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THE STUDY OFLONG NON-CODING RNAs(NAALADL2 AND XIST) GENEEXPRESSION IN DIFFUSE LARGE B CELL LYMPHOMA

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ABSTRACT

Diffuse large B cell lymphoma (DLBCL) is themost predominant type ofNon Hodgkin- Lymphomas,representingmore than one thirdof all recently diagnosedcases.Long non-coding RNAs (lncRNAs) are a class of RNAnot coding for proteinhaving more than 200 nucleotides and have significant roles in tumor formationand they have been investigated in different types of solid and hematologic tumors, including DLBCL.This work aims to studythe level of lncRNAs*(NAALADL2 and XIST)*gene expression in DLBCL.NAALADL2 and XIST geneexpression levelsin 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 expressionin 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 XISTrespectively.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 XISTexpression 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-knownhematologic tumor in adults representing 30–40% of all non-Hodgkin lymphoma cases worldwide (**Swerdlow et al., 2016**).DLBCL is found to bedestructive and complex disease havingvariousclinical,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 sitesmay 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 togenetic profiles; germinal center B-cell-like (GCB) and activated B-cell-like (ABC) **(Rosenwald et al., 2002)**.Tumors having a germinal center phenotype haveremarkablybetter and favorable prognosis than those with theother phenotype**(Scottet al.,2014).**

Long noncoding RNA (lncRNA) is a subtype of noncodingRNA andhas in excess of 200 nucleotidesinlengthandhassignificant biological functions**(Kung et al.,2013).**In latest researches, lncRNA has been shown to be included in chromosomalsilencing 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) familywhichis otherwise called glutamate carboxypeptidase II and N-acetylaspartylglutamate (NAAG) peptidase are described by that theycan 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 familywas found to be amongthe most up regulated lncRNAs in clinical samples from DLBCL patients**(Zhu et al., 2017).**

ThelncRNAX-inactivespecific transcript (XIST)which is a*XIST* gene product, recently have been found differentially expressed inmultiple malignancies as non-small cell lung carcinomaandgastric carcinoma**(chen et al.,2016).**

The aim of this work is to assess the level ofLncRNA(*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 givenby every subject enrolled in the study. The protocol approvalwas 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 study45 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 underwentto: 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 onEastern 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,**3ml** were put and left to clot for 30 minutes at room temperature then centrifugation was donefor 10 minutesat 4000 r.p.m.The resulting serumwas 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 Euromedecine-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).β_2 microglobulin wasmeasured 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 utilizingmiRNeasy Mini Kit (cat. no. 217004), Qiagen, Germany. The purified long non coding RNA productwas put away at −80 °C for reverse transcription step. Reverse transcription (RT) was done toisolated 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 °Cfor inactivationand 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 wassetup 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 reversePrimer of both target and reference gene, 10 ng/ul of RT product and 5ul RNase-free water and using**β-actin** gene as an endogenous reference gene.

Primers were supplied by (Invitrogen):Sequenceof primers of*NAALADL2*Forward primer: GAGCCAACTGGGATAAAGAA,Reverse primer:AGGAAGGCAACTGTCACTCT**.**primers of ***XIST***(**F**: GGATGTCAAAAGATCGGCCC, **R**:GTCCTCAGGTCTCACATGCT) primers of***β-actin*** (**F**:AGCGAGCATCCCCCAAAGTT:,**R**:GGGCACGAAGGCTCATC ATT) **(Zhu et al.,2017)**.Specificity of the primers was verified using Primer BLAST program provided by NCBI. The Real Time PCRinstrument, Applied Biosystems®7500 thermal cycler (Applied Biosystems, Foster City, USA) parameters were first PCR initial denaturation at 95 °C for 10 min followed by50 cycles (denaturation at 95 °C for 15 sec then annealing at 60°C for1 minute then extension at 72°C).

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

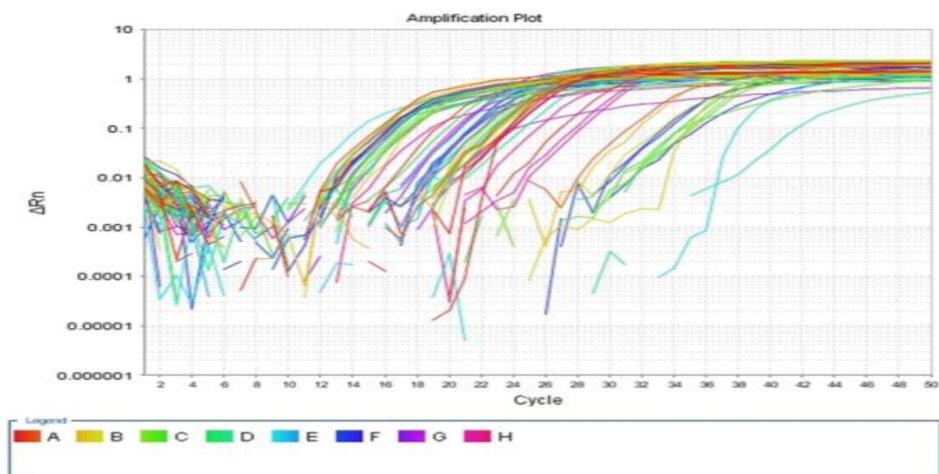
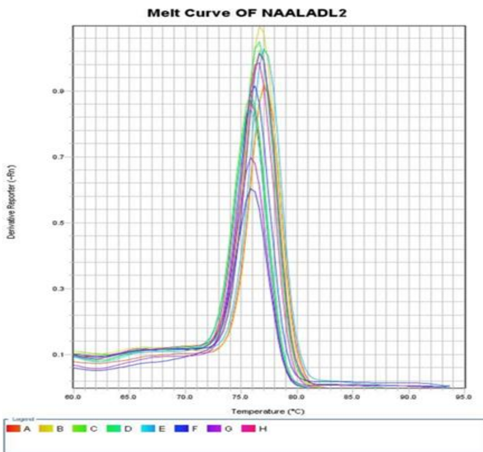


Fig. 1: Amplification plot of NAALADL2 and XIST



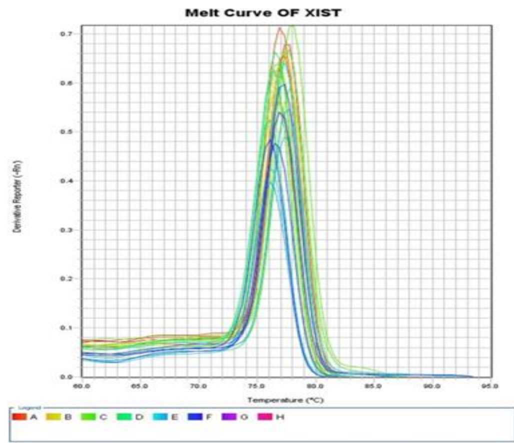


Fig.2: Melting curve of NAALADL2 **Fig.3**: Melting curve of XIST

Statistical analysis: The gathered datawas tabulated and studiedusingSPSS (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 thetwo qualitative variablesrelationship. 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 amongthe two studied groups as regard age, gender, H pylori infectionwhile a significant statistical difference is foundamongthe studied groups as regardBMI, 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 inHb, 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).**

Regardingthe 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 hadpositive 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 versusDLBCL lymphoma patients **(Fig. 4)**.

As regard the relation between mortality and*NAALADL2* and *XIST* expression level; NAALADL2 gene expression wassignificantly 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 stageIII and IV compared with I or II and decrease in *XIST* relative expression level in DLBCL patient with stage III and IV compared withIor 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 IPIscore**(Table8).**

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 thatthemean Progression Free Survival of DLBCL caseswas 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

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

χ^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

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

t: Student t-test, U: Mann Whitney test, ALT: alanine transaminase, AST: aspartate transaminase, Hb: hemoglobin, PLT: platelets, TLC: total leucocytic count LDH: lactate dehydrogenase, ESR: erythrocyte sedimentation rate, B2MICR: B2microglobulin

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

	Patient(n=45)	Control(n=45)	Test of Sig.	P
NAALADL2 Median (Min. – Max.)	23.8(8.1 – 54.4)	2.5(1 – 12.9)	U=56.0*	<0.001*
XIST Median (Min. – Max.)	0.5(0.01 – 2.2)	7.5(0.4 – 12.8)	U=68.50*	<0.001*

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

	No.(%)
Performance status	
0	18(40%)
1	15(33.3%)
2	12(26.7%)
Median (Min. – Max.)	1(0 – 2)
Extra nodal site	20(44.4%)
Liver	3(15%)
Stomach	8(40%)
Lung	2(10%)
Testis	1(5%)
Parotid	1(5%)
Intestine	2(10%)
Suprarenal	2(10%)
Brain	1(5%)
Positive B symptoms	18(40%)
Stage	
I	2(4.4%)
II	18(40%)
III	17(37.8%)
IV	8(17.8%)
IPI score	
0-1(low risk)	18(40%)
2-3 (intermediate risk)	19(42.3%)
4 (high risk)	8(17.7%)
Patient fate	
Live	40(88.9%)
Dead	5(11.1%)
Progression	
Not progressed	33(73.3%)
Progressed	12(26.7%)

IPI:international prognostic index

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

	AUC	P	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
NAALADL2	.972*	<0.001*	.946	.999	>10.3	84.44	82.22	82.6	84.1
XIST	0.966	<0.001*	0.934	0.998	<1.57	95.56	86.67	87.8	95.1

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

	Mortality		Progression	
	Live (n = 40)	Dead (n = 5)	No (n = 33)	Yes (n = 12)
NAALADL2				
Median(Min. – Max.)	21.5(8.1 – 42.2)	36.7(35.3 –54.4)	20(8.1 – 36.4)	35.6(21.5 – 54.4)
U(p)	8.0*(<0.001*)		40.0*(<0.001*)	
XIST				
Median(Min. – Max.)	0.6(0.01 – 2.2)	0.04(0.01 – 0.4)	0.5(0.01 – 2.2)	0.3(0.01 – 1.6)
U(p)	42.50*(0.035*)		186.0(0.771)	

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

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

	Stage		U	P
	I – II (n = 20)	III – IV (n = 25)		
NAALADL2				
Median (Min. – Max.)	19(8.2 – 28.4)	27.1(8.1 – 54.4)	110.50*	0.001*
XIST				
Median (Min. – Max.)	1.04(0.01 – 2.2)	0.10(0.01 – 1.7)	119.50*	0.003*

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

Table (8):Relation betweenIPI and NAALADL2 and XIST in patients

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

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

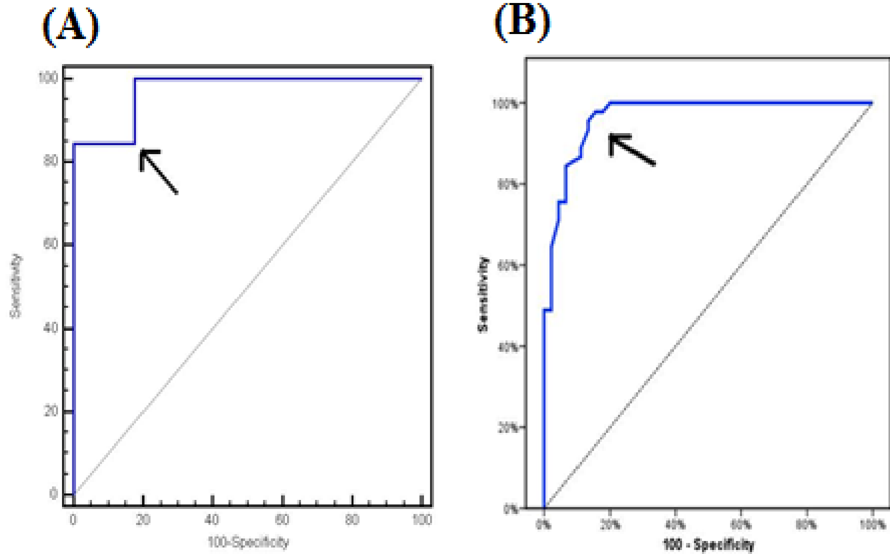


Fig. 4: A. Roc curve of NAALADL2. B. ROC curve of XIST

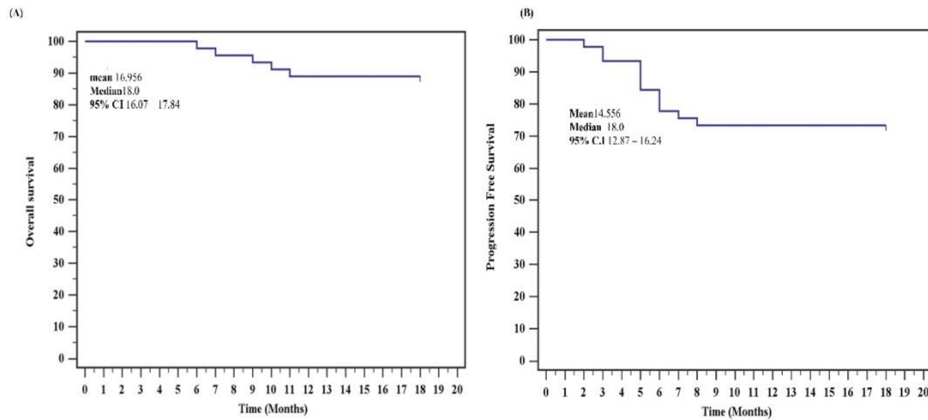


Fig.5:A. Overall survival of DLBCL patientsB. Progression free survival of DLBCL patients

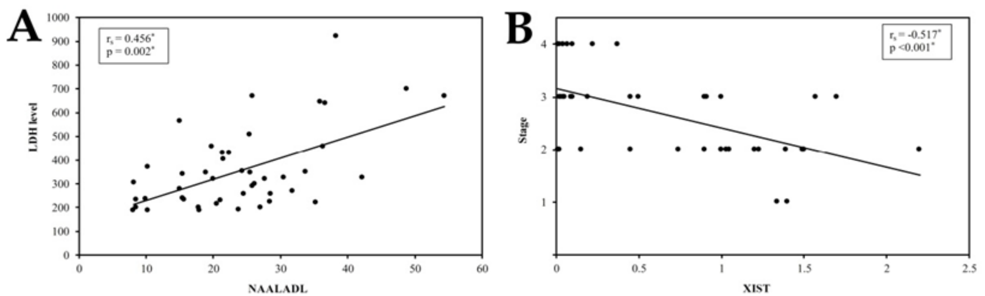


Fig.6:A.Correlation between LDH level and NAALADL2B.Correlation between Stage and XIST.

DISSCUSION

DLBCLrepresentsone-third of all NHL cases and this make it the commonestsubtype of NHL between adults**(Tilly et al.,2015)**.lncRNAcontains more than 200 nucleotides and its mechanism in health and diseases has beenevaluated**(Li et al., 2018).** There are only a few studiesabout lncRNAs expressionin DLBCL or theirassociationwithDLBCL clinical characteristics and prognosis **(Bartonicek et al.,2016).**It has been recommended that lncRNAs 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 wasfound to createa microenvironmenteasy for migration and metastasis

andincreased tumor expression ofNAALADL2 isrelated to poor survival **(Whitaker et al., 2014).**

LncRNA X-inactive specific transcript (XIST) that isa XIST gene product was found to be responsible forcontrol ofmammalian X inactivation, and its gene transcriptiononly 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 achievedand 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 increase the risk for lymphoma overall. Also they reported that, the risk ofDLBCL 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.**Kimet 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, β_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 markerin 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 levelin DLBCL patients compared to control group.This was in accordance with**Gao et al., (2017)**who reportedthat*NAALADL2* was up regulated in DLBCL and alsowas 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**)reportedthat increased *NAALADL2*was associated with a significantly lower 5-year survival.

The present study showeda significant statistical decrease of XIST relative expression level inDLBCL 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 todecrease 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)whofound that down regulation of XIST was related to poor prognosis in HCC patients.**Du et al., (2017)**revealedthat lncXIST was decreased in prostate cancer and decreased expression of XIST was associated with unfavorableoutcome.

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 thancases 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 andlow XIST expression groups respectively.

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

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

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الملخص العربى

دراسة الحمض النووي الريبوزي الطويل الغير مشفر (نالدال٢ و زيست) في ليمفوما الخلايا البائية الكبيرة المنتشرة

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قسمى[١]الكيمياءالحيوية الطبية والبيولوجيا الجزيئية، [٢]علاج الأورام والطب النووي كلية الطب- جامعةالمنوفية

تعد ليمفوما الخلايا البائية الكبيرة المنتشرة أكثر أنواع الليمفوما الغير هودجكينية انتشارا حيث تمثل ٣٠-٤٠% من الحالات حديثة التشخيص. يعد الحمض النووي الريبوزي الطويل الغير مشفر أحد أنواع الاحماض النووية التي تفتقد شفرة لتكوين البروتين ويصل طوله الي أكثر من ٢٠٠ نيوكليتيدة 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 دراسه تعبير الحامض النووى الريبوزي الطويل الغير مشفر(نالدال٢ وزيست) في ليمفوما الخلايا البائية الكبيرة المنتشرة. وتم اجراء الدراسة علي ٤٥ حالة مصابهبسرطان ليمفوما الخلايا البائية الكبيرة المنتشرة و٤٥ شخص كمجموعه ضابطة.وهذه المجموعات قد خضعت لاختبارات معملية علي عينات الدم وذلك لتقييم التعبير عن الحامض النووي الريبوزي الطويل الغير مشفر(نالدال٢ و زيست)عن طريق تفاعل البلمرة المسلسل.**وقد أظهرت النتائج:**ان هناك زيادة ذات دلالة إحصائية في التعبيرالجيني لل(نالدال٢) ونقص ذو دلالة إحصائية في التعبيرالجيني لل(زيست) في مرضي ليمفوما الخلايا البائية الكبيرة المنتشرة بالمقارنة مع مجموعه الاصحاء.وقد أوضح منحني روك أن نقطة القطع للنالدال ٢هي>١٠.٣وللزيست<١.٥٧.وقد وجد ان هناك علاقة طردية بين مستوي التعبير الجيني للنالدال٢ والمراحل المتقدمة من المرض وان هناك علاقة عكسية بين مستوي التعبير الجيني للزيست والمراحل المتقدمة من المرض. **الإستنتاج**: من الواضح ان مستوي التعبير الجيني (نالدال٢) و(زيست) له علاقةبليمفوما الخلاياالبائية الكبيرة المنتشرةكما يمكن ان يحدد تطور هذا المرض.