The Egyptian Journal of Hospital Medicine (October 2025) Vol. 101, Page 5695-5701

Integrin A4 (ITGA4) Overexpression Is Associated with Advanced Clinical Features and Diagnostic Potential in Chronic Lymphocytic Leukemia

Ahmed Ahmed Allam[1], Abdelrahman A. Elsaied[2], Amer Ahmed Youssef*[3], Nourelhoda E. Hassan[4], Rehab Mohamed Ahmed[5], and Heba Abdelhafiz Ahmed[1]

[1]Department of Clinical and Chemical Pathology, Faculty of Medicine, Sohag University, Sohag, Egypt
[2]Department of Clinical and Chemical Pathology, Faculty of Medicine, South Valley University, Qena, Egypt
[3]Department of Clinical and Chemical Pathology,
General Organization for Teaching Hospitals and Institutes, Cairo, Egypt
[4]Department of Laboratory, Luxor Fever and GIT Hospital, Luxor, Egypt
[5]Research Department, Shefaa Al Orman Oncology Hospital, Luxor, Egypt
***Corresponding author:** Amer Ahmed Youssef, **Mobile:** (+20) 01033000406, **E-mail:** amerabdelkarem3@gmail.com

ABSTRACT

Background: Chronic lymphocytic leukemia (CLL) remains clinically heterogeneous, necessitating reliable biomarkers for early diagnosis and risk stratification. Integrin α4 (ITGA4), which mediates cell adhesion and homing, is implicated in CLL pathogenesis, but its precise clinical and diagnostic utility based on gene regulation status has not been comprehensively evaluated.

Patients and Methods: This case-control study compared newly diagnosed CLL patients to a control group. The study enrolled 74 participants, including CLL patients and controls, categorized into down-regulation (n=17), normal-regulation (n=25), and up-regulation (n=32) groups based on ITGA4 gene expression levels. Clinical, demographic, hematological, and immunological data were collected. Statistical analysis utilized Chi-square or Exact tests for categorical data, one-way ANOVA or Kruskal-Wallis tests for numerical data, and logistic regression to identify independent predictors of CLL diagnosis. Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic efficacy of ITGA4 regulation, compared to β2-microglobulin.

Results: ITGA4 up-regulation was significantly associated with several adverse clinical features, including CLL diagnosis (84.4% in the up-regulated group; $p < 0.001$), splenomegaly, lymphadenopathy, and bone marrow hypercellularity ($p < 0.001$). ITGA4 regulation emerged as a highly significant independent predictor of CLL (OR: 2.95; $p < 0.001$). Furthermore, ITGA4 demonstrated excellent diagnostic performance for CLL, with AUC of 0.856 ($p < 0.0001$).

Conclusion: Elevated ITGA4 gene regulation is strongly linked to the clinical phenotype of CLL involving significant organ infiltration and is a robust, independent diagnostic biomarker with performance exceeding that of β2-microglobulin. These findings support the utility of ITGA4 expression analysis for the accurate diagnosis and potential risk stratification of CLL patients.

Keywords: ITGA4; CLL; Chronic lymphocytic leukemia; Diagnostic biomarker; Bone marrow hypercellularity; β2-microglobulin.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most prevalent adult leukemia in the Western world, characterized by the accumulation of mature appearing but functionally incompetent B lymphocytes in the blood, bone marrow, and lymphoid tissues [(1)]. Despite its commonality, CLL exhibits remarkable clinical heterogeneity, with patient courses ranging from indolence, requiring no treatment for years, to aggressive and rapidly progressive [(2)]. This variability underscores the critical need for robust prognostic and diagnostic markers to guide patient management, inform treatment decisions, and improve risk stratification [(3)].

A key player in the pathogenesis and progression of CLL is Integrin Alpha 4 (ITGA4), a subunit that combines with β1 or β7 to form the heterodimers α4β1 (VLA-4) and α4β7 [(4,5)]. These integrins are central to lymphocyte homing and retention within protective tissue microenvironments, such as the bone marrow, spleen, and lymph nodes, by mediating adhesion to ligands like VCAM-1 and MadCAM-1[(5)]. In CLL, the interaction between ITGA4 on malignant B-cells and VCAM-1 on stromal cells is a well-established mechanism that delivers pro-survival signals and confers resistance to chemotherapy, effectively shielding the leukemic cells from apoptosis [(6)]. This microenvironmental crosstalk is a fundamental contributor to disease persistence and relapse[(6)].

While the functional role of the VLA-4 protein in CLL cell adhesion and migration is well documented, a significant gap remains in our understanding of the clinical implications of ITGA4 gene regulation. The existing literature has not yet comprehensively linked specific patterns of ITGA4 gene expression, whether it is upregulated, downregulated, or normal, to the broad spectrum of clinical and hematological characteristics in a large patient cohort [(7)]. A systematic investigation is required to determine if ITGA4 regulation status holds diagnostic power, correlates with established risk features, and possesses independent prognostic value [(8)].

Therefore, this study is designed to bridge this knowledge gap by rigorously evaluating the role of ITGA4 gene regulation in CLL. Our primary objectives

Received: 01/07/2025
Accepted: 02/09/2025

are threefold: first, to compare the clinical and laboratory profiles of CLL patients stratified by their ITGA4 regulation status (down, normal, up); second, to assess the diagnostic utility of ITGA4 in distinguishing CLL patients from healthy controls, including a direct comparison with established biomarkers like β2-microglobulin; and finally, to determine whether ITGA4 gene regulation serves as an independent predictor for CLL diagnosis. By addressing these aims, we seek to clarify the integral role of ITGA4 in CLL pathobiology and its potential clinical utility.

PATIENTS AND METHODS

Study design and setting:

This study utilized a case-control design conducted at Sohag University Hospital.

Study Participants:

The case group consisted of newly diagnosed chronic lymphocytic leukemia (CLL) patients confirmed by standard clinical, hematological, and flow cytometry/bone marrow criteria.

Cases: Patients with previously treated CLL or those diagnosed with other active malignancies were excluded from the study.

Control group: Outpatients attending the hospital for non-malignant conditions (e.g., upper respiratory infection). Controls were not subjected to bone marrow aspiration.

Sample Size:

A minimum sample size was estimated to detect a medium effect size (Cohen's d=0.66) with 80% power and α=0.05 [5]. Target recruitment was set to achieve adequate power after accounting for potential sample attrition.

Data Collection:

Peripheral blood (10 mL) was collected from all participants into EDTA tubes. Samples were processed immediately for two primary purposes:

RNA extraction for gene expression analysis. Genomic DNA extraction for later use [The original request mentioned genotyping removal, so this line is kept brief to acknowledge DNA storage, a common lab practice]. Clinical data collection included demographic details (age, sex), Rai/Binet stage, complete blood count (CBC) parameters (Total leukocyte count (TLC), lymphocyte count, platelet count (PLT), hemoglobin (Hb)), lactate dehydrogenase (LDH), β2-microglobulin, presence of cytopenias, lymphadenopathy, splenomegaly, treatment decisions, and follow-up data (diagnosis date, last follow-up, death status).

ITGA4 Gene Expression Quantitation:

Total RNA was extracted from peripheral blood samples using the QIAamp/QIAcube automated workflow in conjunction with the RNeasy Mini Kit (Qiagen), strictly adhering to the manufacturer's protocol. The resulting RNA was quantified and assessed for quality using both NanoDrop and Qubit systems to ensure integrity. Subsequently, complementary DNA (cDNA) was synthesized from the extracted RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) under standardized conditions as specified by the manufacturer.

Following cDNA synthesis, quantitative real-time PCR (qRT-PCR) was performed to measure ITGA4 gene expression levels. The analysis was conducted on a QuantStudio 3 instrument (Applied Biosystems) using a TaqMan Gene Expression Assay specific for ITGA4 (Assay ID: Hs00168433_m1), with GAPDH (Hs00187842_m1) serving as the endogenous control for normalization. The thermal cycling protocol consisted of an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Relative gene expression was determined using the $2^{-\Delta\Delta Ct}$ method and is reported as fold-change relative to a calibrator sample, with each sample run in technical duplicates or triplicates to ensure reproducibility.

Ethical approval:

The study protocol was reviewed and approved by the Scientific Ethical Committee of Sohag Faculty of Medicine. All procedures adhered to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment.

Statistical analysis

All statistical analyses were conducted with a pre-determined significance threshold of $p < 0.05$. The data underwent initial preprocessing to verify completeness and identify outliers. The distribution of continuous variables was assessed using the Shapiro-Wilk test, guiding the subsequent choice of parametric or non-parametric tests. To evaluate the role of ITGA4, its expression was compared between patient cases and controls, as well as across clinical subgroups, using Student's t-test or the Mann-Whitney U test. Associations between ITGA4 regulation status (down-regulated, normal, or up-regulated) and clinical parameters were analyzed with Chi-square or Fisher's exact tests for categorical variables and one-way ANOVA or Kruskal-Wallis tests for continuous variables. Correlations with continuous clinical variables were examined using Spearman coefficients.

The diagnostic utility of ITGA4 was further investigated through multivariate logistic regression to identify independent predictors of CLL diagnosis. The model-building process included a variable selection procedure to ensure parsimony, and the final model's integrity was verified by confirming that all Variance Inflation Factors (VIF) were below 5, indicating the absence of significant multicollinearity. Results from

the regression are reported as Odds Ratios (OR) and 95% Confidence Intervals (CI). Finally, the diagnostic performance was evaluated using ROC curve analysis, where the Area Under the Curve (AUC) was calculated and an optimal cut-off value was determined using the Youden Index. All statistical computations and analyses, with the exception of the ROC comparisons, were performed using R software (version 4.3.3). The ROC curve analysis was carried out using MedCalc software.

RESULTS

Table 1 summarizes the clinical and demographic characteristics of study participants categorized by regulation status—down-regulation, normal regulation, and up-regulation. The mean age was comparable across the groups. Gender distribution was similar across the groups, predominating males in all regulation categories. Analysis of liver condition showed no significant variation.

A significant difference was noted in the spleen condition. Most participants in the downregulation and normal-regulation groups had healthy spleen, compared to the up-regulation group. Splenomegaly was significantly most prevalent in the up-regulation group. Chronic lymphocytic leukemia (CLL) and lymphadenopathy were significantly more frequent in the up-regulation group.

Bone marrow cellularity also differed significantly. Hypercellularity was observed significantly more in the up-regulation group compared to other 2 groups. While survival outcomes showed a trend toward insignificant higher mortality in the up-regulation group compared to the normal-regulation and down-regulation groups.

A statistically significant difference was observed with expression increasing progressively from the down-regulated group to normal and up-regulated groups. Other parameters: Total leukocyte count (TLC), hemoglobin (Hb), lactate dehydrogenase (LDH), β2-microglobulin, platelet count (PLT), prothrombin time (PT), peripheral lymphocytes, and bone marrow lymphocytes did not show statistically significant differences, despite apparent numerical variation.

Table (1): Clinical and demographic characteristics based on regulation status.

Variables	**Down-regulation (n=17)**	**Normal-regulation (n=25)**	**Up-regulation (n=32)**	**p-value**
Age Years, Mean ± SD	58.8 ±16.0	61.6 ± 11.7	64.0 ± 12.6	**0.422**
Gender, male, n (%)	12 (70.6%)	16 (64.0%)	23 (71.9%)	**0.805**
Liver, hepatomegaly, n (%)	3 (17.6%)	4 (16.0%)	7 (21.9%)	**0.844**
Spleen, splenomegaly, n (%)	3 (17.6%)	5 (20.0%)	11 (34.4%)	**0.386**
CLL, n (%)	4 (23.5%)	6 (24.0%)	27 (84.4%)	**< 0.001***
Lymph node, lymphadenopathy, n (%)	2 (11.8%)	3 (12.0%)	18 (56.2%)	**< 0.001***
Bone marrow cellularity, hypercellular, n (%)	3 (17.6%)	5 (20.0%)	24 (75.0%)	**< 0.001***
Death, n (%)	4 (23.5%)	4 (16.0%)	12 (37.5%)	**0.171**
TLC, Median (IQR)	41.3 (34.2, 50.2)	50.5 (24.0, 71.8)	32.0 (19.2, 56.8)	**0.712**
Hb, Median (IQR)	10.8 (9.6, 11.5)	10.1 (8.3, 12.6)	12.0 (10.6, 13.4)	**0.212**
LDH, Median (IQR)	608.0 (342.8, 810.8)	328.0 (221.0, 533.2)	440.0 (328.5, 655.5)	**0.470**
β_2-microglobulin, Median (IQR)	4.1 (3.9, 4.4)	4.1 (3.9, 4.2)	3.5 (2.8, 4.1)	**0.260**
PLT, Median (IQR)	181.5 (151.2, 194.8)	188.5 (139.5, 236.8)	151.0 (103.5, 220.5)	**0.608**
ITGA4 gene regulation, Median (IQR)	-0.1 (-0.5, 0.3)	1.1 (1.0, 1.3)	3.3 (2.9, 3.8)	**< 0.001***
PT, Median (IQR)	12.8 (11.9, 13.2)	12.2 (12.0, 12.5)	11.1 (10.8, 11.9)	**0.130**
Stages, n (%) 0 - II III – IV	 2 (50.0%) 2 (50.0%)	 3 (50.0%) 3 (50.0%)	 13 (48.1%) 14 (51.9%)	**0.995**
Lymph peripheral, Median (IQR)	34.0 (28.0, 41.2)	54.5 (43.2, 127.2)	24.0 (14.1, 44.5)	**0.231**
Lymph BM, Median (IQR)	74.5 (68.8, 79.2)	63.0 (40.2, 74.5)	70.0 (54.0, 83.5)	**0.353**
*: Significant. SD: Standard deviation.				

Table 2 compares the distribution of qualitative hematological and immunological markers across down-regulated, normal-regulated, and up-regulated groups. While the up-regulated group generally showed numerically higher expression for many surface markers, only a few parameters approached statistical significance, limiting firm conclusions regarding their discriminative utility.

Table (2): Comparative analysis of qualitative hematological and immunological parameters for cases only.

Variables Number (%)	**Down-regulation (n=4)**	**Normal-regulation (n=6)**	**Up- regulation (n=27)**	**p-value**
CD38	2 (50.0%)	1 (16.7%)	8 (29.6%)	0.712
CD79b	2 (50.0%)	2 (33.3%)	13 (48.1%)	0.869
CD22	3 (75.0%)	2 (33.3%)	18 (66.7%)	0.354
CD5	3 (75.0%)	5 (83.3%)	21 (77.8%)	1.000
CD19	4 (100.0%)	6 (100.0%)	23 (85.2%)	0.734
CD.45	4 (100.0%)	6 (100.0%)	27 (100.0%)	1.000
Sigm	2 (50.0%)	2 (33.3%)	10 (37.0%)	0.872
K	1 (25.0%)	3 (50.0%)	16 (59.3%)	0.439
L	2 (50.0%)	2 (33.3%)	7 (25.9%)	0.619
CD20	4 (100.0%)	4 (66.7%)	22 (81.5%)	0.639
FMC7	1 (25.0%)	2 (33.3%)	3 (11.1%)	0.231
CD23	4 (100.0%)	5 (83.3%)	21 (77.8%)	0.812
CD200	0 (0.0%)	1 (16.7%)	9 (33.3%)	0.501

Table 3 shows that age, sex, and β_2-microglobulin were insignificant predictors of chronic lymphocytic leukemia (CLL) diagnosis. Notably, the ITGA4 gene regulation emerged as the only significant predictor. The overall model demonstrated a good fit.

Table (3): Summary for logistic regression model coefficients

Predictor	**Estimate**	**p-value**	**Odds Ratio**	**95% CI Lower**	**95% CI Upper**
(Intercept)	-0.0712	0.951	-	-	-
Age category 25 – 45	-1.0691	0.310	0.343	0.05	2.36
Age category >45 – 65	-1.1649	0.081	0.312	0.088	1.11
Age category > 65	Ref	Ref	Ref	Ref	Ref
Gender Male	-0.7301	0.283	0.482	0.13	1.79
Gender Female	Ref	Ref	Ref	Ref	Ref
ITGA4 gene regulation	1.0831	<0.001	2.95	1.79	4.87
β_2-microglobulin	-0.1366	0.455	0.872	0.61	1.24
Residual deviance = 68.963 , Null deviance = 103.852, Akaike Information Criterion (AIC) = 80.963, VIF < 5					

Figure (1) shows, Spearman correlation analysis with several significant relationships among clinical and biomarker variables. TLC demonstrated a strong positive correlation with peripheral lymphocytes ($r = 0.81$, $p < 0.001$), but a nonsignificant correlation with bone marrow lymphocytes. In contrast, TLC showed a significant negative correlation with platelets ($r = -0.24$, $p = 0.036$). Additionally, ITGA4 gene regulation exhibited significant positive correlations with LDH ($r = 0.49$, $p < 0.001$), TLC ($r = 0.28$, $p = 0.016$), and peripheral lymphocytes ($r = 0.24$, $p = 0.038$), along with a significant negative correlation with platelets ($r = -0.31$, $p = 0.006$). Platelets also displayed significant negative correlations with LDH ($r = -0.30$, $p = 0.008$), TLC ($r = -0.24$, $p = 0.036$), and peripheral lymphocytes ($r = -0.26$, $p = 0.024$). Conversely, several nonsignificant correlations were identified. For example, ITGA4 gene regulation did not correlate significantly with age, bone marrow lymphocytes, hemoglobin (Hb), or β2-microglobulin ($p = 0.182$, 0.974, 0.728, and 0.66, respectively).

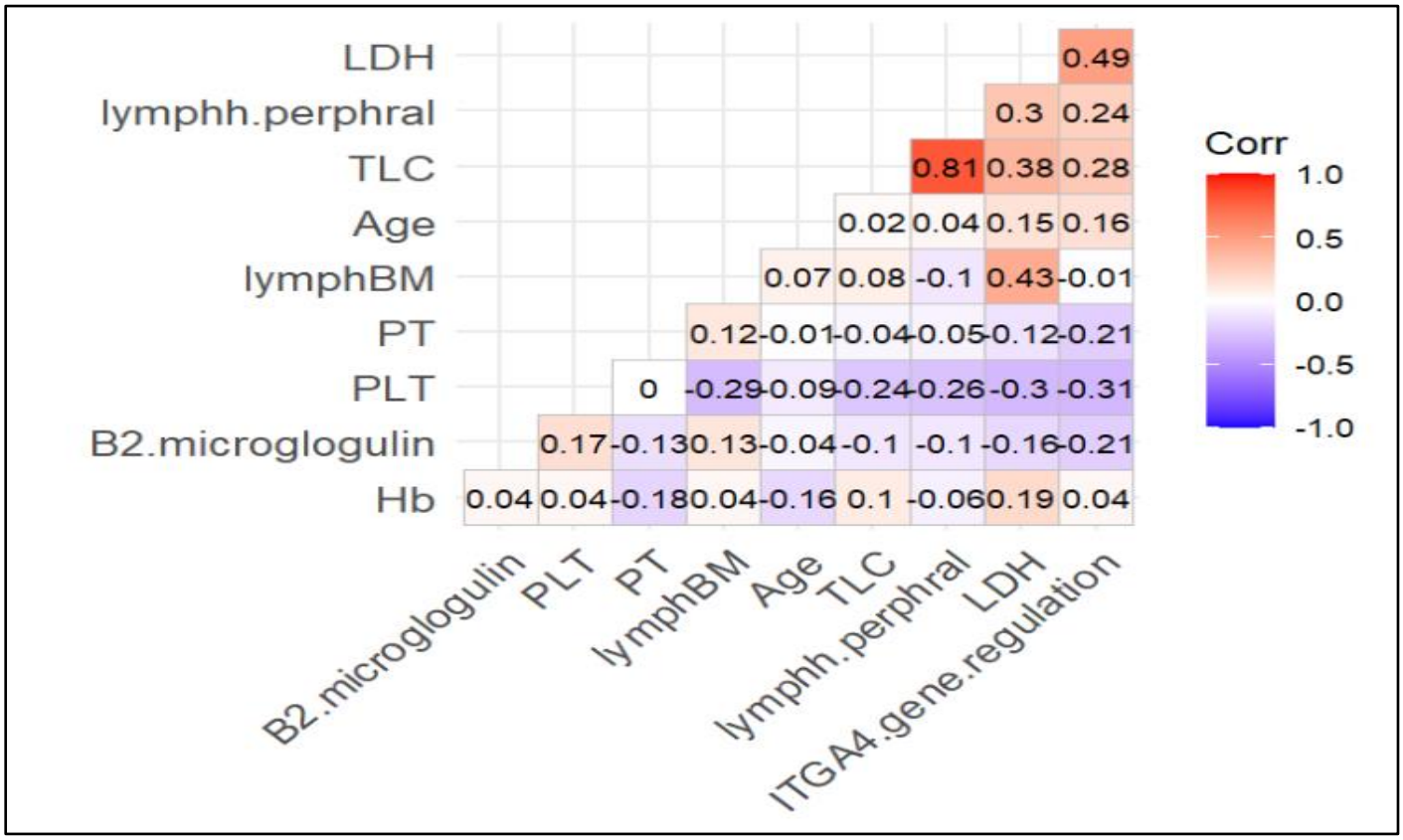

Figure (1): Correlation matrix of ITGA4 regulation and other variables.

Figure 2 presents the ROC analysis for the diagnostic ability of ITGA4 gene regulation in distinguishing between patients with and without CLL. The area under the curve (AUC) was 0.856 (95% CI 0.756 to 0.926, p <0.0001). This value, approaching 1, demonstrates that ITGA4 gene regulation is effective in differentiating between CLL-positive and CLL-negative patients. The Youden Index (J) was 0.6838, corresponds to a criterion value of >0.987, which serves as the classification cut-off for CLL status. At the optimal cut-off value of >1.0498, the test exhibits a sensitivity of 86.11% and a specificity of 82.05%.

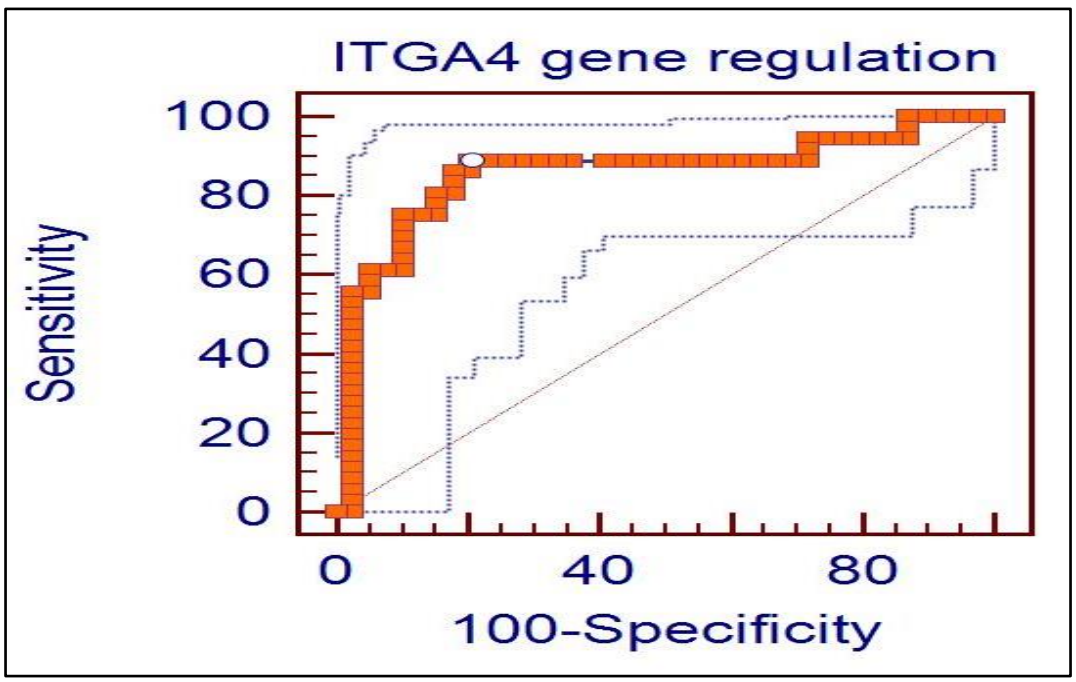

Figure (2): ROC curve illustrating the predictive performance of IGTA4 for CLL diagnosing.

DISCUSSION

This study provides a comprehensive investigation into the role of ITGA4 gene regulation in CLL, confirming its significant impact on the disease's molecular profile, clinical presentation, and diagnostic potential. CLL is a heterogeneous malignancy where genetic and epigenetic aberrations are critical drivers of clinical outcomes. Our findings position ITGA4, which encodes the CD49d protein, as a central player in this process, influencing not only cellular adhesion but also serving as a robust biomarker for disease aggressiveness.

Our research extends the understanding of CLL's molecular landscape by highlighting the significance of ITGA4 polymorphism. Unlike prior research that often focused on protein expression, our study in newly diagnosed cases emphasizes genetic variation as a foundational element [(5)]. This aligns with the established model that DNA mutation and methylation instability are key modulators of gene expression in CLL [(9)]. For instance, **Zucchetto *et al.*** [(10)] demonstrated methylation-dependent overexpression of CD49d in trisomy 12 CLL, implicating epigenetic mechanisms. Our findings corroborate with this, suggesting that ITGA4 polymorphism may be a herald of broader epigenetic dysregulation within the CLL genome. The role of ITGA4 in pathogenesis is further underscored by its involvement in the tumor microenvironment. Previous research has shown that ITGA4/CD49d enhances CLL cell adhesion to stromal cells, promoting survival and conferring resistance against chemotherapy-induced apoptosis [(11)]. Our study provides additional mechanistic evidence, demonstrating that genetic up-regulation of ITGA4 is directly linked to these clinically aggressive traits.

The stratification of patients based on ITGA4 regulation status revealed profound clinical implications. The up-regulated group exhibited a strong association with the presence of CLL itself, alongside features of advanced disease such as lymphadenopathy, splenomegaly, and bone marrow hypercellularity. Specifically, splenomegaly was significantly more prevalent in the up-regulation group, reinforcing the proposed role of ITGA4 in promoting leukemic cell retention and survival in lymphoid tissues [(12)]. Furthermore, the high rate of bone marrow hypercellularity (75.0%) in this group underscores ITGA4's involvement in mediating cellular interactions that support unchecked leukemic proliferation [(13)]. The significant association with lymphadenopathy aligns with the findings of **Attia *et al.*** [(5)]**,** who reported a similar high prevalence in patients with elevated ITGA4 expression, solidifying its role as a hallmark of aggressive disease.

The analysis of hematological parameters provided further diagnostic and prognostic insights. The marked decrease in total leukocyte count (TLC) in the down-regulated group contrasts with the elevated counts in up-regulated patients, aligning with reports that leukocytosis is associated with advanced disease [(14)]. The significant elevation of LDH in the up-regulated group reflects increased cellular turnover and tumor burden. This is consistent with its established role as a marker of adverse prognosis, a point reinforced by studies like that of **Hallek and Al-Sawaf** [(14)] **and Li *et al.*** [(15)], which linked high LDH to poor survival in high-risk genetic subgroups. Concurrently, the significant thrombocytopenia in the up-regulated group parallels previous research emphasizing low PLT as an independent prognostic factor.

Immunologically, the significantly higher positivity rates of CD22, CD5, and CD19 in the up-regulated group highlight their relevance in identifying aggressive disease subsets, corroborating their status as hallmark markers of CLL [(16)]. The high CD45 positivity further underscores its role in immune activation and leukemic cell survival within the tumor microenvironment [(13)].

A key finding of this study is the diagnostic superiority of ITGA4 gene regulation over the conventional biomarker β2-microglobulin. The regression model identified ITGA4 as a powerful predictor, while β2-microglobulin showed no significant association ($p = 0.455$). The model's robustness was evidenced by a substantial reduction in deviance and a low AIC (80.963). This was confirmed by ROC analysis, where ITGA4 demonstrated excellent diagnostic accuracy (AUC = 0.856), whose discriminatory power was akin to chance. This aligns with studies that have identified ITGA4 as a marker of poor prognosis and disease progression [(17)]. It is important to note, however, that while β2-microglobulin may be a poor diagnostic tool in this context, it remains a strong prognostic marker in specific settings like allogeneic stem cell transplantation [(18,19)]. Correlation analysis offered further mechanistic insights, showing a moderate positive correlation between ITGA4 and LDH ($r = 0.49$), linking it to metabolic stress, and a weak negative correlation with PLT ($r = -0.31$), suggesting a role in bone marrow suppression.

STRENGTHS AND LIMITATIONS

This study has several notable strengths. The focus on newly diagnosed CLL patients provides a clear window into the disease's initial molecular state, minimizing confounding effects of prior treatments. The integrated analytical approach, combining polymorphism analysis, gene expression grouping, correlation studies, and advanced regression modeling, provides a multi-faceted validation of ITGA4's role.

However, several limitations must be acknowledged. The case-control design inherently identifies associations rather than establishes causality. The sample size, while sufficient for robust initial findings, may limit the generalizability of the results and the power to detect more subtle associations or fully explore subgroup trends, such as the observed gender

variability in ITGA4 regulation, which may be influenced by sex-specific factors [20]. Future prospective longitudinal studies with larger, multi-center cohorts are necessary to validate these findings and elucidate the precise mechanistic pathways through which ITGA4 polymorphisms influence disease progression and therapy response.

CONCLUSION

In conclusion, this study establishes ITGA4 gene dysregulation as a pivotal element in CLL pathogenesis. It is strongly associated with aggressive clinical phenotypes, adverse laboratory parameters, and serves as a superior diagnostic biomarker compared to β2-microglobulin. The findings underscore the importance of ITGA4 in mediating critical interactions within the tumor microenvironment that promote disease progression. Consequently, ITGA4 emerges as a highly promising biomarker for improving risk stratification at diagnosis and a compelling potential target for novel therapeutic strategies in CLL.

Author contributions: Conceptualization and study design were performed by A.A.A. and H.A.A. Data collection and investigation were carried out by N.E.H. and R.M.A. Data analysis and validation were conducted by A.A.Y. and A.A.E. The original draft was written by A.A.A., and all authors contributed to reviewing and editing the final manuscript.

No funding.

No conflict of interest.

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