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Prevalence and Clinical Correlates of MYD88 L265P Mutation in Sudanese Patients with Chronic Lymphocytic Leukemia

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Abstract

Background: The *MYD88* L265P mutation is well established in lymphoplasmacytic lymphoma and Waldenström macroglobulinemia but is uncommon in chronic lymphocytic leukemia (CLL). Data on its prevalence and clinical significance in African populations are limited.

Objectives: To estimate the prevalence of *MYD88* L265P mutations in Sudanese patients with CLL and evaluate their association with demographic, clinical, hematologic, and morphological features.

Methods: A cross-sectional study was conducted on 100 treatment-naive CLL patients. Clinical staging was assessed using Rai and Binet systems. Mutation detection was performed using allele-specific PCR. Associations between *MYD88* status and demographic, hematologic, and morphological variables were analyzed.

Results: *MYD88* L265P mutations were identified in 6% of patients. Mutated cases were significantly younger than wild-type cases (median 54 vs. 60 years, p=0.041). No association was observed with sex distribution, Rai or Binet stage, white blood cell counts, hemoglobin, platelet counts, or morphology indices, including smudge cells, prolymphocytes, and composite morphology index.

Conclusion: The *MYD88* L265P mutation occurs in a small minority of Sudanese CLL patients, is associated with younger age, but shows no significant clinical or hematological impact. These findings suggest that *MYD88* represents a biologically distinct yet clinically neutral subgroup in CLL. Larger multicenter studies are needed to clarify its prognostic and therapeutic relevance.

Keywords: Chronic Lymphocytic Leukemia; Mutation; Africa

1. introduction

Chronic lymphocytic leukemia (CLL) is a heterogeneous malignancy of mature B lymphocytes, frequently diagnosed in older adults and exhibiting variable clinical progression [1,2]. Traditional risk stratification using Rai and Binet staging systems provides useful prognostic guidance but lacks molecular resolution [3,4]. Increasingly, genetic aberrations—including chromosomal deletions and recurrent somatic mutations—are being integrated into prognostic models to better predict disease course and treatment response [5,6].

Among these, mutations in the *MYD88* gene—particularly the L265P hotspot—have attracted growing interest. *MYD88* encodes an adaptor protein that mediates Toll-like receptor (TLR) and interleukin-1 receptor signaling, culminating in

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NF-κB activation [7]. The L265P variant leads to constitutive activation of downstream pathways, contributing to lymphocyte survival and proliferation. While *MYD88* L265P is most characteristic of Waldenström's macroglobulinemia and ABC-type diffuse large B-cell lymphoma, it is also reported in 2–8% of CLL cases [8–10].

Emerging evidence suggests that *MYD88*-mutated CLL may represent a biologically and clinically distinct subtype. Studies have reported associations with mutated IGHV status, indolent progression, and lower leukemic burden [11]. However, other findings suggest possible resistance to standard chemoimmunotherapy, and the prognostic significance of *MYD88* L265P in CLL remains debated [12,13]. Furthermore, regional data - particularly from underrepresented populations such as North and Sub-Saharan Africa - are sparse.

This study aimed to estimate the prevalence of the *MYD88* L265P mutation among Sudanese patients newly diagnosed with CLL and evaluate its clinical correlations, particularly with Rai and Binet staging at diagnosis.

2. Materials and Methods

2.1. Study Design and Setting

This retrospective cohort study was conducted at the Radiation and Isotope Centre Khartoum (RICK) between January and December 2021. The cohort comprised adult patients (≥18 years) newly diagnosed with CLL, confirmed through sustained lymphocytosis and typical morphologic features. Flow cytometry was not universally performed due to resource limitations. Patients previously treated for CLL or with other hematologic malignancies were excluded.

2.2. Clinical and Laboratory Data Collection

A structured clinical form was used to collect data on demographics, family history, clinical symptoms, and physical examination findings. Staging at diagnosis was recorded using both the Rai and Binet classification systems. Baseline laboratory investigations included complete blood count (CBC), differential, and peripheral smear assessment. Morphologic variables (e.g., smudge cells, atypical lymphocytes, and prolymphocytes) were graded and used to compute a composite morphology index (CMI).

2.3. Sample Collection and DNA Extraction

Venous blood samples (7.5 mL) were collected into K_2-EDTA tubes. Genomic DNA was extracted from ~5 mL of whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Germany), with elution in 100 µL AE buffer. DNA concentration and purity were assessed using a NanoDrop ND-1000 spectrophotometer (acceptable A260/A280: 1.8–2.0).

2.4. Mutation Detection by qRT-PCR

Detection of the *MYD88* L265P mutation was performed using allele-specific real-time PCR (qRT-PCR) based on TaqMan chemistry, with GAPDH serving as an internal control. Primers and probes were designed as follows:

Table 1 Primers and probes

Target	Primer/Probe	Sequence (5′→3′)	Amplicon Size	Reference
MYD88 L265P	Forward	CAGAGGACTTGGGACTGCTG	112 bp	[14]
	Reverse	AGGGTCTCCTGTTGACTGGA		
	Probe	FAM-TTTGAGGCCAGACTTCCAAAC-BHQ1		
GAPDH (Control)	Forward	GAAGGTGAAGGTCGGAGTCA	140 bp	[15]
	Reverse	GACAAGCTTCCCGTTCTCAG		
	Probe	FAM-CCAGCCTGCACCACCAACTGCTT-BHQ1		

PCR amplification was conducted using an ABI 7500 platform (Applied Biosystems, USA) with the following thermal profile: 50 °C for 2 min, 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 s and 60 °C for 60 s. A Ct value <35 was considered positive. Inconclusive results were retested in duplicate.

2.5. Sanger Sequencing Validation

To confirm qPCR findings, 20% of samples (randomly selected from both positive and negative results) underwent Sanger sequencing targeting exon 5 of *MYD88* using BigDye Terminator v3.1. PCR reactions were cleaned using ExoSAP-IT and run on an ABI 3500 Genetic Analyzer.

2.6. Statistical Analysis

Statistical analyses were performed using SPSS v25 and GraphPad Prism v8. Categorical variables were compared using chi-square or Fisher's exact test; continuous variables with Welch's t-test or Mann–Whitney U test as appropriate. A logistic regression model was built to assess predictors of advanced disease (Rai stage III–IV), including age, sex, hemoglobin, WBC, and *MYD88* status. Model validation included ROC curve analysis and bootstrap internal validation.

2.7. Ethical Approval

The study was approved by the RICK Institutional Ethics Committee (Ref. 2021-CLL-01). All participants provided written informed consent. Laboratory procedures adhered to standard molecular diagnostic protocols, with internal quality control for each qPCR run.

3. Results

3.1. Demographics

A total of 100 treatment-naive Sudanese patients with CLL were included. The mean age was 59.0 ± 11.2 years (range 33–85), with a male predominance (62%). Two-thirds of patients resided in urban areas, and occupational status was heterogeneous. Table 2 summarizes the baseline demographics of the cohort.

Table 2 Baseline cohort characteristics of study participants (N=100)

Variable	Value
Age, mean ± SD (range)	59.0 ± 11.2 (33–85)
Male sex, n (%)	62 (62.0)
Female sex, n (%)	38 (38.0)
Urban residence, n (%)	67 (67.0)
Rural residence, n (%)	33 (33.0)
Employment status	Employed 38 Other/unemployed 62

3.2. Clinical Staging

By Rai staging, 73% of patients were stage 0–II and 27% were stage III–IV. Binet staging classified 52% as A, 31% as B, and 17% as C. MYD88 status was not associated with Rai stage. Both crude (P = 0.67) and adjusted (P = 0.69) models confirmed stage neutrality. Table 3 shows the stage distribution.

Table 3 Clinical staging distribution among participants

Staging system	Distribution
Rai stage 0–II	73 (73.0%)
Rai stage III–IV	27 (27.0%)
Binet A	52 (52.0%)
Binet B	31 (31.0%)
Binet C	17 (17.0%)

3.3. *MYD88 L265P* Mutation Prevalence

MYD88 L265P mutations were present in 6% of patients (6/100). Median Ct values were 29.2 (IQR 27.8–30.1), as illustrated in Figure 1.

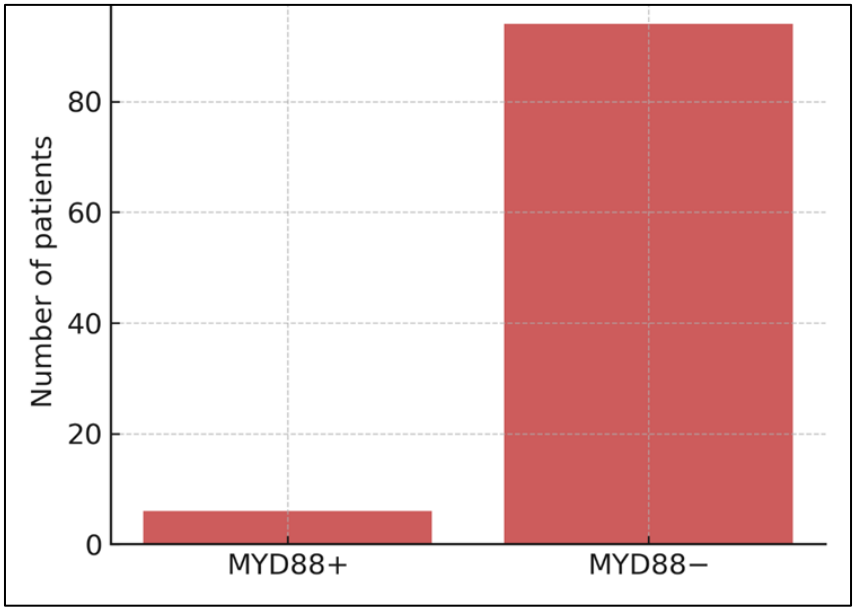

Figure 1 Prevalence of MYD88 gene mutation among participants

3.4. Age and Sex Associations

Patients with MYD88 mutations were significantly younger than wild-type patients (median 54 vs 60 years, p=0.041). There were no differences in sex distribution. Table 4 shows the age comparison.

Table 4 Age by MYD88 status

Group	n (%)	Median Age (IQR)	p-value
MYD88+	6 (6.0)	54 (49–58)	0.041
MYD88–	94 (94.0)	60 (54–67)	

3.5. CBC Indices

No significant associations were observed between MYD88 status and WBC (P = 0.77), hemoglobin (P = 0.43), or platelets (P = 0.56) as illustrated in Table 5.

Table 5 CBC indices by MYD88 mutation status

Parameter	MYD88+ mean ± SD	MYD88– mean ± SD	Δ (95% CI)	p-value
WBC ($\times 10^9$/L)	68.5 ± 22.7	71.6 ± 29.2	−3.1 (−21.0 to 15.0)	0.77
Hb (g/dL)	12.2 ± 1.8	11.6 ± 2.1	+0.6 (−0.9 to 2.1)	0.43
Platelets ($\times 10^9$/L)	182 ± 61	167 ± 75	+15 (−38 to 68)	0.56

3.6. Morphology

Severe smudge-cell fields (Score 2) were frequent (44%), whereas marked prolymphocytic change (Score 2) was less common (12%). A composite morphology index (CMI) classified 22% as high aberrancy, 36% intermediate, and 42% low. Inter-rater agreement was substantial in a blinded subset: $\kappa = 0.78$ (95% CI 0.60–0.96) for smudge Score 2 and $\kappa = 0.71$ (0.50–0.92) for prolymphocytes Score 2, supporting the internal validity of morphology endpoints. Compared with

Rai 0–II, advanced Rai III–IV showed more marked prolymphocytic change (Score 2: OR 4.77, 95% CI 1.45–16.0; Fisher p = 0.006) and more high-aberrancy CMI (OR 2.94, 1.12–7.70; p = 0.023), alongside fewer severe smudge fields (OR 0.36, 0.14–0.90; p = 0.024). MYD88 mutation was not associated with smudge-cell severity, prolymphocyte score, or CMI as illustrated in Table 6.

Table 6 Morphology features by MYD88 status

Feature	MYD88+ (%)	MYD88− (%)	OR (95% CI)	p-value
Smudge cells score-2	33.3	41.9	0.70 (0.12–4.02)	0.68
Prolymphocytes score-2	16.7	14.9	1.14 (0.12–10.7)	0.90
High CMI (≥3 Score-2)	16.7	21.3	0.75 (0.08–6.87)	0.79

4. Discussion

In this Sudanese cohort of 100 treatment-naïve CLL patients, the prevalence of the *MYD88* L265P mutation was 6%. This is consistent with reports showing that *MYD88* mutations occur in a small subset of CLL, generally under 10% of cases, in contrast to their high prevalence in lymphoplasmacytic lymphoma and Waldenstrom macroglobulinemia [16].

Unlike *SF3B1* and *NOTCH1*, which are strongly linked to adverse prognostic features, *MYD88* mutations in CLL have shown more heterogeneous associations. In the current study, the mutation was significantly associated with younger age but showed no correlation with sex, clinical stage, CBC indices, or morphology. This supports findings from prior studies that described *MYD88*-mutated CLL as a biologically distinct but clinically indolent subgroup [17,18].

A Taiwanese sequencing study highlighted poor outcomes in certain *MYD88* variants (notably V217F), but found no clear prognostic effect for L265P, which aligns with the current observation of stage neutrality [19]. Similarly, analyses from larger genomic datasets indicate that *MYD88* L265P CLL lacks the aggressive behavior conferred by other driver mutations and often arises in younger patients with mutated IGHV [20].

The current data showed no significant differences in WBC, hemoglobin, or platelet counts between mutated and unmutated patients. Morphological indices, including smudge cells, prolymphocytes, and CMI, were also similar. This is consistent with prior work demonstrating that *MYD88* mutations do not shape the morphological profile of CLL [21].

Interestingly, although *MYD88* mutations can drive NF-κB signaling and B-cell receptor activation, their clinical impact in CLL appears muted compared to other B-cell malignancies. This may reflect the broader mutational landscape of CLL, where *MYD88* rarely occurs as the dominant driver [22].

The clinical utility of testing for *MYD88* mutations in CLL remains limited at present. While in Waldenstrom macroglobulinemia the detection of *MYD88 L265P* is diagnostic, in CLL it does not currently guide therapeutic decisions. However, as new targeted therapies are developed to exploit NF-κB signaling or MYD88-dependent pathways, identifying this subgroup could become relevant [23].

4.1. Limitations and strengths

The main limitation of the current study is the small number of *MYD88*-positive cases (n=6), restricting the ability to detect subtle clinical correlations. Nevertheless, strengths include uniform molecular testing, comprehensive staging, and integration of morphology scoring with clinical data.

5. Conclusion

The current findings confirm that *MYD88 L265P* mutations occur in a minority of Sudanese CLL patients, are associated with younger age, but have no impact on staging, blood counts, or morphology. These results reinforce the view that *MYD88* mutation in CLL represents a biologically distinct yet clinically neutral entity. Larger multicenter studies are needed to define its long-term prognostic relevance and potential role in future precision therapies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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