Plasma Cell-Free DNA (cfDNA) as a Non-Invasive Biomarker in Patients with Diffuse Large B-Cell Lymphoma (DLBCL)

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Background

DLBCL is among the most prevalent lymphomas, significantly burdening patients, communities, and healthcare systems. Routine management of these tumors involves procedures like tissue biopsies, Computed Tomography, and Positron Emission Tomography scans, which are invasive, expensive, and radiation-heavy. Also, these procedures are not always available in low-resource settings and have different disadvantages and limitations. These prompted the exploration of simpler tests, such as plasma fDNA.

The term cfDNA describes any DNA existing in the extracellular environment. It is released from cells into the circulation in both physiological and pathological conditions by passive and active mechanisms. Many studies showed its elevation in pathological conditions such as sepsis, cancer, & acute myocardial infarction.

Taken together, with the easy repeatedly obtaining of cfDNA by minimally invasive procedures, have indicated the tremendous potential for cfDNA as a non-invasive biomarker for the screening & management of DLBCL.

Objectives

To compare cfDNA levels between DLBCL patients & controls, to study the correlations of these levels with tumor stage and prognostic determinants, and to evaluate cfDNA alteration after 3 cycles of treatment.

Material &Methods

The study included 64 newly-diagnosed DLBCL patients and 28 apparently healthy controls. In addition, 41 patients were followed-up to evaluate treatment response after 3 cycles of chemotherapy.

Tumor stage, prognosis determinants, uric acid, creatinine, LDH, LDH ratio, and cfDNA were evaluated at diagnosis (baseline) and after 3 cycles of chemotherapy.

Using *QIAamp DNA Mini Kit* (Qiagen, Hilden, Germany), cfDNA was extracted from blood samples taken from controls and from DLBCL patients (at diagnosis from all participants and before the 4th cycle of chemotherapy from 41 DLBCL patients). Extracted cfDNA was measured by real-time qPCR using Sybrmaster mix (Jena Bioscience GmbH, Löbstedter, Jena, Germany) with two primers flanking beta globin gene (Alpha DNA, Montreal, Canada).

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Results

At diagnosis (baseline), cfDNA levels (ng/ml) was significantly (P<0.0001) higher in DLBCL patients (12.71±7.20) compared to controls (7.07±3.76). These levels were significantly correlated with baseline levels of LDH, LDH ratio, and size of the largest tumor mass but not with performance status [according to Zubrod / Eastern Cooperative Oncology Group (ECOG) scale]; tumor stage (using the Ann Arbor or Lugano classification); prognosis [based on NCCN-International Prognosis Index (NCCN-IPI) scores]; the presence of bulky disease (lymph nodes larger than 7.5 cm); or differences in sex and age.

At follow-up, cfDNA levels decreased significantly after 3 cycles of chemotherapy (from 12.33±6.74 to 3.19±1.99). These levels were correlated with the significant reduction in tumor size. In contrast, neither HDL, nor HDL ratio changes after treatment was significant or correlated with mass size change after 3 cycles of chemotherapy .

Discussion

Current study showed the importance of cfDNA as a non-invasive biomarker in the course of DLBCL. It appears as an important diagnostic and predictive biomarker. Both odd ratio (1.2; P=0.007) and area under the curve were comparable to, if not better than, those related to LDH.

The current results also showed the importance of cfDNA in the monitoring of DLBCL treatment response, which is the most important use of this biomarker till now.

Fig. 1. Comparison of cfDNA Concentration between control and DLBCL groups.

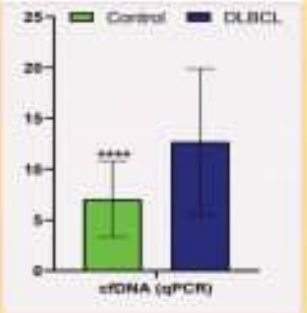
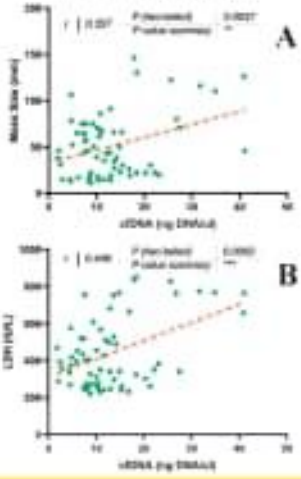


Fig. 2. CfDNA Corelations with mass size (A) & LDH (B) in DLBCL groups.



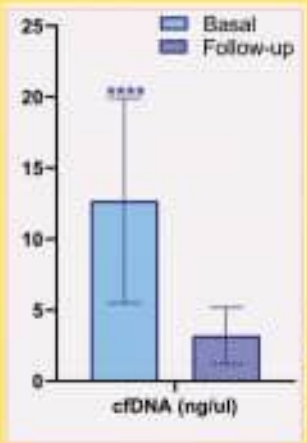


Fig. 3. Comparison of cfDNA Concentration between baseline and 4th cycle of chemotherapy.