THE STUDY OF LONG NON-CODING RNAs (NAALADL2 AND XIST) GENE EXPRESSION IN DIFFUSE LARGE B CELL LYMPHOMA

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ABSTRACT

Diffuse large B cell lymphoma (DLBCL) is the most predominant type of Non Hodgkin- Lymphomas, representing more than one third of all recently diagnosed cases. Long non-coding RNAs (lncRNAs) are a class of RNAs not coding for protein having more than 200 nucleotides and have significant roles in tumor formation and they have been investigated in different types of solid and hematologic tumors, including DLBCL. This work aims to study the level of lncRNAs (NAALADL2 and XIST) gene expression in DLBCL. NAALADL2 and XIST gene expression levels in blood were performed by real time qPCR technique. Forty five DLBCL patients and forty five age and gender matched healthy controls were included in this study. There was significant statistical increase in the expression of NAALADL2 and decrease of XIST expression in patients compared to control group (p value < 0.001). From this study, ROC curve analysis revealed cut-off values >10.3 and <1.57 for NAALADL2 and XIST respectively. There was significant positive correlation between NAALADL2 and advanced stage and a significant negative correlation is found between XIST and advanced stage. It may be concluded that NAALADL2 and XIST expression levels were obviously associated with DLBCL and might determine the disease progression.

Keywords: DLBCL; lncRNAs (NAALADL2 and XIST); real-time PCR.

*This was taken from thesis submitted to partial fulfillment of MSc degree in Medical Biochemistry by Fatma Ahmed Mohammed.

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most well-known hematologic tumor in adults representing 30–40% of all non-Hodgkin lymphoma cases worldwide (Swerdlow et al., 2016). DLBCL is found to be a destructive and complex disease having various clinical, phenotypic and molecular forms (Pan et al., 2015).

Patients with DLBCL typically present with a painless rapidly growing mass, which may be nodal or extranodal. The nodal sites may be cervical, axillary, inguinal/femoral regions and are more common than extranodal sites (Lopez-Guillermo et al., 2005).

There are two types of DLBCL according to genetic profiles; germinal center B-cell-like (GCB) and activated B-cell-like (ABC) (Rosenwald et al., 2002). Tumors having a germinal center phenotype have remarkably better and favorable prognosis than those with the other phenotype (Scottet al., 2014).

Long noncoding RNA (lncRNA) is a subtype of noncoding RNA and has in excess of 200 nucleotides in length and has significant biological functions (Kung et al., 2013). In latest researches, lncRNA has been shown to be included in chromosomal silencing in cells of tumor, chromatin modification, genomic imprinting and transcription regulation (Mathias et al., 2019).

The N-acetyl-L-aspartyl-L-glutamate peptidase I (NAALADase) family, which is otherwise called glutamate carboxypeptidase II and N-acetylaspartylglutamate (NAAG) peptidase are described by that they can act as M28 membrane metalloproteases catalyzing NAAG hydrolysis to glutamate and N-acetylaspartate (Mesters et al., 2006).

NAALADL2 which is one of the members of NAALADase family was found to be among the most up regulated lncRNAs in clinical samples from DLBCL patients (Zhu et al., 2017).

The lncRNA X-inactive specific transcript (XIST) which is a XIST gene product, recently have been found differentially expressed in multiple malignancies as non-small cell lung carcinoma and gastric carcinoma (Chen et al., 2016).

The aim of this work is to assess the level of lncRNA (NAALADL2 and XIST) gene expression and evaluate their relations with DLBCL.
MATERIALS AND METHODS
This study was achieved by cooperation between Medical Biochemistry & Molecular Biology department and Clinical Oncology department, Faculty of Medicine, Menoufia University. Patients selected from Clinical Oncology department, Menoufia University Hospital in the period from August 2018 to February 2019. DLBCL patients were diagnosed by tissue biopsy: lymph node, surgical excisional biopsy of any peripheral accessible lesion or image guided tissue biopsy of any inaccessible lesion, any other diagnostic procedure as upper gastrointestinal endoscopic biopsy or bone marrow biopsy was acceptable (Freedman et al., 2015).

An informed written consent was given by every subject enrolled in the study. The protocol approval was done by the Ethical Committee of Medical Research, Faculty of Medicine, Menoufia University. Ninety (90) subjects were enrolled in the study; they were 45 DLBCL patients (Group I); 23 males and 22 females with mean age X ± SD was 52.6 ± 12.6 and 45 age and gender matched healthy controls (Group II); 17 males and 28 females with mean age of 50.1 ± 13.9.

Exclusion criteria: Patients with personal history of other types of cancer and Patients with associated major comorbidities were excluded from this study.

The sample size was calculated using Epi Info (2000) program depending on previous studies (Chen et al., 2018) and we need to study 45 cases and 45 controls with 1 control per a case. The sample size was calculated at power 85%, 95% CI and alpha margin of error is 5%.

All studied subjects underwent: Full history taking and General clinical examination. Abdominal ultrasound and CT-scan were done for all cases, Metastatic work up: chest x-ray and bone scan, performance status was estimated based on Eastern Cooperative Oncology Group (ECOG) classification and International Prognostic Index (IPI) for DLBCL (Foussard et al., 1997). Tumor staging depended on Ann Arbor staging system (Cheson et al., 2014).

Samples collection: Ten milliliters (10 ml) of venous blood were withdrawn from every subject and distributed as follows: in a plain tube, 3 ml were put and left to clot for 30 minutes at room temperature then centrifugation was done for 10 minutes at 4000 r.p.m. The resulting serum was separated and stored at -80°C till determination of serum liver enzymes, urea and creatinine, 2 ml of blood were put in an
EDTA tube for complete blood count (CBC), 1.6 ml of blood into a tube containing 0.4 ml of sodium citrate for ESR, 3 ml blood was transferred into plain tube for determination of serum LDH and B2M and the remaining of blood were delivered in a vacutainer plastic tube containing EDTA for detection of NAALADL2 and XIST expression by RT-PCR.

**Assay methods:** Laboratory investigations (CBC counting in whole blood sample (EDTA tube) with Pentra-80 automated blood counter (ABX–Franc–Paris Euromedicine-BP-7290.34184 Montpellier-Cedex 4), ALT and AST using kinetic UV optimized method IFCC (LTEC Kit, England) and renal function tests (urea and creatinine) via colorimetric techniques using (DIAMOND diagnostics kits, Germany) were performed, serum lactate dehydrogenase (LDH) measurement by LDH (P-L) kit (Mod. IFCC method), β2 microglobulin was measured by Human beta 2-Microglobulin ELISA Kit from GenWay Biotech, Inc (United States).

**Detection & quantitation of long non coding RNA (NAALADL2 and XIST) gene expression:**
Long non coding RNA was first successfully isolated from whole blood by utilizing miRNeasy Mini Kit (cat. no. 217004), Qiagen, Germany. The purified long non coding RNA product was put away at −80 °C for reverse transcription step. Reverse transcription (RT) was done to isolated long non coding RNA for single-stranded cDNA synthesis utilizing Kit given by (Thermofisher Scientific, Applied Biosystem).

The cDNA Reverse Transcription reaction was prepared10 ul of 2x RT master mix was placed into each tube and 10 ng/ul of RNA sample, pipetting was up and down two times to mix. The RT master mix contained 10x Reverse Transcription Buffer (2.0 ul), 100 mM dNTPs with dTTP (0.8 ul), RNase inhibitor (1.0 ul), MultiScribe™ Reverse Transcriptase (1.0 ul), 10x RT random primers (2 ul) and nuclease-free water (3.2 ul)]. The thermal cycler 2720 Singapore was adjusted to run long non coding RNA RT reaction (10 min for primer annealing at 25 °C, 120 min at 37 °C, 5 min at 85 °C for inactivation and 4 °C for ∞) for one cycle. Storage of the RT product was done at −20 °C for real-time PCR step.

The PCR reaction plate was setup by apportioning 20 μL of the entire PCR master mix (10μl Syber green master mix with low ROX dye; Thermo fisher Scientific), 1 ul Forward Primer of both target and
reference gene, 1 ul reverse primer of both target and reference gene, 10 ng/ul of RT product and 5 ul RNase-free water and using β-actin gene as an endogenous reference gene. **Primers** were supplied by (Invitrogen): Sequence of primers of NAALADL2 Forward primer: GAGCCAACCTGGGATAAAGAA, Reverse primer: AGGAAGGCAACTGTCACTCT. Primers of **XIST** (F: GGATGTCAAAAAGATCGGCCC, R: GTCTGCAGGTCTCACATGCT) primers of β-actin (F: AGCGAGCATCCCCCAAGTT; R: GGGCACGAAGGCTCATCATT) (Zhu et al., 2017).

Specificity of the primers was verified using Primer BLAST program provided by NCBI. The Real Time PCR instrument, Applied Biosystems® 7500 thermal cycler (Applied Biosystems, Foster City, USA) parameters were first PCR initial denaturation at 95 °C for 10 min followed by 50 cycles (denaturation at 95 °C for 15 sec then annealing at 60°C for 1 minute then extension at 72°C).

Analysis of data was done by utilizing Comparative ΔΔ Ct Cycle threshold technique for relative quantitation RQ (Livak and Schmittgen, 2001), where measurement of target (NAALADL2 and XIST) are standardized to housekeeping gene (β-actin) and comparative to control (Fig. 1), Melting curve was done to validate the specific generation of the expected PCR product and to avoid primers dimmers (Fig. 2 and Fig. 3).

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**Fig. 1:** Amplification plot of NAALADL2 and XIST
Fig. 2: Melting curve of NAALADL2  Fig. 3: Melting curve of XIST

**Statistical analysis:** The gathered data was tabulated and studied using SPSS (statistical package for the social science) software version 20. To verify the distribution normality, Kolmogorov-Smirnov test was applied. Chi-square test is applied to study the two qualitative variables relationship. Student’s t test was utilized to assess the statistical significance of parametric data. Mann Whitney test was used for quantitative variables which are abnormally distributed, to compare between two studied groups. P values are considered significant when they are less than 0.05.

**RESULTS**

The results show a non-significant statistical difference among the two studied groups as regard age, gender, H pylori infection while a significant statistical difference is found among the studied groups as regard BMI, farmer job, HCV infection and smoking (Table 1). There is non-significant statistical difference between the two studied groups regarding ALT, AST, urea and creatinine, while there is significant statistical difference in Hb, PLT, TLC, LDH, ESR and B2 microglobulin (Table 2). There is increase of NAALADL2 and decrease of XIST expression in patients group compared to the controls group (Table 3). Regarding the clinicopathological factors, 40% of patients group had performance status 0 and performance status 1 and 2 were 33.3%, 26.7%
respectively, 44.4% presented with extra nodal site, 40% of patients had positive BSymptoms, 44.4% of patients were presented with early stage, 55.6% of patients were presented with advanced stage, 40% of cases were in low risk, 88.9% of DLBCL patients were alive and 73.3% of cases are not progressed (Table 4).

It revealed that the best cutoff point for NAALADL2 is >10.3 and for XIST is <1.57. NAALADL2 had a diagnostic sensitivity of 84.44%, specificity of 82.22% (*P < 0.001), while XIST had a diagnostic sensitivity of 95.56%, specificity of 86.67% (*P < 0.001) (Table 5).

The ROC curve was applied to assess the diagnostic utility of NAALADL2 and XIST lncRNA expression in control versus DLBCL lymphoma patients (Fig. 4).

As regard the relation between mortality and NAALADL2 and XIST expression level; NAALADL2 gene expression was significantly increased and XIST expression significantly decreased in patients with dead fate than live one and also NAALADL2 expression significantly increased in patients with bad prognosis than patients with good one, while there was no significant decrease of XIST gene expression in patients with bad prognosis (Table 6).

There was significant statistical increase in NAALADL2 relative expression level in DLBCL patient with stage III and IV compared with I or II and decrease in XIST relative expression level in DLBCL patient with stage III and IV compared with I or II stage (Table 7).

There was significant statistical increase in NAALADL2 relative expression level in DLBCL patient with high IPI score, while there was no significant statistical decrease in XIST relative expression level in DLBCL patient with high IPI score (Table 8).

It was revealed that the mean overall survival of DLBCL cases was 16.956 months and median 18 months with 95% confidence interval 16.07 – 17.84. Furthermore, it was found that the mean Progression Free Survival of DLBCL cases was 14.556 months and median 18 with 95% confidence interval 12.87 – 16.24 (Fig. 5).

There was significant positive correlation between NAALADL2 relative expression level in DLBCL group and LDH level and there was significant statistical negative correlation between XIST expression level in DLBCL group and stage of the tumor (Fig. 6).
Table (1): Demographic characteristics of the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Patient(n=45)</th>
<th>Control(n=45)</th>
<th>Test of Sig.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23(51.1%)</td>
<td>17(37.8%)</td>
<td>(\chi^2 = 1.620)</td>
<td>0.203</td>
</tr>
<tr>
<td>Female</td>
<td>22(48.9%)</td>
<td>28(62.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td>(t=0.874)</td>
<td>0.385</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>52.6 ± 12.6</td>
<td>50.1 ± 13.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI(kg/m2)</td>
<td></td>
<td></td>
<td>(t=3.414^*)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>27 ± 3.6</td>
<td>30 ± 4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>30(66.7%)</td>
<td>5(11.1%)</td>
<td>(\chi^2=29.221^*)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>H pylori</td>
<td>13(28.9%)</td>
<td>7(15.6%)</td>
<td>(\chi^2=2.314)</td>
<td>0.128</td>
</tr>
<tr>
<td>Smoking</td>
<td>10(22.2%)</td>
<td>2(4.4%)</td>
<td>(\chi^2=6.154^*)</td>
<td>0.013*</td>
</tr>
</tbody>
</table>

\(\chi^2\): Chi square test, \(t\): Student t-test, * p less than 0.05 is statistically significant, BMI: body mass index, HCV: hepatitis c virus, H Pylori: helicobacter pylori

Table (2): Laboratory data of the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Patient(n=45)</th>
<th>Control(n=45)</th>
<th>Test of Sig.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT(U/L)</td>
<td></td>
<td></td>
<td>(t=829.0)</td>
<td>0.138</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>29.4 ± 7.8</td>
<td>27.2 ± 7.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST(U/L)</td>
<td></td>
<td></td>
<td>(t=826.0)</td>
<td>0.131</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>32 ± 7.8</td>
<td>29.5 ± 8.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea(mg/dl)</td>
<td></td>
<td></td>
<td>(t=1.260)</td>
<td>0.211</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>34.8 ± 6.3</td>
<td>32.3 ± 4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine(mg/dl)</td>
<td></td>
<td></td>
<td>(U=878.50)</td>
<td>0.266</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>1 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td></td>
<td></td>
<td>(t=4.691^*)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>10.6 ± 1.3</td>
<td>11.8 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLT(Thousands/cmm)</td>
<td></td>
<td></td>
<td>(U=704.5^*)</td>
<td>0.013*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>255.1 ± 110.9</td>
<td>304.6 ± 51.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLC(Thousands/cmm)</td>
<td></td>
<td></td>
<td>(t=2.550^*)</td>
<td>0.013*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>6.5 ± 2</td>
<td>7.4 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH level(U/L)</td>
<td></td>
<td></td>
<td>(U=51.0^*)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>356.1 ± 169.4</td>
<td>157.6 ± 35.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR(mm/hr)</td>
<td></td>
<td></td>
<td>(t=11.860^*)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>90.3 ± 37.2</td>
<td>24 ± 4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2MICR(mg/L)</td>
<td></td>
<td></td>
<td>(U=44.0^*)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean ± SD.</td>
<td>5.7 ± 3.4</td>
<td>2.1 ± 0.5</td>
<td></td>
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</tr>
</tbody>
</table>

THE STUDY OF LONG NON-CODING RNAs (NAALADL2 ........

Table (3): Statistical comparison of NAALADL2 and XIST expressions between both studied groups

<table>
<thead>
<tr>
<th></th>
<th>Patient (n=45)</th>
<th>Control (n=45)</th>
<th>Test of Sig.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAALADL2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Min. – Max.)</td>
<td>23.8 (8.1 – 54.4)</td>
<td>2.5 (1 – 12.9)</td>
<td>U=56.0*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>XIST</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Min. – Max.)</td>
<td>0.5 (0.01 – 2.2)</td>
<td>7.5 (0.4 – 12.8)</td>
<td>U=68.50*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

U: Mann Whitney test, NAALADL2: N-acetylated alpha-linked acidic dipeptidase-like2, XIST: X-inactive specific transcript

Table (4): Distribution of the cases according to clinicopathological factors in DLBCL

<table>
<thead>
<tr>
<th>No., (%)</th>
<th>Performance status</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18 (40%)</td>
</tr>
<tr>
<td>1</td>
<td>15 (33.3%)</td>
</tr>
<tr>
<td>2</td>
<td>12 (26.7%)</td>
</tr>
<tr>
<td>Median (Min. – Max.)</td>
<td>1 (0 – 2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extra nodal site</th>
<th>No., (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Stomach</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Lung</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Testis</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Parotid</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Intestine</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Suprarenal</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Brain</td>
<td>1 (5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Positive B symptoms</th>
<th>No., (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18 (40%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage</th>
<th>No., (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2 (4.4%)</td>
</tr>
<tr>
<td>II</td>
<td>18 (40%)</td>
</tr>
<tr>
<td>III</td>
<td>17 (37.8%)</td>
</tr>
<tr>
<td>IV</td>
<td>8 (17.8%)</td>
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<table>
<thead>
<tr>
<th>IPI score</th>
<th>No., (%)</th>
</tr>
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<tbody>
<tr>
<td>0-1 (low risk)</td>
<td>18 (40%)</td>
</tr>
<tr>
<td>2-3 (intermediate risk)</td>
<td>19 (42.3%)</td>
</tr>
<tr>
<td>4 (high risk)</td>
<td>8 (17.7%)</td>
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</table>

<table>
<thead>
<tr>
<th>Patient fate</th>
<th>No., (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live</td>
<td>40 (88.9%)</td>
</tr>
<tr>
<td>Dead</td>
<td>5 (11.1%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progression</th>
<th>No., (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not progressed</td>
<td>33 (73.3%)</td>
</tr>
<tr>
<td>Progressed</td>
<td>12 (26.7%)</td>
</tr>
</tbody>
</table>

IPI: international prognostic index
Table (5): Performance characteristics of NAALADL2 and XIST in differentiating between DLBCL patients and controls

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>P</th>
<th>95% C.I.</th>
<th>Cut off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
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<tbody>
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</tr>
<tr>
<td>NAALADL2</td>
<td>.972*</td>
<td>&lt;0.001*</td>
<td>.946</td>
<td>.999</td>
<td>&gt;10.3</td>
<td>84.44</td>
<td>82.22</td>
<td>82.6</td>
</tr>
<tr>
<td>XIST</td>
<td>0.966</td>
<td>&lt;0.001*</td>
<td>0.934</td>
<td>0.998</td>
<td>&lt;1.57</td>
<td>95.56</td>
<td>86.67</td>
<td>87.8</td>
</tr>
</tbody>
</table>

AUC: Area Under the Curve, PPV: positive predictive value, NPV: negative predictive value

Table (6): Relation between mortality and progression and NAALADL2 and XIST in patients group

<table>
<thead>
<tr>
<th></th>
<th>Mortality</th>
<th>Progression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Live (n = 40)</td>
<td>Dead (n = 5)</td>
</tr>
<tr>
<td>NAALADL2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median(Min. – Max.)</td>
<td>21.5(8.1 – 42.2)</td>
<td>36.7(35.3 – 54.4)</td>
</tr>
<tr>
<td>U(p)</td>
<td>8.0* (&lt;0.001*)</td>
<td>40.0* (&lt;0.001*)</td>
</tr>
<tr>
<td>XIST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median(Min. – Max.)</td>
<td>0.6(0.01 – 2.2)</td>
<td>0.04(0.01 – 0.4)</td>
</tr>
<tr>
<td>U(p)</td>
<td>42.50* (0.035*)</td>
<td>186.0(0.771)</td>
</tr>
</tbody>
</table>


Table (7): Relation between stage and NAALADL2 and XIST in patients group

<table>
<thead>
<tr>
<th></th>
<th>Stage</th>
<th>U</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I – II (n = 20)</td>
<td>III – IV (n = 25)</td>
<td></td>
</tr>
<tr>
<td>NAALADL2</td>
<td>Median (Min. – Max.)</td>
<td>19(8.2 – 28.4)</td>
<td>27.1(8.1 – 54.4)</td>
</tr>
<tr>
<td>XIST</td>
<td>Median (Min. – Max.)</td>
<td>1.04(0.01 – 2.2)</td>
<td>0.10(0.01 – 1.7)</td>
</tr>
</tbody>
</table>

U: Mann Whitney test, NAALADL2: N-acetylated alpha-linked acidic dipeptidase –like2, XIST: X-inactive specific transcript
Table (8): Relation between IPI and NAALADL2 and XIST in patients

<table>
<thead>
<tr>
<th></th>
<th>IPI</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (0 – 1)</td>
<td>Intermediate (2 – 3)</td>
<td>High (4 – 5)</td>
<td>H</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>(n= 18)</td>
<td>(n= 19)</td>
<td>(n= 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAALADL2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Min. – Max.)</td>
<td>15.6 (8.1 – 27.7)</td>
<td>25.8 (10.3 – 48.8)</td>
<td>32.8 (24.3 – 54.4)</td>
<td>22.436*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>XIST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Min. – Max.)</td>
<td>0.6 (0.01 – 1.5)</td>
<td>0.5 (0.02 – 2.2)</td>
<td>0.1 (0.01 – 1.6)</td>
<td>1.691</td>
<td>0.429</td>
</tr>
</tbody>
</table>

H: H for Kruskal Wallis test, IPI: international prognostic index

Fig. 4: A. ROC curve of NAALADL2. B. ROC curve of XIST
Fig. 5: A. Overall survival of DLBCL patients B. Progression free survival of DLBCL patients

Fig. 6: A. Correlation between LDH level and NAALADL2 B. Correlation between Stage and XIST.

DISCUSSION

DLBCL represents one-third of all NHL cases and this makes it the commonest subtype of NHL between adults (Tilly et al., 2015). IncRNA contains more than 200 nucleotides and its mechanism in health and diseases has been evaluated (Li et al., 2018). There are only a few studies about IncRNAs expression in DLBCL or their association with DLBCL clinical characteristics and prognosis (Bartonicek et al., 2016). It has been recommended that IncRNAs may play a vital role in the chromosome breaks involved in typical gene rearrangements in hematologic malignancies (Lu et al., 2015).

NAALADL2 which is a member of NAALADase family was found to create a microenvironment easy for migration and metastasis.
and increased tumor expression of NAALADL2 is related to poor survival (Whitaker et al., 2014).

LncRNA X-inactive specific transcript (XIST) that is a XIST gene product was found to be responsible for control of mammalian X inactivation, and its gene transcription only occurs from the inactive X chromosome (Tantai et al., 2015).

The current study showed that, matching of age and gender between cases and controls group was achieved and also showed predominance of male gender 51.1% than female gender 48.9% of DLBCL cases and DLBCL was common in old age with mean age 52.56±12.64 years.

These results come in agreement with those reported by (Tobias and Hochhauser, 2015) who reported that, old age increases the risk for lymphoma overall. Also they reported that, the risk of DLBCL is greater in males than in females.

The present study showed that, there is significant increased rate of DLBCL in farmers (40% of cases were farmers) and these results agree with those reported by (Hohenadel et al., 2011) who reported that DLBCL incidence increase with pesticide exposure which commonly occurs in farmers.

The present study revealed that, a significant statistical increased rate of smoking in cases than control and these results agree with Cerhan et al., (2014) who showed that, an increased risk of DLBCL was associated with smoking.

Cigarette smoking may possibly affect survival of DLBCL via many mechanisms comprising a direct carcinogen effect; carcinogens activation through metabolizing enzymes induction; effect on immunologic function, involving increasing pro-inflammatory cytokines and bcl-2 overexpression which can inhibit apoptosis (Gritz et al., 2008).

The present study showed that, a significant statistical increased rate of hepatitis C virus infection (HCV) in cases than control, 66.7% of cases were HCV positive patients and this was in accordance with (Khaled et al., 2017) who stated that, DLBCL lymphoma is intensely associated with HCV. On the other hand, Kaya et al., (2002) suggest that HCV is not a causative factor in the pathogenesis of NHL.
The current study reported that, there is a significant decrease in hemoglobin, platelets and white blood cells count in DLBCL patients when compared to control group. Naoum (2016) agreed with our results. Kim et al.,(2012) reported that thrombocytopenia affect results of survival in DLBCL patients having bone marrow metastasis. This study showed that, there is a highly significant increase in ESR, $\beta_2$ microglobulin (B2MG) and LDH in DLBCL patients when compared with the controls group. Oana et al., (2018), reported that, high ESR is a prognostic factor in NonHodgkin’s disease and also has been shown to predict gastrointestinal lymphomas survival. Ji et al.,(2018) reported that serum LDH is an ideal tumor marker in DLBCL patients. Jiang et al., (2016), showed that the serum B2MG level has been extensively assessed for its prognostic value in several hematological conditions.

This study found that there was highly significant statistical increase of NAALADL2 relative expression level in DLBCL patients compared to control group. This was in accordance with Gao et al., (2017) who reported that NAALADL2 was up regulated in DLBCL and also was found to be involved in p53 (a tumor suppressor gene), NF-kB pathway and JAK/STAT signaling pathways. This study show significant increased mortality rate and progression of tumor among patients with higher NAALADL2 expression when compared to the rest of patients. Whitaker et al., (2014) agree with our results and reported that NAALADL2 adjust the expression of CAMP-binding protein of cellular processes implicated in malignant growth and development. Taylor et al., (2010) reported that increased NAALADL2 was associated with a significantly lower 5-year survival.

The present study showed a significant statistical decrease of XIST relative expression level in DLBCL patients compared to control. These results are agreed by Richard and Ogawa, (2016) who found that XIST has been downregulated in DLBCL to certain degree. XIST has been shown to decrease HCC spread and metastasis by regulating XIST/miR-181a/PTEN signaling pathway and increase the E-cadherin (an EMT-induced marker) expression (Chang et al.,2017).

The present study revealed that that there was significant increased mortality rate among patients with lower XIST expression when compared with the rest of patients. These come in agreement with Ma.
et al., (2017) who found that down regulation of XIST was related to poor prognosis in HCC patients. Du et al., (2017) revealed that IncXIST was decreased in prostate cancer and decreased expression of XIST was associated with unfavorable outcome.

The overall survival is significantly higher among the cases with lower NAALADL2 expression values than cases with higher expression values and survival significantly higher among the cases with higher XIST expression values than cases with lower expression values. Kobayashi et al., (2016) detected that the 4-year overall survival rates of cervical squamous cell carcinoma patients were 87.1% and 54.4% in the high and low XIST expression groups respectively.

Ma et al., (2017) revealed that XIST act as a tumor suppressor gene and decreased its expression in the tissues of HCC or cervical cell carcinoma was correlated with lower overall survival (OS).

Conclusions: Overall, LncRNA (NAALADL2 and XIST) were obviously related to DLBCL and might predict the outcome of this disease.

REFERENCES


الملخص العربي

دراسة الحمض النووي الريبوزي الطويل الغير مشفر (نالدال 2 وزيست) في ليمفوما الخلايا البائية الكبيرة المنتشرة

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تعد ليمفوما الخلايا البائية الكبيرة المنتشرة أكثر أنواع الميمفوما غير Hodgkinية انتشارًا حيث تمثل ٣٠-٤٠% من الحالات حديثة التشخيص. يعد الحمض النووي الريبوزي الطويل الغير مشفر أحد أنواع الأحماض النووية التي تفتقد شفرة لتكوين البروتين ويصل طوله إلى أكثر من ١٠٠ نوكليوتيد وله دور مهم في حدوث وتطور الأورام وقد تم دراسته في العديد من الأورام الدموية ومنها ليمفوما الخلايا البائية الكبيرة المنتشرة. 

الهدف من البحث: هو دراسة تعبير الحامض النووي الريبوزي الطويل الغير مشفر (نالدال 2 وزيست) في ليمفوما الخلايا البائية الكبيرة المنتشرة. وتم إجراء الدراسة على ٥٤ حالة مصاب بسرطان ليمفوما الخلايا البائية الكبيرة المنتشرة و٥٥ شخص كمجموعة ضابطة. وهذه المجموعات قد خضعت لاختبارات عمي عينات الدم وذلك لتقدير التعبير عن الحامض النووي الريبوزي الطويل الغير مشفر (نالدال 2 و زيست) عن طريق تفاعل البممرة المسمول. وقد أظهرت النتائج: أن هناك زيادة ذات دلالة إحصائية في تعبير (نالدال) على طريقة تفاعل اللمیرة المسلسل وكانت ٣٣.٣% لليمندال ٢ و ٣٣.٣% لزيست. ونقص ذو دلالة إحصائية في تعبير (زيست) في مرضى ليمفوما الخلايا البائية الكبيرة المنتشرة.خفض نتيجة الاصحاء. وقد أوضح منحني روك أن نقطة القطع لليمندال ١ هو الحد المورث > ٥٤، والزيست > ٥٤. ووجد أن هناك علاقة طردية بين مستوى التعبير الجيني للليمندال ٢ والمراحل المتقدمة من المرض، وأن هناك علاقة عكسية بين مستوى التعبير الجيني لزيست والمراحل المتقدمة من المرض. الإستنتاج: من الواضح أن مستوى التعبير الجيني (ليمندال ٢ وزيست) له علاقة بليفو الميمفوما الخلايا البائية الكبيرة المنتشرة. يمكن أن يحدد تطور هذا المرض.

Fatma A Mohammed et all

38