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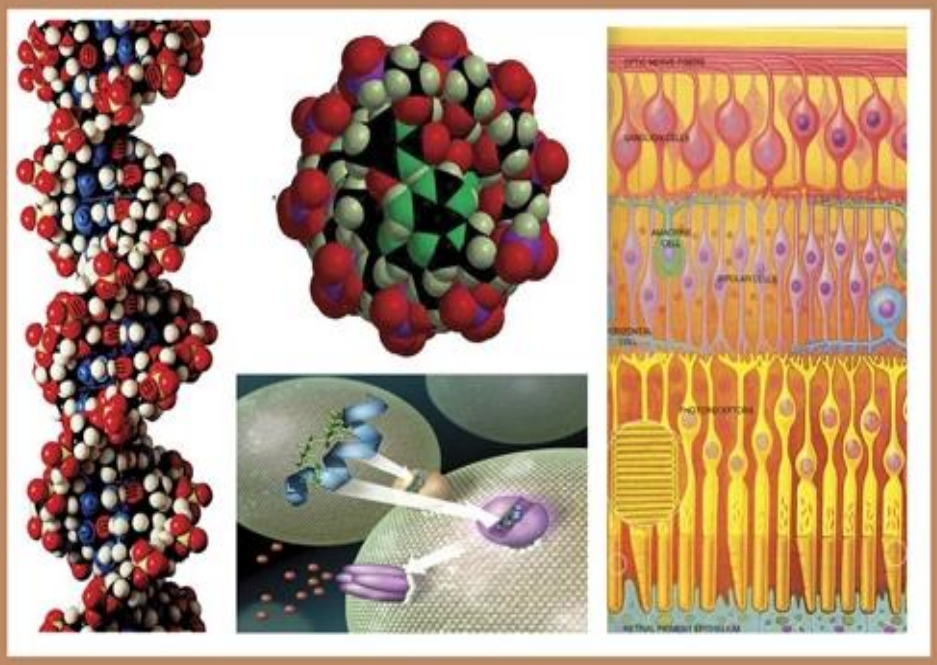

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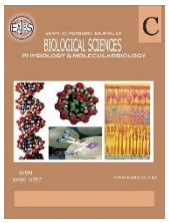

Molecular Evaluation of Long Non-Coding RNA (TUG1) in Systemic Lupus Erythematosus

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ABSTRACT

Long non-coding RNAs play an important role in systemic lupus erythematosus. Our work purpose is to evaluate the level of LNC (TUG1) in SLE patients. This research includes 20 control and 70 SLE subjects. The serum level of LNC (TUG1) was measured by polymerase chain reaction. Down-expression of LNC (TUG1) was observed in SLE patients more than in control. A significant AUC of TUG1 level was recorded, which recommended that (TUG1) can act as a new biomarker for SLE determination.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic immune-related disorder described by a lack of tolerance to self-antigens and the over-secretion of autoantibodies against host DNA and other cellular elements. This disorder mostly affects females of reproductive age. SLE has a broad range of clinical manifestations that affect several organs. Approximately 50% of SLE cases will have serious and life-threatening complications such as nephritis, vasculitis, pulmonary hypertension, interstitial lung disorder and cerebral stroke (Ramos *et al.*,2010).

Long non-coding RNAs (lncRNAs) are a group of RNAs with a length of longer than 200 nt and do not encode any proteins (Xiao *et al.,* 2018).

In addition to having key roles in acquired and innate immunity, lncRNAs are also implicated in the differentiation and activation of immune cells and in the regulation of the development of SLE (Wu *et al.,* 2015).

Taurine up-regulated gene 1 (TUG1) has a length of approximately 7.1 kb and is located on human chromosome 22q12.2 (Niu *et al.,* 2017).

MATERIALS AND METHODS

Study Population and Samples Collection:

This study contained 20 control and 70 sick people with SLE, samples were collected from all members and took 3 ml of blood and handled into serum by using a centrifuge at 1500 xg for ten minutes. The aliquots of samples were put away at −80°C until utilize. In addition, another blood sample was collected in an EDTA tube for other biochemical examinations.

Laboratory Investigations:

Erythrocyte sedimentation rate (ESR), using Westergren's method. Complete blood count measured by hematology analyzer, Anticardiolipin antibodies (Acl-IgG) and C reactive protein (CRP) were done quantitatively with ELISA technique.

Isolation of LNC-TUG1:

The miRNeasy Mini Kit (Qiagen, Valencia, CA, USA) was used to isolate the LNC-RNA from the serum samples according to the manufacturer's instructions of the kit. Reverse transcription (RT) was carried out on total RNA, RT reactions using the miScript II RT kit, according to the manufacturer's instructions of the kit.

Quantitative Real-time PCR for Detection of LNC-TUG1:

This step was carried out using miScript SYBR® Green PCR kit and protocol for LNC-TUG1 by using real-time. The forward and reverse primers of used RNAs: long non-coding (TUG1) (5′-TAGCAGTTCCCCAATCCTTG-3′) forward and (5′- CACAAATTCCCATCATTCCC-3′) reverse, (GAPDH) for quantification of (TUG1) and the forward primer of (GAPDH) is (5′-CCCACTCCTCCACCTTTGAC -3′) and reverse primer is (5′-TGGTCCAGGGGTCTTACTCC -3′) (Rashad *et al.*, 2022).

The mixture measurement for LNC-TUG1 by PCR technique contained 20 microliters, which consisting of 10 microliters of 2xQuantiTect SYBR Green PCR Master Mix, one microliter (TUG1-primer) and 2.5 microliters of template complementary DNA, with RNase-free water 6.5 microliters. The cycling conditions were: 95°C for ten minutes, followed by forty-five cycles of 95°C for fifteen seconds, finally forty-five cycles of 60°C for sixty seconds, and finally forty cycles of 70°C for thirty seconds. Data analysis is calculated by mathematical equations.

Statistical Analysis:

Data analysis was done by SPSS, version 17 analyzed as (mean ± SD). comparison between groups was done by using a t-test and one-way ANOVA. The correlation coefficient (r) was determined to estimate correlations among numerical variables.

RESULTS

I. Laboratory Parameters:

A. Blood Picture:

The mean total leucocyte count (TLC) for control was 11.17±7.93 $x10^3$/mL, while that for SLE patients was 19.91±47.32 $x10^3$/mL. No statistical difference in total leucocyte count ($p>0.05$) among both groups (Table 1).

The mean platelet count (Plt) for control was 296.69±95.62 $x10^3$/mL, and that for SLE patients was 263.002±118.46 $x10^3$/mL. No statistical difference in platelet count *($p= 0.33$)* among both groups (Table 1).

B. Erythrocyte Sedimentation Rate:

The mean ESR for the control was 48.09±31.68 mm/hr, while that for SLE patient group was 57.27±42.76 mm/hr. P value of ESR between both groups *($p=0.43$)* (Table 1).

C. C Reactive Protein:

The mean (CRP) value for patients with SLE was 25.88±29.16 mg/dL, while that for control was 4.88±3.99 mg/dL. P value is not significant for (CRP) *($p=0.14$)* between the two groups (Table 1).

Table 1: Laboratory parameters for both SLE patient groups.

Parameter		Unit	SLE patients (n=70)	control (n=20)	P value
TLC	Mean ± SD	$x10^3$/mL	11.17±7.93	19.91±47.32	0.31(NS)
Plt	Mean ± SD	$x10^3$/mL	296.69±95.62	263.002±118.46	0.23(NS)
ESR	Mean ± SD	mm/hr	48.09±31.68	57.27±42.76	0.53(NS)
CRP	Mean ± SD	mg/dL	25.88±29.16	4.88±3.99	0.24(NS)

NS: not significant.

LNC-TUG1:

The mean overall of long non-coding (TUG1) For SLE (n=70), was 0.476±0.32 points and control (n=20) was 0.63±0.25 points. No statistical difference for (TUG1) between the two groups (p=0.075) (Table 2).

Table 2: TUG1 level in SLE and control groups.

parameter	SLE patients (n=70)	Control (n=20)	P value
(TUG1) Mean ± SD	0.476±0.32	0.63±0.25	0.075(NS)

NS: not significant. **: highly significant.

Table 3: Correlation of laboratory and diagnostic parameters in SLE patients.

		C3	CRP	C4	ESR	TLC	TUG1
CRP	**r**	-0.53					
	p	0.04*					
C4	**r**	+0.55	+0.34				
	p	0.003**	0.196				
ESR	**r**	-0.66	+0.11	-0.5			
	p	<0.001**	0.63	0.006**			
TLC	**r**	-0.29	-0.05	-0.12	+0.13		
	p	0.14	0.83	0.55	0.38		
TUG1	**r**	-0.23	+0.63	-0.14	-0.12	-0.08	
	p	0.23	0.002**	0.49	0.43	0.59	

*: significant. **: highly significant.

In all systemic lupus patients, there was a negative correlation among C3 and CRP (r= -0.53) *(p=0.04)* and significant P value, and also a negative correlation among C3 and ESR (r= -0.66) *(p<0.001)* with highly significant P value, while a positive correlation among C3 and C4 (r= +0.55) *(p=0.003)*. (Table 3)

In all systemic lupus patients, there was a positive correlation between CRP and TUG1 (r= +0.63) *(p=0.002)*, There was a negative correlation among C4 and ESR (r= -0.5) *(p=0.006)* and P value was significant. (Table 3).

DISCUSSION

Systemic lupus erythematosus is a complicated autoimmune illness; it may be mortal when accompanied by specific organ harm or damage. There are a lot of factors, which may be the cause of SLE appearance and development such as genetic factors, hormonal and other reasons (Alexander *et al.*, 2015). There are a lot of features of SLE disease such as more production of B and T lymphocytes, which lead to the overproduction of autoantibodies and then the accumulation of these autoantibodies inside different tissues causing organ harm (Giles *et al.*, 2013). Lupus-nephritis is one of the most dangerous developments of SLE and around 40% of lupus patients developed lupus nephritis (Hoover *et al.*, 2016).

Approximately 10% of lupus nephritis suffer from irreversible kidney deterioration and end-stage kidney illness. Long-term misdiagnosis can be the cause of lupus nephritis, which is a serious complication and is the cause of a four-fold increase in mortality among lupus erythematosus patients (Pakchotanon *et al.,* 2018).

LNC-RNAs are a cluster of RNAs that have a length of more than 200 nucleotides, and do not translate to protein (Xiao *et al.,* 2018). As well as, long non-coding RNAs having important functions in acquired and innate immunity, LNC-RNAs also play an important role in the differentiation and activation of many immune cells and the development of lupus erythematosus (Wu *et al.,* 2015).

The aim of this study was to recognize the ability of TUG1 in SLE patients. In this work, we have illustrated the down-regulation of LNC-TUG1 in systemic erythematosus patients against healthy people among the Egyptian populace.

Our study is harmonic with those of (Cao et al., 2020) who illustrated the down-expression of LNC-TUG1 in lupus erythematosus patients against control, and it is expression was more decreased in lupus-nephritis (P <0 .05).

Also, (Zhang *et al.,* 2018) detailed that the expression of LNC-TUG1 in LPS-induced damage of myocardial cells was very low, while over-expression of it may be able to moderate that damage.

In addition, the other study reported that LNC-TUG1 expression in liver cells undergoes reduction when uncovered to cold situations, whereas LNC-TUG1 over-expression can reduce cell apoptosis, oxidative stress and irritation, in this manner securing liver cells from damage that occur by cold conditions (Su *et al.,* 2016). In addition, there is another study that illustrated that LNC-TUG1 can decrease the level of pro-inflammatory cytokines in lipopolysaccharides which activate osteogenic arthritis, and also decrease cell apoptosis (Liang *et al.,* 2018).

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