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Original Research Paper

Clinical and Laboratory Insights into Leukemia Subtypes: An Integrated Analysis of ALL and CLL

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Abstract: This study comprehensively analyzed hematological and biochemical parameters in 246 participants (ALL: n=90, CLL: n=66, HC: n=90) to refine leukemia diagnosis, monitoring, and disease understanding. Multi-parameter flow cytometry precisely classified ALL and CLL cases by characterizing aberrant leukocyte profiles, which proved superior to routine CBCs for initial diagnosis. However, CBCs remained valuable for monitoring disease progression. Notably, both ALL and CLL cohorts exhibited elevated liver and renal function parameters compared to healthy controls, highlighting a systemic impact of the diseases or their treatments, and underscoring the need for continuous organ function monitoring. Demographically, ALL patients were predominantly pediatric, while CLL exclusively affected older adults, consistent with known epidemiology. Hypertension was significantly less prevalent in ALL patients (2.22%) than in CLL patients (15.15%) and healthy controls (13.33%). Autoimmune disease prevalence was low in ALL (3.66%) but higher and comparable in CLL (6.5%) and healthy controls (6.5%), reinforcing the known link between CLL and autoimmunity. This integrated approach, combining advanced cellular phenotyping with routine blood and biochemical analyses, offers a more complete picture of patient physiological status, crucial for guiding clinical management and improving patient outcomes in leukemia.

Keywords: ALL, CLL, HC, CBC and autoimmune disease

1. Introduction

Leukemia, a cancer characterized by the production of abnormal leukocytes, is broadly categorized based on its proliferation speed (acute or chronic) and cellular origin (myeloid or lymphoid). Among the predominant subtypes are Acute Lymphoblastic Leukemia (ALL) and Chronic Lymphocytic Leukemia (CLL) [1, 2]. CLL is the most prevalent leukemia subtype, with approximately 4.1 cases per 100,000 adults in the United States, leading to about 4,500 annual deaths [3]. In contrast, ALL primarily affects children aged 1–4 years, although it also impacts adults, with roughly 60% of the 6,000 annual US cases occurring in patients under 20 years old, boasting a pediatric survival rate exceeding 90% [4].

The incidence of the acute lymphocytic leukemia as the new studies in 2021 shown that there are approximately 46,306 new reported cases globally and the children group are the main the affected genera and the men are more affected by the disease where the percentage of the men are 75% than women where their percentage is 25% this is due to the decrease in the women incidence in the last period [5, 6]. ALL is predominantly a pediatric cancer, with the highest incidence observed in children aged 0–4 years and approximately 6% of new ALL cases

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occurred in individuals aged 75 and over. Notably, incidence rates peak in early childhood and decrease sharply through adolescence and young adulthood. A secondary, smaller peak in incidence is observed in individuals around 50 years of age [7]. Furthermore, the region variation incidence of ALL indicate that east Asia reported the highest number of childhood ALL cases, significantly exceeding other regions and Hispanic/Latino individuals have significantly higher age-adjusted incidence rates (AAIRs) compared to non-Hispanic Whites, particularly for B-cell ALL[8, 9]. Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults in Western countries, globally, the age-standardized incidence rate (ASIR) of CLL is approximately 4.92 per 100,000 person-years in developed countries, but significantly lower in many low- and middle-income nations[10]. The Global Cancer Observatory (GLOBOCAN) 2020 data reports about 61,000 new cases of CLL annually worldwide [11]. The overall aim of this study was to thoroughly investigate and compare hematological and biochemical parameters in patients with Acute Lymphoblastic Leukemia (ALL) and Chronic Lymphocytic Leukemia (CLL) against healthy controls (HC). other major leukemia subtypes, and how it is influenced by age, sex, and years living with the disease.

2. Methodology

Study Design and Population

This cross-sectional study was conducted at the in National Hospital for Oncology and Blood Diseases in Najaf, between Sep 1, 2024, and January 28, 2025. The study aimed to investigate the clinical profiles of patients with ALL or CLL and focus on detailed clinical stratification. A total of 246 participants were recruited consecutively following the provision of informed consent. The study protocol was approved by the ethical approval Committee (Approval No. 0012, dated Sept 24, 2024) the ethical principles outlined in the Declaration of Helsinki.

Participant Classification

Participants were categorized into three groups based on clinical and laboratory assessments, according to the diagnostic criteria of the World Health Organization (WHO): Group 1 ALL : Individuals diagnosed with Acute Lymphoblastic Leukemia (ALL) (n = 90). The specific criteria used for the diagnosis of ALL should be explicitly stated here (e.g., Flow cytometry parameters). Group 2 CLL: Individuals diagnosed with and Chronic Lymphocytic Leukemia (CLL) (n = 66). Group 3 (Healthy Controls, HC): Age- and gender-matched healthy individuals without ALL or CLL (n = 90). The criteria for "healthy" should be defined there is absence of metabolic disorders.

Data Collection

For each participant, comprehensive clinical and demographic data were collected using standardized protocols and validated data collection forms. The following data were included: Age: Recorded in years and categorized for analysis as ≤15 years or >15 years. Gender: Male or Female. Autoimmune Disease Status: Presence or absence of a documented clinical diagnosis of any autoimmune disorder. The specific autoimmune diseases considered in the study should be listed (e.g., rheumatoid arthritis, Hashimoto's thyroiditis). Hypertension Status: Presence or absence of a clinical diagnosis of hypertension, defined according to established criteria (e.g., systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥80 mmHg, or use of antihypertensive medication). The method of blood pressure measurement should be specified. Clinical diagnoses were established through standard laboratory assessments, physical examination, and a thorough review of patient medical history by qualified endocrinologists. The physical examination should be briefly described, including any relevant components.

3. Results and discussion

Integrated Analysis of Hematological and Biochemical Parameters in Leukemia Subtypes: A Comprehensive Study

This study aimed to thoroughly investigate hematological and biochemical parameters in patients with Acute Lymphoblastic Leukemia (ALL) and Chronic Lymphocytic Leukemia (CLL), comparing them to healthy controls (HC). Recognizing the complex nature of leukemia and the systemic effects often associated with its treatment, a detailed evaluation integrating cellular phenotyping with routine blood work was

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deemed essential for accurate diagnosis, effective monitoring, and a deeper understanding of the disease's impact. A total of 246 biological samples were collected from September 2nd, 2024, to January 2nd, 2025, encompassing participants diagnosed with ALL (n=90), CLL (n=66), and healthy controls (n=90). From each participant, both whole blood and serum samples were obtained. Peripheral blood mononuclear cells (PBMCs) were promptly isolated from whole blood, and serum samples were immediately cryopreserved for subsequent biochemical analyses. All whole blood samples underwent routine complete blood count (CBC) and microscopic blood smear analysis to provide general hematological insights. For definitive classification and precise characterization of ALL and CLL cases, multi-parameter flow cytometry was rigorously employed. This involved incubating whole blood samples with a specific panel of diagnostic monoclonal antibodies targeting key markers: CD19, CD20, CD23, CD45, CD34, CD10, CD38, CD22, CD79a, and CD79b. Following manufacturer's instructions, cells underwent three crucial washing steps to ensure the removal of unbound antibodies prior to analysis on a BD flow cytometer, utilizing reagents and disposables confirmed for instrument compatibility. Flow cytometric results are presented as Mean Fluorescence Intensity (MFI), categorizing antigen expression levels as dim, moderate, or bright. All the samples have been confirmed by using flow cytometry for diagnosis. Many studies have been used the same parameter for diagnosis either CLL or ALL [12-14]. In parallel, liver and renal function parameters were meticulously assessed from the collected serum samples. Flow cytometric analysis, with its comprehensive panel of lymphoid and myeloid markers, served as the definitive method for classifying ALL and CLL cases, precisely characterizing aberrant leukocyte populations based on their specific antigen expression profiles (dim, moderate, bright MFI). This robust cellular phenotyping was paramount for accurate diagnostic confirmation, particularly given that standard CBCs proved to be non-specific in distinguishing between ALL and CLL. Despite the precise diagnosis achieved by flow cytometry, initial CBC and blood smear analysis (Fig 1) revealed that the percentages of lymphocytes, dendritic cells, immature cells, and monocytes were largely within or near normal reference ranges across all study groups (ALL, CLL, and healthy controls), these results were agreed with [15]. While CBC lacked diagnostic specificity for these leukemia subtypes, its utility for monitoring disease prognosis throughout treatment remains significant. Despite its limitations in initial diagnosis, our study correctly points out that CBCs remain significant for monitoring disease prognosis throughout treatment. This is a widely accepted principle in leukemia management[15, 16]. Changes in red blood cell count (anemia), platelet count (thrombocytopenia), and neutrophil count (neutropenia) on CBC can indicate bone marrow suppression due to the disease or chemotherapy, treatment effectiveness, or potential complications like infection or bleeding. Therefore, while not a diagnostic tool for ALL/CLL differentiation, CBC provides essential, real-time insights into the patient's hematological status and the impact of therapeutic interventions.

Conversely, a notable and consistent finding was the elevation of liver and renal function parameters in both treated and untreated ALL and CLL patient cohorts when compared to healthy controls (Fig 2). This observation strongly suggests a systemic impact of either the disease itself or its therapeutic interventions on these vital organ systems [17]. Specifically, the elevated liver enzymes may stem from multiple factors, including the hepatotoxic effects of chemotherapy, disease-related cellular infiltration, or direct cellular dysfunction within the liver due to the neoplastic process [18]. These integrated findings critically underscore the importance of and continuous and comprehensive monitoring of liver and renal function in patients diagnosed with ALL and CLL. This is particular crucial for individuals undergoing chemotherapy, where treatment-induced organ damage can exacerbate disease-related complications. Ultimately, the combined. The reliance on flow cytometry for definitive diagnosis and the nuanced interpretation of CBC alongside biochemical markers provide a more complete picture of the patient's physiological status, guiding clinical management and potentially improving patient outcomes.

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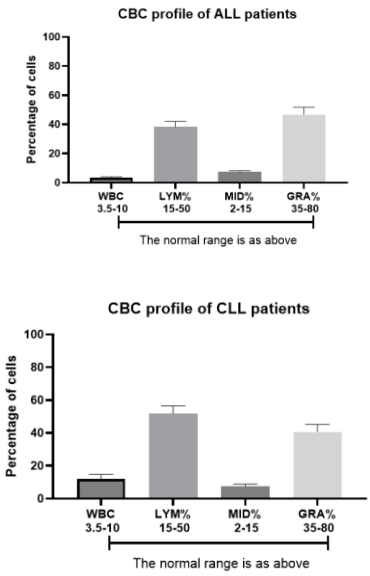

Fig1: Blood Parameters in Leukemia Patients. White Blood Cell Count, Lymphocytes cells, monocytes, granulocytes percentages. Upper panel patients with Acute Lymphoblastic Leukemia (ALL) and Chronic Lymphocytic Leukemia (CLL), The CBC profile has been measured by Urit for all patients.

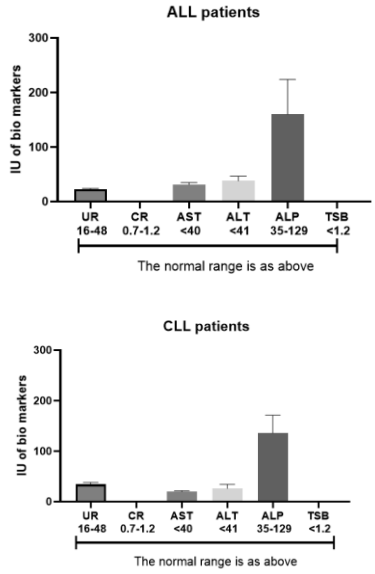

Fig 2: Lower panel is [specific parameter of renal function test urea and creatinine, and liver function test GOT,GPT,ALP, and bilirubin].

Age Distribution of Study Participants

A total of 246 participants, encompassing both patients and controls, were included in this study. As shown in Table 1, the demographic analysis revealed distinct age distributions across the healthy control group, acute lymphoblastic leukemia (ALL) patients, and chronic lymphocytic leukemia (CLL) patients. The healthy control cohort exhibited a predominant representation of younger individuals, with 57% of participants aged ≤15 years and 33% in the >15 years age group. Consistent with established epidemiological patterns, ALL demonstrated a significant predilection for pediatric populations, with 79% of ALL patients being ≤15 years old. Conversely, only 11% of ALL patients were older than 15 years, reinforcing the understanding of ALL as primarily a childhood malignancy. In stark contrast, CLL was exclusively observed in the older age demographic within this dataset. Specifically, 60% of CLL patients were aged >15 years, with no representation in the ≤15 years age group. This finding is consistent with the known epidemiology of CLL, which predominantly affects adults, particularly older adults [19, 20].

Table 1: Age Distribution Analysis (Percentage of Whole Sample).

Age	Healthy (Count)	ALL Patients (Count)	CLL Patients (Count)	Healthy (% of Total)	ALL Patients (% of Total)	CLL Patients (% of Total)
≤15	57	79	0	23.75	32.92	0.00
>15	33	11	60	13.75	4.58	25.00

Gender Distribution of Study Participants

As described in Table 2The study sample demonstrated a notable gender imbalance across the healthy control group and patient cohorts. Females constituted a larger proportion of the healthy group, accounting for 23.33% of the total sample, while males represented 14.17%. Among patients with Acute Lymphoblastic Leukemia (ALL), females comprised a higher proportion (27.50% of the total sample) compared to males (10.00%). This suggests a potential female predominance within the ALL patient subset in this study. Conversely, Chronic Lymphocytic Leukemia (CLL) exhibited a male predominance, with males constituting 14.58% of the total sample compared to females at 10.42%. These findings align with known epidemiological trends for CLL, which often shows a higher incidence in males[6, 21, 22].

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Table 2: Gender Distribution Analysis (Percentage of Whole Sample)

Gender	Healthy (Count)	ALL Patients (Count)	CLL Patients (Count)	Healthy (% of Total)	ALL Patients (% of Total)	CLL Patients (% of Total)
Female	56	66	25	23.33	27.50	10.42
Male	34	24	35	14.17	10.00	14.58

Prevalence of Hypertension Across Study Cohorts

Our analysis, detailed in Table 3, investigated the prevalence of hypertension among Acute Lymphoblastic Leukemia (ALL) patients, Chronic Lymphocytic Leukemia (CLL) patients, and healthy controls (HC), revealing notable differences and similarities across these groups. A remarkably low percentage of ALL patients (2.22%) presented with hypertension. This figure stands in stark contrast to both the CLL patient group and the healthy control cohort, potentially suggesting an inverse association or a protective factor against hypertension within the ALL population.

Conversely, CLL patients exhibited a higher prevalence of hypertension at 15.15%. Interestingly, this percentage closely mirrored that observed in the healthy control group, where 13.33% of individuals had hypertension. This striking similarity between CLL patients and healthy controls regarding hypertension prevalence might indicate that CLL itself does not significantly alter the risk of hypertension beyond what is observed in the general healthy population. Furthermore, the vast majority of ALL patients (97.78%) did not have hypertension[23]. For CLL patients, 84.85% were free of hypertension, a figure closely aligned with the 86.67% of healthy controls without hypertension. These findings collectively underscore the distinct hypertension profile of ALL patients when compared to both CLL patients and the healthy population [24].

Table 3: Hypertension Status Analysis

Hypertension status	ALL (%)	CLL (%)	HC (%)
With hypertension	2.22	15.15	13.33
Without hypertension	97.78	84.85	86.67

Prevalence of Autoimmune Diseases Across Study Cohorts

This section details the distribution of individuals with and without autoimmune diseases across the Acute Lymphoblastic Leukemia (ALL), Chronic Lymphocytic Leukemia (CLL), and healthy control (HC) groups, relative to the total study sample of 246 participants. These findings, as summarized in Table 4, offer insights into the co-occurrence of autoimmune conditions within these distinct populations. A relatively small percentage of the total study population, 3.66%, comprised ALL patients who also had an autoimmune disease. This suggests that while autoimmune conditions can coexist with ALL, they represent a minor segment of this patient group. In contrast, CLL patients with an autoimmune disease constituted a larger share of the total sample at 6.5%. This observation aligns with the documented higher propensity for autoimmune complications in CLL. Notably, healthy controls with an autoimmune disease comprised the same percentage (6.5%) of the total sample as CLL patients with an autoimmune disease. This provides a valuable baseline for the prevalence of autoimmune conditions within a general healthy population in the context of this study. The largest single category within the entire study sample was ALL patients without an autoimmune disease, accounting for 32.93%. This indicates that the vast majority of ALL patients in this dataset did not have a co-existing autoimmune condition, forming a significant portion of the overall study population. A substantial portion of the total sample, 20.33%, comprised CLL patients who did not have an autoimmune disease. While CLL is associated with a higher prevalence of autoimmunity, a larger segment of CLL patients in this cohort did not experience these co-morbidities [24, 25]. Finally, healthy controls without an autoimmune disease represented the second largest category in the total sample, at 30.08%. This group reflects the majority of the healthy population within this study that did not report autoimmune conditions [26, 27].

Table 4: Distribution of Autoimmune Disease Status Across Groups (Percentage of Whole Sample)

Category	Count	Percentage of Whole Sample (%)

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ALL-With autoimmune disease	9	3.66
CLL-With autoimmune disease	16	6.5
HC-With autoimmune disease	16	6.5
ALL-Without autoimmune disease	81	32.93
CLL-Without autoimmune disease	50	20.33
HC - Without autoimmune disease	74	30.08

Conclusion

This study provides an integrated analysis of hematological, biochemical, and clinical parameters in ALL and CLL patients versus healthy controls. We found multi-parameter flow cytometry crucial for accurate leukemia diagnosis, while CBCs remained essential for disease monitoring. Significantly, both ALL and CLL patients exhibited elevated liver and renal function, underscoring the systemic impact of these diseases or their treatments and the need for continuous biochemical surveillance. Our demographic findings reinforced known epidemiological patterns for ALL (pediatric) and CLL (adult). We also observed distinct comorbidity profiles, with low hypertension prevalence in ALL patients and a higher, comparable rate in CLL and healthy controls. Furthermore, the study confirmed a greater propensity for autoimmune diseases in CLL compared to ALL. Ultimately, our comprehensive approach, combining advanced cellular phenotyping with routine hematological and biochemical assessments, offers a more complete understanding of patient status, critical for guiding clinical management and improving outcomes in leukemia.

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Author's Contributions

Both authors conceived and designed the experiments, performed the experimental work, analyzed the data, and wrote the manuscript. Affiliation: Department of Laboratory Investigations, Faculty of Science, University of Kufa, Najaf, Iraq.

Ethics

This study was conducted under approval by the medical ethics committee at the University of Kufa (2017). Verbal and written consent was provided by parents and agreement for publication was obtained from both participants and researchers.

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