The Egyptian Journal of Hospital Medicine (October 2021) Vol. 85 (1), Page 3247-3250

# The Regulatory Role of CD26 And Its Expression in Chronic Lymphocytic Leukemia and B-Cell Non-Hodgkin Lymphoma

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## ABSTRACT
**Background:** Chronic lymphocytic leukemia (CLL) is a monoclonal disorder characterized by uncontrolled proliferation of mature B lymphocytes. CD26 is a multi-functional type II cell surface glycoprotein, which is expressed mainly by T lymphocyte and it has a regulatory role in progression and also in migration and metastasis of tumour.
**Objective:** The aim of the work was to assess the regulatory role and expression of CD26 in patients with B-CLL and B-cell non-Hodgkin lymphoma (B-NHL) and the relation to disease staging and progression.
**Methods:** This study was conducted on 6 patients with B-CLL and 5 patients with NHL who were attending at National Cancer Institute. We detected functional role and specificity of CD26 by flow cytometry during the period from December 2019 to March 2020. The ages of patients ranged from 42 to 79 years old. Besides, 5 healthy individuals worked as control group. **Results:** A significant difference was found as regards expression of CD26 in CLL cases in comparison to control cases (p.0001). Also there was a significant difference between the NHL group and control group in expression of CD26 (p.0001). But no significant difference was detected between the NHL groups and CLL groups in expression of CD26 (p.052). **Conclusion:** This study confirmed that CD26 expression in CLL and NHL is variable and didn't correlate with other prognostic factors. In addition, there was no significant difference between the NHL groups and CLL groups in expression of CD26.
**Keywords:** CD26, Chronic lymphocytic leukemia, B-cell non-Hodgkin lymphoma.

## INTRODUCTION
Chronic lymphocytic leukemia (CLL) is a monoclonal disorder characterized by uncontrolled proliferation of mature B lymphocytes with a distinct morphological and immunological pattern [(1)]. CLL is due to the accumulation of monoclonal B-cells, this accumulation is due inhibition of apoptosis rather than cell proliferation [(2)].

CD26 is a type II cell surface glycoprotein, which is expressed by T lymphocytes and natural killer cells. It has a regulatory role in progression of neoplasm as a result of its ability to bind extracellular matrix proteins [(3)]. B-CLL undergoes a slowly progressive clinical course and others enter advanced phase [(4)]. We must identify molecular parameters to detect high risk patients [(5)]. A large number of factors help predict the outcome of the patients with B-CLL. These include clinical stage, cell morphology, and cytogenic abnormalities [(6)].

**Typical feature of CLL (**called Gumprecht phenomenon) is presence of basket cells on the smears, reflecting fragility and distortion during preparation of the smear on the glass slide [(7)].

**Atypical CLL** is about 15% [(8)]. Active disease is defined by The National Cancer Institute Working Group (NCI-WG). The guide lines include: - progressive marrow failure, splenomegaly, progressive lymphadenopathy (>10 cm in longest diameter), lymphocytosis with an increase of > 50% over a 2-month period, and anemia and/or thrombocytopenia [(10)].
- Additionally, one of the following must be present: weight loss of ≥ 10% within the previous 6 months, fatigue, fevers of > 38.0 ºC for 2 weeks [(11)].

## METHODS
This study was done on 6 patients with B-CLL and 5 patients with NHL who were attending at National Cancer Institute. We detect functional role and specificity of CD26 by flowcytometry, during the period from December 2019 to March 2020. Their ages ranged from 42 to 79 years old. 5 healthy individuals worked as control group.

**Ethical approval:**
**The study was approved by the Ethics Board of Al-Azhar University and an informed written consent was taken from each participant in the study. This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.**

All patients and controls were subjected to the following except for (4) was done for patients only:
1. Full history and clinical examination for the presence of lymphadenopathy, splenomegaly or hepatomegaly.
2. Abdominoplevic ultrasound for lymphadenopathy and /or organomeglay
3. Laboratory investigations including:
   - Complete blood count (CBC)
   - Leishman-stained peripheral blood (PB) smears.
4. Leishman stained bone marrow (BM) aspiration smears were examined for lymphocyte percentage and morphology [(12)].
5. Detection of CD26 by flow cytometry.





Received: 3 /6 /2021
Accepted:29/7 /2021

6. **Patients fulfilling all the following criteria were selected:**

   a. Lymphocytosis (> $5x10^9$/L) for at least 3 months [13].

   b. Lactate dehydrogenase enzyme concentration is measured by fully automated machine (Synchron CX4).

**SAMPLE COLLECTION:**

1. 2 ml of PB were collected on (K-EDTA) for CBC and preparation of Leishman-stained PB smears.
2. 1 ml PB or BM sample on EDTA for detection of CD26 by flow cytometry. Bone marrow aspirates or PB samples were processed within 24 hours of collection, being preserved at room temperature.

**CD26 Expression Analysis:**

1. For each sample, two tubes were labeled for the antibody used and the negative control
2. 100 µL of diluted samples were delivered in each tube.
3. 20 µL of anti CD26 was added to tubes of sample
4. 2 mL lysing solution was added to each tube.
5. The tubes were incubated and centrifuged
6. The supernatant was discarded and cells were suspended in PBS for flow cytometer.

**Results were expressed as:**

- The percentage of lymphocytes carrying CD26 within the gated population of lymphocytes.

***Statistical analysis***

SPSS statistical software was used. Data are presented as mean ± SD. In analysis we used t test and Chi square test. P value ≤ 0.05 was considered significant.

**RESULTS**

**Laboratory Data of the Studied CLL Patients (Table 1):**

As for the laboratory data, the mean of TLC was 137.2 ± 18.3 $x10^9$/L, the mean lymphocytic count was 78.4 ± 19.5%.

The mean of Hb was 10.1 ± 2.1 g/dL, the mean platelets count was 153.4.0 ± 28.9 $x10^9$/L, The mean of bone marrow lymphocytes was 74.5% ±16.8% and mean of LDH concentration was 694.2 ± 151.1 IU/L**.**

**Table (**1**):** Description of lab data among CLL cases

| | **Mean** | **±SD** | **Median** |
|---|---|---|---|
| TLC($x10^9$/L) | 137.2 $x10^9$ | 18.3 | 125.9 |
| Lymph (%) | 78.4 | 9.5 | 66.5 |
| HB (g/dl) | 10.1 | 2.1 | 7.9 |
| Plts ($x10^9$/L) | 153.4 $x10^9$ | 28.9 | 137.0 |
| BM lymph (%) | 74.6 | 6.8 | 67.0 |
| LDH (IU/L) | 694.2 | 51.1 | 620 |

**CD26 expression by flow cytometry in CONTROL cases:**

CD26 expression was negative in all control cases (100%) with a mean of 1.09 ± .18 (Table 2).

**Table (2):** Description of CD26 among controls

| | Mean | ± SD | Median |
|---|---|---|---|
| CD26 | 1.09 | 0.01 | 0.86 |

**CD26 EXPRESSION by FLOW CYTOMETRY in CLL cases (Table 3, 4):**

A high significant difference was found as regards expression of CD26 in CLL cases in comparison to control cases (p.0001)

**Table (3):** Comparison between CLL and control group

| | CLL=6 cases | | | Control=5 cases | | | P | Sig |
|---|---|---|---|---|---|---|---|---|
| | Mean | ± SD | Median | Mean | ± SD | Median | | |
| CD 26 | 7.43 | 1.67 | 6.61 | 1.09 | 0.18 | 0.86 | .0001 | HS |

Mann Whitney test

Based on cut off ≥ 10%, (16.67%) were considered positive expression of CD26 among CLL cases. While, 83.33% are considered negative (< 10 %).

**Table (**4**):** Description of CD26 positive among CLL cases

| | Mean | ± SD | Median |
|---|---|---|---|
| CD26 | 7.43 | 1.67 | 6.61 |

**Table (**5**):** Comparison between positive and negative CD26 CLL cases

| | | **cd26 <10** | **cd26 ≥ 10** | P | Sig |
|---|---|---|---|---|---|
| | | 83.33% | 16.67 % | | |
| | | % | % | | |
| Sex | Male | 40.0% | 75.0% | .300 | NS |
| | Female | 60.0% | 25.0% | | |
| Age | Mean ± SD | 11.2 | 13.4 | .862 | NS |
| LN | Negative | 30% | 50.0% | 1.0 | NS |
| | Positive | 70 % | 50.0% | | |
| Splee n | Negative | 35% | 25.0% | 1.0 | NS |
| | Positive | 65 % | 75.0% | | |
| Liver | Negative | 50 % | 75.0% | .121 | NS |
| | Positive | 50% | 25.0% | | |
| Stage | I | 25% | 25.0% | 1.0 | NS |
| | II | 25% | 25.0% | | |
| | III | 20 % | 25.0% | | |
| | IV | 30.0% | 25.0% | | |
| HB | Median | 7.9 | 7.3 | .988 | NS |
| Plts | Median | 137 | 130.65 | .556 | NS |
| TLC | Median | 125.9 | 121.38 | .467 | NS |
| PBL | Median | 67.0 | 61 | .408 | NS |
| LDH | Median | 620 | 587.3 | .483 | NS |

*Fisher exact test **student t test

**Laboratory data of the studied NHL patients (Table 6).**

As for the laboratory data, the mean Hb was 9.7 g/dL ± 2.4, the mean platelet count was 171.2 ± 41.1 $x10^9$/L, the mean of TLC 41.9 ± 10.1 $x10^9$/L, the mean of relative lymphocytic count was 64.4 ± 16.1 and the mean bone marrow lymphocytes was 53.7% ±12.2.

**Table (**6**):** Description of lab data among NHL cases

| | Mean | ± SD | Median |
|---|---|---|---|
| HB(g/dL) | 9.7 | 2.4 | 10.2 |
| Plts ($x10^9$/L) | 171.2 $x10^9$ | 4.1 | 143.0 |
| TLC($x10^9$/L) | 41.9 $x10^9$ | 10.1 | 36.8 |
| Lymph (%) | 64.4 | 6.1 | 55.0 |
| BM lymph (%) | 53.7 | 1.2 | 48.0 |

**CD26 in NHL cases:**

CD26 expression was positive in NHL cases with a mean of 17.11± 4.08 **(Table 7)**.

**Table (7):** Description of CD26 among NHL cases

| n :5 | Mean | ± SD | Median |
|---|---|---|---|
| CD 26 | 17.11 | 4.08 | 15.73 |

There was a high significant difference between the NHL group and control as regards expression of CD26 (p.0001) as shown in table (8).

**Table (**8**):** Comparison between NHL and control groups as regard CD26

| | Group | | | | | | P | Sig |
|---|---|---|---|---|---|---|---|---|
| | NHL=5 cases | | | Control=5 cases | | | | |
| | Mean | ±SD | Median | Mean | ±SD | Median | | |
| CD26 | 17.11 | 4.08 | 15.73 | 1.09 | 0.18 | 0.86 | .0001 | HS |

*Mann Whitney test

As regards cut off 10 %, cases of NHL were divided into 2groups, positive CD26 expression (≥ 10 %) and negative CD26. This study showed positive CD26 expression (45%) and showed negative CD26 expression (55%), which was not statistically significant.

There was non-significant difference between the NHL groups and CLL groups in CD26 (p.052) as shown in table (9).

**Table (9):** Comparison between NHL and CLL groups as regard CD26

| | | **Non Hodgkin=5 cases** | **CLL =6 cases** | **Chi-square test** | |
|---|---|---|---|---|---|
| | | **%** | **%** | **$X^2$** | **P-value** |
| CD 26 | negative | 55.00% | 83.33% | 2.956 | 0.052 |
| | positive | 45.00% | 16.67% | | |

## DISCUSSION

Patients with CLL have different clinical courses, some live a long-time without therapy, while others progress to advanced stages and die despite aggressive treatment [14]. Many markers were found to detect the progression of the disease like age, sex, lymphocyte morphology and number and recently cytogenetic findings [15, 16].

This study was conducted on 6 B-CLL patients and 5 NHL patients where CD26 expression was measured by flowcytometry to detect their regulatory role.

The results of the present study showed that CD 26 expression in CLL patients were highly significant as compared to control group (p .0001). In contrary, **Molica *et al.*** [18], found that levels of CD26 measured by ELISA technique were not different between CLL patients and control (p .0793). Our results are in agreement with **Cro *et al.*** [17], who explained different expression of CD26 and found that CD26 expression was variable in CLLs and lymphomas

Based on cutoff value of ≥10%, only 16.67%) among CLL patients showed **positive CD26**, while 83.33% were **negative CD 26** expression.

The present study showed insignificant correlation between positive CD26 expression with age and sex (p > 0.05). This agrees with **Molica *et al.*** [18], who found that CD26 levels didn't relate to patients' age (p 0.589) and sex (p 0.296) in CLL patients.

This study showed non-significant correlation between positive CD26 expression and lymphadenopathy, splenomegaly or hepatomegaly among CLL patients (p > 0.05). There was insignificant correlation between **positive CD26** regarding lower Hb levels or high TLC count in peripheral blood or high lymphocytes in BM or lower plts and Rai staging and LDH level (p > 0.05). **Molica *et al.*** [19], failed to correlate CD26 with Rai stages, hemoglobin, lymphocyte count, platelet count, LDH or cytogenetic abnormalities. Our results are in agreement with **Carbone *et al.*** [20], who explained insignificant differences in mean LDH levels between CD26- and CD 26+ cases.

As regards NHL cases, this study found a high significant difference between the NHL group and control as regards CD26 expression.

This study showed non-significant correlation between CLL cases and NHL cases as regards CD26 expression (p 0.05). This agrees with **Verstovsek *et al.*** [21], who found that CD26 was not detected in B-cell lymphomas and was rarely found in NHL cases. **Cro *et al.*** [9], found variable expression of CD26 in certain types of B-CLL and lymphoma but in this study we didn't classify types of NHL.

## CONCLUSION

This study confirmed that CD26 expression in CLL and NHL is variable and didn't correlate with other prognostic factors. There was no significant difference between NHL and CLL in detection of CD26.

**Financial support and sponsorship: Nil.**
**Conflict of interest: Nil.**

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