(4), 718-730. https://doi.org./10.3390/hemato.3040048
29. Lee, J. H., Cho, Y. H., Kim, H. J., Kim, S. J., Kim, D. W. and Jang, S. Y. (2021). Gene expression profiling of chronic myeloid leukemia patients and healthy controls. *Genes & Genomics,* 43(10), 707-715.
30. Czeten, M. and Fulda, S. (2020). BCL-2 family wrestling: crosstalk at the crossroads of life and death. *Cell Death & Differentiation*, 27(10), 2609-2622.
31. Zhang, W., Hu, X., Chen, S., Sun, Y. and Liu, Z. (2023). BCL-2 family proteins and drug resistance in leukemia. *Journal of Experimental & Clinical Cancer Research*, 42(1), 7.

QUESTIONNAIRE/CONSENT FORM

ASSESSMENT OF BCL-2 GENE MUTATION BY ITS EXPRESSION LEVEL IN HAEMATOLOGIC MALIGNANCIES IN ILE IFE, OSUN STATE

Good day, Sir/Ma. This questionnaire is being administered to you to determine the importance of, ASSESSMENT OF BCL-2 GENE MUTATION BY ITS EXPRESSION LEVEL IN HAEMATOLOGIC MALIGNANCIES (CML) IN ILE IFE, OSUN STATE Please respond honestly to the question below by ticking the appropriate response (√). The confidentiality of your response is guaranteed.

DATE…… I.D NUMBER……..

SECTION A (SOCIO-DEMOGRAPHIC CHARACTERISTICS)

Age (years)…….. Gender: (Male) (Female)

SECTION B (MEDICAL HISTORY)

When were you diagnosed with CML? ……or Any other types……Specify…….

Are you using any drugs currently? Yes ( ) No ( )

If Yes please state the name of the drug …….

When did you commence the use of this drug?.....

Are you being treated for any other medical condition? Yes ( ) No ( )

If yes please explain briefly_____

_____

Are you aware of BCL2 mutation? (Yes) (No)

If yes, Has the test been conducted on you before? (Yes) (No)

If yes, kindly give a short detail of the test's outcome_____

_____

___

Thanks for your co-operation.

Socio-demographic characteristics of CML patients and Control subjects

	Chronic Myeloid Leukemia Patients		Control Group	
Variable	**Frequency (N=50)**	**Percentage**	**Frequency (N=50)**	**Percentage**
Age				
1-20	3	6.0	3	6.0
21-40	17	34.0	28	56.0
41-60	21	42.0	19	38.0
61-80	8	16.0	0	0.0
81-100	1	2.0	0	0.0
Gender				
Male	21	42.0	30	60.0
Female	29	58.0	20	40.0
Education				
None	3	6.0	0	0.0
Primary	2	4.0	4	8.0
Secondary	2	4.0	24	48.0
Tertiary	43	86.0	22	44.0
Year of onset of disease				
2008-2012	8	16.0	Not Applicable	
2013-2016	7	14.0		
2017-2020	9	18.0		
2021-2024	26	52.0		
Year of Placement on Treatment	8	16.0	Not Applicable	
2008-2012	7	14.0		
2013-2016	9	18.0		
2017-2020	26	52.0		
2021-2024				