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Clinicopathological Significance of A20 Genetic Mutation in Diffuse Large B-Cell Lymphoma

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ABSTRACT

Background: Activation of pathway of nuclear factor kappaB (NF-κB) which is caused by genetic alterations has been reported in B cell lymphoma. A20 gene is considered main regulator component of NF-κB signaling. Its function is mainly suppression of this pathway. Deletions and/or mutations in A20 gene cause inactivation of this pathway which were found in various hematologic malignancies.

Objective: The current study aims to determine the prevalence of A20 gene mutations and their relationship with the clinical and laboratory profile in diffuse large B-cell lymphoma (DLBCL).

Patients and methods: A total 100 DLBCL patients were investigated for A20 gene mutation by real time polymerase chain reaction. **Results:** A20 gene mutation GA mutant genotype was found in 18% of the patients, where 77.8% of them were ABC-DLBCL subtypes. GA heterozygous genotype was frequently associated with stage IV and extra-nodal infiltration, and shorter overall survival (OS).

Conclusion: Our study suggests a role of A20 gene mutation in DLBCL pathogenesis as well as its prognosis.

Keywords: Tumor necrosis factor alpha induced protein3, Nuclear Factor kB, Diffuse large B-cell lymphoma, Case series, Mansoura University.

INTRODUCTION

DLBCL is considered as the most frequent type of B cell lymphoma, representing 40% of non-Hodgkin lymphomas [(1)].

DLBCLis considered a heterogeneous tumor with variable genetic changes, clinical background, treatment outcome and prognosis [(2)]. NF-κB signaling is a very important part for activated B cells diffuse large lymphoma (ABC-DLBCL) to achieve survival [(3)]. NF-κB signaling interruption could induce apoptosis of lymphoma cells [(4)].

Tumor necrosis factor alpha induced protein3 which is also known as (A 20), an enzyme modifying ubiquitin which negatively regulates NF-κB. Somatic mutations in A 20 gene cause inhibition of its function which is seen in most cases of the disease of study [(5)]**.** The incidence of this gene mutation in ABC subtype is significantly increased [(4)]. Thus, the current study aims to determine the prevalence of A20 gene mutation with clinical and laboratory parameters in DLBCL patients besides its role in the prognosis of the disease.

PATIENTS and METHODS

This is an observational cohort study conducted on 100 DLBCL cases. These cases were selected from inpatient and outpatient clinics of Oncology Center Mansoura University from December 2019 to September 2022.

Any DLBCL patients with a history of other cancers or autoimmune diseases were excluded. Complete history taking and staging according to the Rai stage were done for all cases. Laboratory investigations were carried out as CBC and flow cytometry and bone marrow examination for infiltration. Diagnosis of DLBCL was done by IHC according to Hans algorithm [(6)].

DNA extraction:

Fresh lymph nodes were removed from all patients then delivered into a container containing saline to be stored in -80 C until time for genotyping. DNA was extracted from lymph nodes by QIAamp DNA Mini Kit from Qiagen (Catalog No. 51304, Germany) following the instruction of the manufacturer. The DNA quality was confirmed using Nanodrop2000.

Detection of A20 gene mutation was done by taqman SNP genotyping assay real time PCR using master mix preparation (Applied biosystems, lot 00723780, Foster, USA). Sequence-specific primers and two probes for the SNPs were also used amplify the targeted sequence.

The probes for A20 gene: One was labelled with "VIC" dye for the Allele 1 (A) sequence & the other was labelled with "FAM" dye for the other Allele (G) sequence. The SNP ID is rs143002189 and the chromosomal location is Chr6:137881310. The context Sequence [VIC/FAM] was: ACGAATGCTTTCAGTTCAAGCAGAT[A/G]TATGG CTAACCGGAAACAGGTGGGT

Allelic interpretation was performed by computing fluorescence intensity. The analysis of the results were done by "SDS software1.7" (Appllied Biosystem, Foster, USA) and genotyping was determined. Each sample is interpreted according to the 2 alleles & Genotypes (Homozygous or heterozygous).

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Ethical Approval:

This study was ethically approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Mansoura University. Written informed consent was obtained from all participants. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical Analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 20 for windows. Frequencies of genotypes and alleles were assessed by direct counting. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as mean and standard deviation (SD) or median and range. Mann-Whitney test was the test of choice for comparison between groups non-parametric data. Disease-free survival (**DFS**) was detected from the diagnosis date to disease progression date or death. Overall survival (OS) was from the diagnosis date to the last follow-up or death. OS and DFS were calculated using the curve of Kaplan Meier. P value ≤0.05 was considered to be statistically significant.

RESULTS

This study was performed on 100 newly diagnosed lymphoma patients subdivided into 42 females and 58 males, aged from 23 to 75 years with median 57 years attending Oncology Center Mansoura University (OCMU). About 57% of them were GC, as regard disease stage; 71.0% were stage III, extra-nodal involvement detected in 50%, bone marrow (BM) infiltration detected in 28% of cases. Regarding A20 gene mutation, 18% of the patients were GA heterozygous mutant genotype.

Up to 14 out of those 18 patients (77.8%) were ABC-DLBCL and only 4 (22.2%) were GC. Hb was significantly diminished in cases with GA heterozygous genotype versus those with GG wild genotype also, stage IV, and extra-nodal infiltration were significantly more frequent in patients with GA heterozygous genotype versus cases with GG wild genotype (**Tables 1 and 2**).

Table (1): Comparison of clinicopathological characteristics as regard A20 gene genotype

Parameters			GG (n=82)	GA (n=18)	P-value
Age		**Median (Min-Max)**	**57 (30-75)**	**56 (23-75)**	0.726
Gender	**Male**	**Count (%)**	**46 (56.1%)**	**12 (66.7%)**	0.411
	Female		**36 (43.9%)**	**6 (33.3%)**	
	Stage III		**62 (75.6%)**	**9 (50.0%)**	
	Stage IV		**20 (24.4%)**	**7 (38.9%)**	
ECOG score	**<2**		**45 (54.9%)**	**9 (50.0%)**	0.707
	≥2		**37 (45.1%)**	**9 (50.0%)**	
IPI	**Low-risk**		**2 (2.4%)**	**0 (0.0%)**	**0.010**
	Low-intermediate risk		**68 (82.9%)**	**9 (50.0%)**	
	High-intermediate risk		**11 (13.4%)**	**8 (44.4%)**	
	Higher risk		**1 (1.3%)**	**1 (5.6%)**	
Extra-nodal involvement			**37 (45.1%)**	**13 (72.2%)**	**0.037**
B symptoms			**34 (41.5%)**	**11 (61.1%)**	0.129

Significant (P-value <0.05).

Table (2): Comparison of laboratory findings as regard A20 gene genotypes.

Parameters			GG (n=82)	GA (n=18)	P-value
WBCS $\times 10^9$/L		**Median (Min-Max)**	**6.2 (2.2-102.0)**	**6.6 (2.6-102.0)**	0.683
Hemoglobin g/dl		**Median (Min-Max)**	**12.9 (6.5-13.9)**	**10.6 (7.0-13.5)**	**0.010**
PLT $\times 10^9$/L		**Median (Min-Max)**	**169.0 (55-412)**	**146.0 (85-233)**	0.805
LDH		**Median (Min-Max)**	**263.0 (160-1548)**	**297.5 (197-797)**	0.229
Type	**GC**	**Count (%)**	**39 (47.6%)**	**4 (22.2%)**	0.066
	Non-GC	**Count (%)**	**43 (52.4%)**	**14 (77.8%)**	
BM infiltration		**Count (%)**	**22 (26.8%)**	**6 (33.3%)**	0.578

Significant (P value ≤0.05).

Regarding survival analysis correlation with gene A20 mutation the follow-up time across the entire 24 months, a significant correlation was found in OS **(P=0.045)** and DFS **(P<0.001)** with patients of GA mutant genotype (**Figure 1)**.

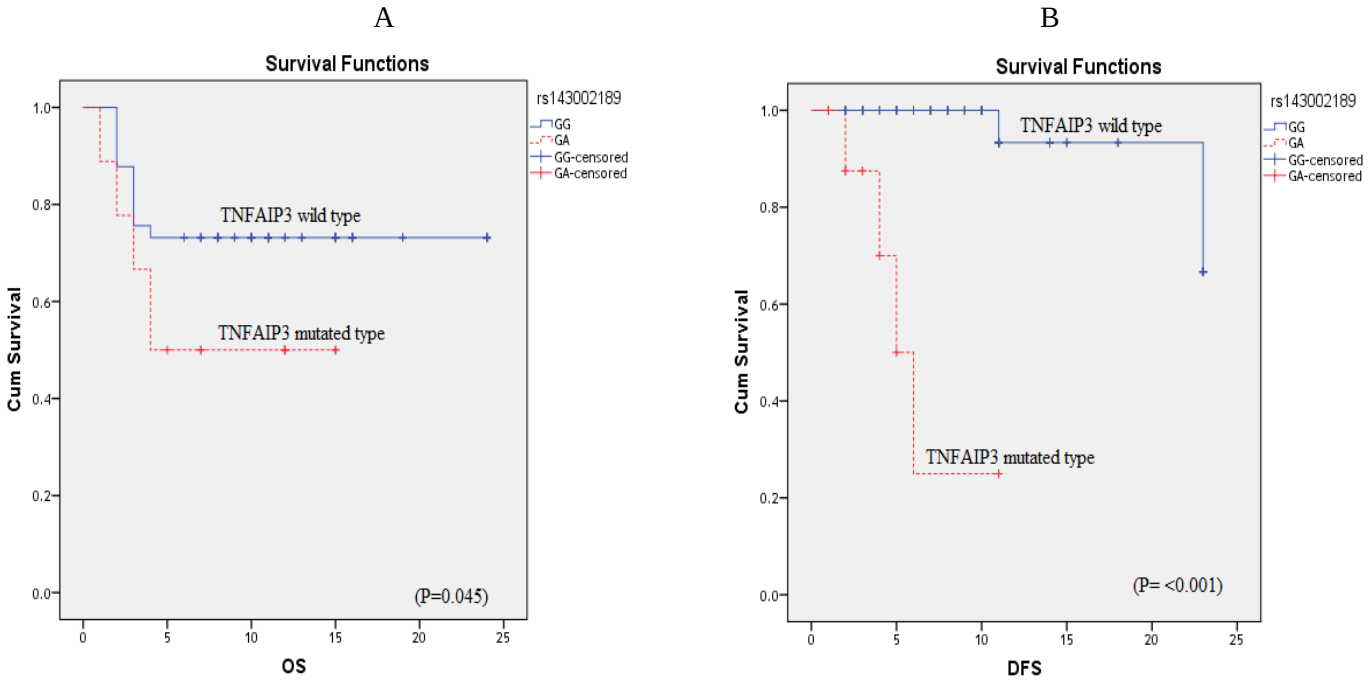

Figure (1): Kaplan-Meier survival curves for A 20 gene mutation in DLBCL. (A) Overall survival A20 gene in lymphoma patients. (B) Disease-free survival of A20 gene, OS and DFS time is represented in months.

DISCUSSION

The role of A 20 as a functioning tumor suppressor gene has been studied in many B cell lymphoma types and sub-types. Inactivation of A20 gene by methylation process of the promoter sequence, gene-deletion different mechanisms and also mutation was found in NHLs [7].

A 20 gene mutations through the studied pathway are related to a wide spectrum of diseases of humans as MALT lymphoma of the salivary gland, DLBCL, and primary mediastinal B-cell lymphoma [8]. Besides that, A20 is associated with other diseases of autoimmune pathogenesis as systemic lupus erythromatosus which significantly is in increased association with the B cell lymphoma [8].

Most of A 20 somatic mutations in B cell lymphoma are found to be frameshift, nonsense besides missense changes. These changes cause inhibition of its activity which leads to hyper-activation of the NF-κB pathway [8].

We found in our study that frequency for A20 gene mutation GA genotype was found in 18% of patients. Stage IV, higher IPI groups, and extra-nodal infiltration were significantly more frequent in GA genotype. We found that this mutation was associated with a shorter OS and DFS.

Rosenquist *et al.* [9] performed a genetic study by sequencing on 224 DLBCL tissue samples and found A20 gene mutation in 6% of patients. Also, **Cao *et al.*** [10] found that this mutation was 7.14% of 196 cases.

Wenzl *et al.* [1] found a higher incidence of A20 mutation (2.97%) in DLBCL patients considering an effect of this mutation in the lymphogenesis in DLBCL cases.

Another study by **Dubois *et al.*** [11] found that A20 gene mutation presented in 17% of cases where 15% of them are ABC origin and 11% are GC-DLBC. This study also showed a significant association of A20 gene mutation and shorter OS**.**

In contrast to our study, **Cen *et al.*** [4] revealed that A20 mutation occurs more frequently (29%) of 67 DLBCL in ABC subtype. But no difference in PFS and OS was related to A 20 mutations. Besides to that most of coding exons of A20 gene analysis was done by **Montsino *et al.*** [12] and found that mutations in 20% of extranodal DLBCL did not differ from nodal DLBCL. One mutation was detected in 20% (2/10) of extranodal DLBCL. They found that extranodal DLBCL showed inhibiting mutations for A 20 gene, roughly equivalent to nodal DLBCL.

Bu and collaborators [13] analyzed the mutations in A20 in the 150 DLBCL cases and found the mutation in 4.6% of 150 DLBCL samples. However, A20 gene mutation was not correlated with overall survival**.** This difference may be related to different numbers of cases and techniques.

Paik *et al.* [14] revealed that high IPI expression were independent prognostic factors, while A 20 mutation was not a prognostic factor.

CONCLUSION

A20 gene mutation has a higher incidence in DLBCL. It can be guessed that this mutation has an effect on B cell lymphoma induction in patients with DLBCL. This mutation also could be one of the factors for poor prognosis in DLBCL cases.

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- **Availability of data**: The paper itself contains the data sets that are used to support the findings.
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