

Evaluation of heat shock protein 70(HSP-70) & heat shock protein 90(HSP-90) in diffuse large B-cell non-Hodgkin's lymphoma patients

Abstract

Back ground: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease with recognized variability in clinical outcome, genetic features, and cells of origin. It is the most common lymphoid malignancy in adults, comprising almost 40% of all lymphoid tumors.

Objective: To evaluate heat shock protein (70) & heat shock protein (90) in diffuse large B cell non-Hodgkin lymphoma patients.

Patients and method: This is a case control study conducted during the period from (April 2015 to December 2015) on 55 patients (19 patients before treatment & 36 patients after treatment). Their ages ranged between 14 and 80 years and diagnosed to have diffuse large B cell non-Hodgkin lymphoma patients who attended the Baghdad teaching Hospital in medical city complex & Emmammain kadhmain medical city Hospital. In addition to control group consisted of 30 apparently healthy volunteer. Both patient and control groups were examined and reviewed clinically and assessed by basic laboratory investigation as well as measurement of heat shock protein (70) & heat shock protein (90) via Elisa technique.

Results: HSP 70 showed a highly significant difference between patients and control groups (P value=0.001) as well as showed a highly significant difference (P value=0.001) between limited and advanced stage DLBCL. This difference is persistently reported in advanced disease patient whether before treatment or after treatment course (P value=0.001), as well as shown significantly (P value=0.014) in limited stage disease before and after treatment. HSP 90 shows significant difference between patients and control (P value=0.004) which is also highly significant difference (P value=0.001) for both limited and advanced stage as well as in relation to treatment courses (P value=0.001).

Conclusion: HSP-70,HSP-90 are involved in pathogenesis of NHL in comparison with healthy people Which may indicate the abnormal cell growth and abnormal stability of tumor cells this may monitored and reduced after response to treatment.

Keywords: DLBCL Heat shock protein 70 (HSP-70), Heat Shock Protein 90 (HSP-90), proteins, hormone receptors

Volume 6 Issue 4 - 2018

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Received: March 23, 2018 | **Published:** July 09, 2018

Introduction

Heat shock proteins family represents one of the largest stress protein families. They are most highly expressed cellular proteins across all species, and they can protect cells from stress by elevated temperatures. They account for 1–2% of total protein in unstressed cells which may get increased up to 4–6% when cells are heated. The 70 kilo Dalton heat shock proteins (Hsp70s) are a family of conserved ubiquitously expressed heat shock proteins.² It aids in trans membrane transport of proteins, by stabilizing them in a partially folded state and can act to protect cells from thermal or oxidative injury. These stresses normally act to damage proteins, causing partial unfolding and possible aggregation. By temporarily binding to hydrophobic residues exposed by stress, Hsp70 prevents these partially denatured proteins from aggregating, and allows them to refold again.² Hsp70 seems to be able to participate in disposal of damaged or defective proteins.³ In addition it can improve overall protein integrity and directly inhibits apoptosis.⁴ but without being involved in Fas-ligand-mediated apoptosis. Therefore, HSP-70 not only saves important components of the cell but also directly saves the cell as a whole. The 2 essential HSPs are HSP 70 and HSP 90. HSP 70 is overexpressed in different malignancies like melanoma⁵ while it is under expressed

in renal cell cancer.⁶ They participate in the folding of client proteins, such as tyrosine-kinases, serine-threonine kinases, hormone receptors, Inhibition of apoptosis, activation of proteasomes & NF kappa B pathway.⁷

Hsp90 (heat shock protein 90) is a part of a multi-protein complex that assists in the folding and stabilization of client proteins which implicated in oncogenesis, like AKT, and mutant p53 etc.⁸ It is therefore; considered as potential element in growth and survival of cancer cell and may be target for therapy.⁸

In diffuse large B-cell lymphomas (DLBCLs), there are HSP90 isoforms defined by their transcriptional profiles and consensus clusters as 'B-cell receptor (BCR)', 'Oxidative Phosphorylation (Ox Phos)' and 'Host response (HR)' DLBCLs.⁹

In a case control study, fifty five patients with DLBCL, (19) of them were before treatment, while the rest 36 assessed after treatment were enrolled. They are diagnosed and followed by consultant hematologist from hematology unit of both Baghdad teaching Hospital in medical city complex & Emmammain kadhmain medical city Hospital during the period of from (April 2015 to December 2015). They were between the age (14-80)years and they interviewed and assessed

for the baselines clinical characteristics (age, gender, presenting compliant, past medical history as well as the method of histological diagnosis & radiological staging of the disease). All patients were classified according to their stages as

- a. Limited stage disease (stage I A,II A) and
- b. Advanced stage disease (I B,II B, III A,III B, IV A and IV B)¹⁰
- c. All were received treated with R-CHOP chemotherapy every 21days, for eight cycles over 3-4months duration.^{11,12}
- d. Those patients were subdivided into 2 further groups which are
 - i. Group 1 (before treatment group): including all patient at time of diagnosis before receiving any treatment or those just received first cycle of chemotherapy.
 - ii. Group 2 (at after treatment group): including those patient who were received 6-8cycles of chemotherapy (for advanced stage disease).
 - iii. In addition to Group 3 who considered as a (Control group) included 30 apparently healthy volunteer individuals (with equivalent age and gender).

All groups were assessed for the same laboratory parameters

Inclusion criteria were any case of definitive diagnosis of DLBCL NHL confirmed by histopathology of LN biopsy or BM biopsy with typical immunopheno typing markers by either immunohistochemistry (IHC) or flow cytometry. Exclusion criteria included all other types of non-Hodgkin lymphoma like (T cell type NHL, Follicular type NHL, Marginal zone NHL, Mantle cell lymphoma, Chronic lymphocytic leukemia or small lymphocytic lymphoma) or relapsed DLBCL cases that received multiple prior courses of treatment.

All patients were assessed according to international prognostic score IPI which include Five adverse prognostic risk factors:

- a) Age>60 years

- b) Ann Arbor stage III/IV
- c) >1 extra nodal site
- d) Serum lactate dehydrogenase (LDH) level>normal
- e) Eastern Cooperative Oncology Group (ECOG) performance status≥2

The total score will be classify the patient prognosis into 4 groups that help in predicting their survival as: Low risk=0-1 (five-year overall survival of 73%), Low-Intermediate=2, High-intermediate=3 and High=4-5 (fiveyear overall survival of only 26 %).¹²

In addition to basic laboratory parameters, HSP 70 and 90 were estimated using Elisa test.

Methodology of ELISA¹⁸

We used the ELISA instrument to detect HSP-70 & HSP-90 by standard curve. HSP-70 & HSP-90 based on biotin substance double antibody sandwich to inspect the test. Put HSP-70 & HSP-90 to wells that are coated with HSP-70 & HSP-90 monoclonal antibody, formerly incubate. After incubation, additional anti HSP-70 & HSP-90 antibodies branded with biotin substance to bind with streptavidin-horse reddish peroxidase, which formulae complex of immune. By washing, Disregard unbound enzymes and stay bound enzymes to get this test, formerly add substrate color reagent which prepare from of A reagent and B reagent. Solution will go blue which change from blue to yellow color with the outcome by acid. Shadow of solution and the concentration of Human HSP-70 & HSP-90 are positively allied.

Results

Sample description

Fifty five patients enrolled in this study their age ranged for (14-80) years with Mean±SD 51.6+18.6yr as shown in Table 1.

Patient group was comparable with thirty control healthy volunteer who had equivalent age & gender with no statistical significance Table 1.

Table 1 Demographic and clinical parameters for patients and control groups

Parameter		Patients (N=55)	Control (N=30)	P value
Age (year)	Mean±SD	51.64+18.62	51.37+16.81	0.946
	Range	14-85	16-80	
Gender	Male, N(%)	30 (54.55)	20(66,67)	0.358
	Female, N(%)	25 (45.45)	10 (33.33)	
Stages	Limited (I A, II A) N (%)	7 (12.73)		
	Advanced (I B, II B, III B, IVB, III A, IVA) N (%)	48 (87.27)		
Extra nodal involvement	Present N (%)	11(20)		
Bulky diseases	Present N (%)	4 (7.27)		
International prognostic Index (IPI) score	Low IPI=0-1	17 (30.90)		
	Low- Intermediate IPI=2	23 (41.83)		
	Intermediate-High IPI=3	11 (20)		
	High IPI=4-5	4 (7.27)		
Hypertension	Present N(%)	17 (30.91)		
Diabetes mellitus	Present N(%)	10 (18.18)		

Clinical characteristic of patient group

Majority of patients with NHL were in advanced stage (IB, IIB, IIIB, IVB, IIIA, IVA) 87.27%. Most patients were found to have nodal involvement while none of them presented with extra nodal site as shown in Table 1. Only 4 patients (7%) had bulky disease (as large big LN> 10 cm).Concerning IPI score thirty four patients (61.83%) were in intermediate stage Table 1. Medical history confirmed the presence of hypertension in 30 % and diabetes mellitus in 18%.

HSP 70 & HSP 90 in DLBC NHL

Expression of both proteins is high in patient when compared with control (P value=0.001, 0.004 respectively). Their levels are significantly different between limited and advanced stage (P value=0.001) and significantly reduced when compared in case of (after treatment) with those recorded in case of (before treatment) in both limited and advanced patient (P value =0.014 and 0.001 respectively). These levels may be related to stage of disease and effect of treatment as shown as in Table 2 & 3 see Figure 1 & 2.

Table 2: Comparison of HSP 70 and HSP 90 between patients and controls by unpaired T test

Parameters	Patients n=55Mean±SD(Range)	Control n=30Mean±SD(Range)	P value
HSP 70(ng/ml)	2510.91±719.58 (1251-3677)	1939.3±463.2 (1251-3550)	0.001
HSP 90(ng/ml)	2225.25±732.25 (1160-3751)	1849.63±432.83 (1038-3297)	0.004

Table 3: Comparison of HSP 70 and HSP 90 among patients groups by ANOVA

Parameter	Limited stages		Advanced stages		P value
	Before Rxn=3 Mean±SD(Range)	After Rxn=4 Mean±SD(Range)	Before Rxn=16 Mean±SD(Range)	After Rxn=32 Mean±SD(Range)	
HSP 70 (ng/ml)	3125.33±295.81 (3065-3647)	1872.75±623.14 (1344-2766)	3251.5±516.36 (1950-3677)	2144.03±437.01 (1251-2251)	0.001
	0.014		0.001		
HSP 90 (ng/ml)	2862.67±669.48 (2248-3576)	1916.0±437.18 (1358-2425)	3010.81±725.13 (1160-3751)	1811.38±291.85 (1173-2872)	0.001
	0.071		0.001		

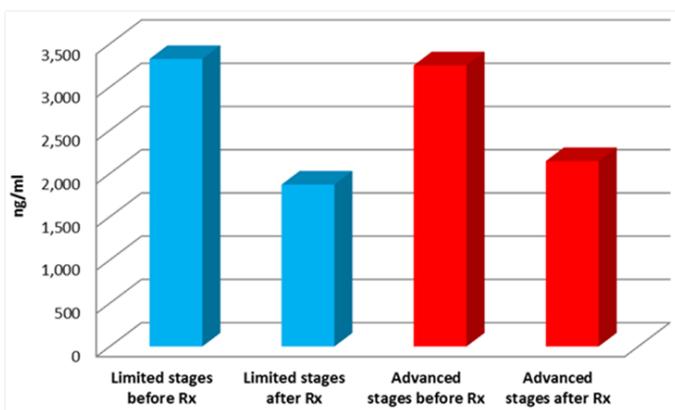


Figure 1 HSP 70 in different patients groups.

Discussion

In this study, it is shown that the mean of level of HSP 70 (ng/ml) in serum of DLBCL patients is significantly higher than its mean level in control group (2510.91±719.58) (1939.3±463.2) respectively which is highly significant (P value=0.001). This result in agreement with previous studies like Gallardo et al.¹⁴ The level of HSP-70 in patients is more than the level of control, that may indicate the role of HSP-70 in maintaining the survival of tumor cells by protection from thermal

oxidative stress² & direct inhibition of apoptosis.⁴ Similarly, HSP-90 shows a statistically significant difference between DLBCL patients in contrast to control group (2225.25±732.25 and 1849.63±432.83ng/ml) with p value 0.004.

Both HSP70 and HSP90 are related to each other and can block the apoptotic pathway at different levels.¹⁵ Normally, HSP90 is expressed at relatively low levels and does not form complexes with other chaperone proteins. However, it may also present in latent form in normal cells. While in process of neoplasia, HSP90 gets higher expression up to 10 folds more, suggesting its role in survival advantage for neoplastic cells.¹⁶

The mean level of HSP-70 and HSP-90 show a statistically significant difference between limited stage and advanced stage (P value = 0.001 and 0.001 respectively). In limited stage DLBCL the effect of treatment showed lower significant difference for both type of HSP 70 and 90 (P value=0.014 and 0.071 respectively) unlike the clear effect of treatment consequences as there is high significant difference in advanced stage diseases (P value=0.001 and 0.001 respectively).

Therefore; persistence HSP70 over expression after end of treatment can provide a selective survival advantage to tumor cells due to its ability to inhibit multiple pathways of cell death and may indicate failure of complete response with chance of progressive disease.¹⁴

The present study is agreement with Cerchietti et al.,¹⁷ which reported that the HSP 90 works in facilitating folding of proteins, thus, the advanced stage is tumor progression which contain large tumor.¹⁷

The result obtained in this study is agreement with previous studies Cerchietti et al.¹⁷ Attempts had started to develop HSP90 inhibitors in order to act by competing with ATP for binding to the N-terminal domain of HSP90.¹⁷ The first inhibitor suitable for clinical trials, 17-allylamino-17-desmethoxygeldanamycin (17-AAG), was shown in preclinical studies to induce apoptosis in cell lines derived from solid and hematologic neoplasms like NHL.¹ The role of HSP90 in tumor genesis has been studied in solid tumors, lymphomas. Increasing suggestion has shown that specific inhibition of HSP90 in neoplastic cells can lead to cell cycle arrest and apoptosis. However, has analytically surveyed HSP90 expression in a variety of NHL types as defined in the WHO classification. The role of HSP90 in tumor genesis has been studied in solid tumors.^{16,18}

Acknowledgements

None.

Conflict of interest

Author declares that there is no conflict of interest.

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