DIAGNOSTIC AND PROGNOSTIC VALUE OF NOTCH-2 MUTATIONS IN DIFFUSE LARGE B CELL LYMPHOMA IN EGYPTIAN PATIENTS INFECTED WITH HEPATITIS C VIRUS GENOTYPE 4

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Introduction

Notch pathway has been reported to have both oncogenic and tumor suppressor roles, dependent on the cancer cell type. It is an evolutionally conserved signaling pathway, consisting a family of transmembrane receptors.These notch receptors will communicate and regulate other cells, which have a specific ligand for their receptors. This highly coordinated signaling system controls many aspects of cell biology including differentiation, proliferation and death.Notch-2 gene mutation causes deregulation of the signaling pathways such as notch2 receptor and IL-2 receptor (Toll- like receptor) these will lead to increased activation of nuclear factor kappa B (NFκB), which leads to increased survival of malignant cells. The notch inhibitors in malignant cells have the potential to slow cell proliferation, cause apoptosis and induce differentiation. These effects are surprisingly given an extensive cross- through of notch with major malignant pathways such as NFκB, Ras and Akt. Notch pathway is a particularly powerful target of tumor stem cell subsets, that is resistant to standard therapy.

Aim of the work

The aim of this study is to determine the diagnostic and prognostic implications of notch-2 gene mutations in hepatitis C virus, genotype4, in diffuse large B cell lymphoma. In addition, the study will evaluate the hepatitis C viral load as well as Ki67 as an indicator of cell proliferation.

Subjects and Methods

Molecular studies for detection of NOTCH-2 gene (exon 34 mutations reference NM. 024408) by PCR amplification and direct Sanger sequencing. Sanger sequencing analysis of Notch-2 gene mutation was done using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Thermo Fisher Scientific Inc.) on Applied Biosystems ABi 3500xL genetic analyzer in 40 newly diagnosed DLBCL patients [HCV positive [n = 20] and HCV negative [n = 20].

Results

The prevalence of Notch-2 gene mutation was higher in the HCV positive DLBCL patients (10%) than their HCV negative counterpart (5%). Notch2 gene mutation was detected in both ABC subtype and GCB subtype [p = 1.000]. Patients with notch2 gene mutation had higher incidence of extranodal involvement, advanced stages disease, and higher IPI score as well as high Ki67 index. Besides, Patients with the notch2 gene mutation revealed an inferior overall response to therapy . In HCV positive group, notch2 gene mutation displayed a positive correlation with the viral load.

Table 1: The rate of Notch2 gene mutation among the HCV positive and HCV negative DLBCL patients

NOTCH 2 gene Mutation	Groups		Test of significance p
	HCV +ve DLBCL (n=20)	HCV -ve DLBCL (n=20)	
Unmutated (n=37) (92.50%)			
- n	18	19	$c^2_{(Y)(df=1)}$=0.000
- % Within Group	90.00%	95.00%	p=1.000 NS
Mutated (n=15) (50.00%)			
- n	2	1	
- % Within Group	10.00%	5.00%	

Table 2: Clinic-pathologic features of mutated notch2 gene among the HCV positive and HCV negative DLBCL patients (n=3)

	Case no.1 (HCV positive)	Case no. 2 (HCV positive)	Case no.3 (HCV negative)
Age	58 years	55 years	51 years
Viral load (IU/ml)	1,247900	762,004	N/A
Hb (g/dl)	10.00	10.00	8.90
Platelet (x10³/µL)	130.00	210.00	269
WBC (x10³/µL)	8.5	4.2	7.5
AST (U/L)	17	20	35
ALT (U/L)	33	22	42
Creatinine (mg/dL)	0.5	0.9	0.8
Bone marrow infiltration	Present	Absent	present
Cell of origin	GCB	GCB	ABC
Ann Arbor stage	IV	I	IV
Zubrod/ ECOG	2	1	2
IPI score	Low intermediate	Low	Low intermediate
Response to therapy	Refractory	Partial response	Partial response

Conclusion

The Notch-2 gene pathway was found to be recurrently mutated in diffuse large B-cell lymphoma (DLBCL). In the present study, mutated notch-2 gene was evaluated as both diagnostic and prognostic relevance to DLBCL. It was found to be associated with bad prognosis and may occur in both GCB and ABC DLBCL subtypes. Besides this notch-2 gene mutation showed a higher expression in HCV positive DLBCL compared to HCV negative group. It occurred obviously in 10% of HCV positive DLBCL as compared to HCV negative DLBCL group of patients (5%). In the HCV positive group, the viral load displayed a positive correlation with Notch2 gene mutational expression. Simultaneous administration of direct acting anti-C virus (DAA) therapy in combination with CHOP disclosed a better response and high tolerability.