ASSESSMENT OF CIRCULATING CELL FREE DNA IN EGYPTIAN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the single most common type of lymphoma worldwide. [1] It is a clinically aggressive cancer that rapidly grows and usually metastasizes throughout the body at early stages yet it's potentially curable; therefore, it requires early diagnosis, urgent treatment and close monitoring. In DLBCL, the diagnosis, prognosis and monitoring of the disease are currently based on clinical assessment, invasive biopsies, CT scans and PET scans. Those methods are highly valuable; however, there's an urgent need for a simpler tool to assess prognosis, closely monitor the patient's response to treatment and allow earlier detection of relapse. Circulating cell free DNA (ccfDNA) is this promising biomarker; the liquid gold that must be further studied to obtain such a simple accessible biomarker that can reflect so much information about the underlying malignant clone. Circulating cfDNA is mainly composed of double stranded DNA fragments circulating in the bloodstream of both healthy and diseased individuals. Normally, ccfDNA is present in low concentrations; however, ccfDNA levels are increased in certain conditions like pregnancy, malignant diseases and non-malignant diseases such as inflammatory conditions, autoimmune diseases, trauma, myocardial infarction stroke, sepsis and allograft transplant rejection. It has been found that circulating cfDNA in cancer patients show a different fragmentation profile from cfDNA in healthy individuals; therefore, the integrity of cfDNA has been investigated as a potential diagnostic and prognostic marker in several cancers.

Aim of the work

The aim of the study was to assess the concentration of cfDNA and DNA integrity index in DLBCL patients and to correlate them with the international prognostic index of DLBCL.

Subjects

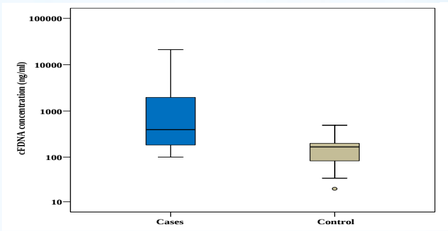
After approval of the Ethics Committee of the Faculty of Medicine, Alexandria University, this study was conducted on 30 patients with newly diagnosed diffuse large B-cell lymphoma and 30 sex and age matched normal healthy individuals as a control group. Patients who were pregnant or had any other malignancy, autoimmune disease, myocardial infarction, hepatic insufficiency or renal insufficiency were excluded from the study.

Methods

Circulating cell free DNA was extracted from peripheral whole blood samples using Magcore® circulating DNA kit, (RBC Bioscience Corp., Belgium). The extracted cfDNA was quantified using Qubit dsDNA HS Assay kit using the Invitrogen Qubit fluorometer (ThermoFisher Scientific, USA). The DNA integrity index was assessed by Quantitative Real time PCR using the Rotor-gene Q (Qiagen, Germany). The QuantiNova SYBR Green PCR kit (catalogue numbers: 208052) was used to amplify ALU 111 and ALU 260 primers. The absolute concentrations of shorter and longer cfDNA fragments were determined using serial dilutions of a standard human genomic DNA G304A (Promega, USA). The DNA integrity index (DII) was calculated as the ratio of the absolute concentration of longer cfDNA fragments to the absolute concentration of shorter cfDNA fragments (ALU260/ALU111).

Results

Our study showed that DLBCL newly diagnosed cases showed significantly higher circulating cell free DNA concentrations (ccfDNA) as compared to healthy controls with a P value <0.001 as shown in figure (1). Furthermore, a valid cut off value was obtained to discriminate cases from controls that defines cases as having cfDNA concentrations greater than 227 ng/ml with an AUC of 0.821, 70% sensitivity and 90% specificity which indicates a very good diagnostic power as shown in figure (2).Moreover, our study showed that DLBCL cases showed significantly lower DNA integrity index (DII) as compared to healthy controls with a P value of 0.001 as shown in figure (3). A cut off value to discriminate cases from controls was obtained that defines cases as having DII equal to or less than 0.1933 with an AUC of 0.742, 66.67% sensitivity and 83.33% specificity which indicates a good diagnostic power as shown in figure (4). There was a statistically significant association between the cfDNA concentrations and adverse prognostic factors like performance status scores (P value is <0.001), stage at diagnosis (P value is 0.001), IPI score (P value is <0.001), the presence of B-symptoms (P value=0.021), the presence of bulky disease (P value=0.044) and levels of LDH (P value is <0.001) as shown in table (1). Additionally, there was a statistically significant association between the cfDNA concentrations and the levels of blood urea (P value is 0.048), hemoglobin levels (P value is 0.001) and WBC counts (P value is 0.004). There was no statistically significant correlation between DII of DLBCL cases and any of those adverse prognostic factors or laboratory parameters.



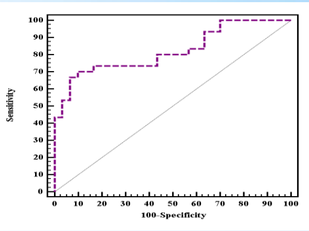
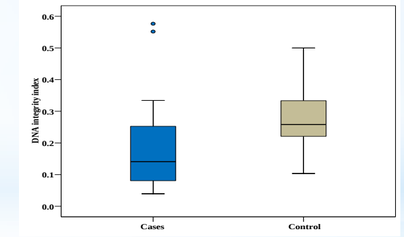


Figure (1):Comparison between the two studied groups according to cFDNA concentration
Figure (2):ROC curve for cFDNA concentration to discriminate cases (n = 30) from control (n = 30)



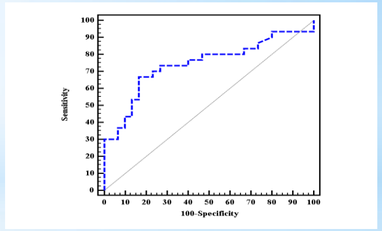


Figure (4):ROC curve for DNA integrity index to discriminate cases (n = 30) from control (n = 30)
Figure (3):Comparison between the two studied groups according to DNA integrity index

Table (1): Correlation between cfDNA concentration with different parameters in cases group (n=30)

	cFDNA concentration (ng/ml)	
	r_s	p
Performance status	0.733*	<0.001*
Stage at diagnosis	0.591*	0.001*
IPI	0.806*	<0.001*
Urea	0.365*	0.048*
LDH	0.681*	<0.001*
Hemoglobin	-0.560*	0.001*
WBCs	0.508*	0.004*

Conclusion

Circulating cfDNA concentration as well as DNA integrity index are promising diagnostic noninvasive tools for DLBCL. Also, cfDNA concentration is considered a reliable prognostic marker for DLBCL as it correlates with IPI and other adverse prognostic factors of DLBCL.