LNCRNA LY86-AS1 EXPRESSION ON T2DM AGED 40 YEARS OVER IN MUNGGU SOCIETY

Ayu Saka Laksmita W
Bali International University, Denpasar, Indonesia
sakalaksmita@gmail.com

Ni Putu Widayanti
Bali International University, Denpasar, Indonesia
wida.yantisp@gmail.com

Desak Putu Risky Vidika Apriyanthi
Bali International University, Denpasar, Indonesia
riskyvidika@gmail.com

I G K Nyoman Arijana
Department of Histology Faculty of Medicine Udayana University, Denpasar, Indonesia
nyomanarijana@yahoo.com

Abstract

Diabetes mellitus type 2 (T2DM) is the most common health problem found in almost all over the world. T2DM sufferers need long-term care and treatment to prolong life and improve their life quality. Long noncoding RNA (lncRNA) is a part of noncoding RNA that was recently created with various human diseases. This research aims to explore the analysis of lncRNA LY86-AS1 as a diagnostic marker for T2DM. This study is quantitative descriptive using simple random sampling technique. Blood samples were taken from 10 respondents of Munggu society. Extracted RNA was used to synthesize complementary DNA with Reverse Transcription Kit. Quantitative real-time PCR has doneby SYBR Green. PCR analysis was performed with

quantitative analysis by conducting 10 samples. The result found was the decrease in lncRNA LY86-AS1 expression. From this it can be concluded the possibility of using lncRNA LY86-AS1 for detecting diagnostic markers of T2DM sufferers for Munggu society.

Keywords

Long Non-Coding, lncRNA LY86-AS1, Diabetes, T2DM

1. Introduction

Diabetes milletus is one of problem the highest number of sufferers worldwide. According to data from the World Diabetes Federation, there were 194 million DM sufferers worldwide in 2009 and there were 8.4 million DM sufferers in Indonesia. Diabetes mellitus has become the fourth largest cause of death in the world. Among several types of diabetes, type 2 diabetes mellitus (T2DM) is the most common diabetes found in patients compared to other types of diabetes, because almost 90% of all diabetes cases are T2DM (Gonzales et al., 2009). Blood glucose will increase with age. The number of people with T2DM is increasing worldwide, especially in developing countries due to genetic factors, demographic factors (increasing population, urbanization, age over 40 years) and lifestyle change factors (Kusnadi et al., 2017). The risk of disease is determined by a combination of genetic, environmental and behavioral factors (Sun and Wong 2016). T2DM results from interactions between genetics and the environment (Olokoba et al., 2012).

According to World Health Organization projection, the prevalence of diabetes increase by 35%. Diabetes Mellitus is a major health problem throughout the world and is the third most common disease in the world next to cardiovascular diasease and oncological diseases (Sankhari, 2019). In recent years, large-scale Genome-Wide Association Studies identified around 80 Single Nucleotide Polymorphisms related to T2DM (Billings and Jose, 2010). Long non-coding RNA transcript has a length of more than 200 nucleotides but does not encode a protein (Mao et al., 2015). It is well known that lncRNAs shows potential as a direct target for therapeutic interventions in liver disease (Takhashi, 2014). In Sri Lanka, it was found that one in five adults are diabeties (Senevirathne et al., 2015).

Efforts to detect early T2DM by molecular examination in Indonesia have not been done much. Along with the aging process, more and more elderly people are at risk for the occurrence of T2DM (Nugroho, 2009). DM in the elderly is generally asymptomatic, although there are symptoms often in the form of non-typical symptoms such as weakness, lethargy, changes in

behavior, decreased cognitive status or functional ability. This causes the diagnosis of T2DM in the elderly rather late (Kurniawan, 2010). Because of this background, a research is needed to determine the pathogenesis indication of T2DM by conducting research on long non-coding RNA (lncRNA) gene specifically the lncRNA LY86-AS1 target gene at the age of 40 years and over, as an early detection of the pathogenesis of T2DM disease in Munggu society.

2. Material and Methods

2.1 Design and Participants

This study used 10 probandus from T2DM patients in Munggu village. The Probandus are 40 years old and above. The selected probandus are those who regularly go to Mengwi II Health Center as outpatients. Probandus were first given a briefing and explanation of the purpose of the research and the benefits of the research for both the probandus and the community in general (informed consent). If they agree, a blood sample is taken. Sampling is based on inclusion and exclusion criteria. Inclusion criteria used were respondents diagnosed with T2DM, with a minimum duration of 2 months, men and women over 40 years of age, able to communicate verbally well and were willing to become research respondents. Meanwhile, the exclusion criteria were sufferers of T2DM which is comorbid with other diseases.

2.2 Methodology Sample

Sampling in this study is using a simple random sampling technique that is determining the sample of the study by selecting respondents DMT2 patients who visited the Puskesmas Mengwi II according to the random lottery method so that the sample can represent the characteristics of the population of Munggu village that had been known previously. This research uses the Slouin formula, namely:

$$n = \frac{N}{1 + N \cdot e^2}$$

n: Number of samples

N: Total population

e: error (0.05)

Based on the data obtained, obtained a total population of 11 people. Therefore, the study will continue to use a sample of 10 out of 11 respondents representing the local population. The existence of technological advances to detect molecular T2DM disease with RNA long non-

coding, it was concluded that the possibility of using LY86-AS1 lncRNA can detect diagnostic markers of T2DM sufferers for the Munggu community in particular. The limitation of this research is the lack of research funding from the government so that the samples used are not many.

2.3 Sampling Technique

Blood samples were taken from 10 probandus. They were taken from each probandus using a 3 ml EDTA tube. Homogenization of the tissue was carried out, and TRI-zol was added. The TRI-zol LS reagent complies with the manufacturer's protocol. Briefly, after administration of TRI-zol, chloroform was added to the sample and the sample was centrifuged at $12,000 \times g$ for 15 minutes. Quantitative real-time PCR technique was performed in which RNA was extracted to synthesize complementary DNA with a Reverse Transcription kit. Quantitative real-time PCR was carried out by SYBR Green. Fluorescence data was measured using Real Time thermocycler (MiniOpticon BioRad).

2.4 Ethical Approval

This study was approved by the Ethics Commission of the Faculty of Medicine Udayana University (No: 2550 / UN14.2.2.VII.14 / LP / 2019).

3. Results and Discussion

3.1 Sampling Result

This study used 10 blood samples from people with type 2 diabetes in Munggu village. Expression of LY86-AS1 in patients T2DM was carried out by quantification of gene expression. It was done through real time PCR using comparative CT method which uses a mathematical formula to calculate differences in the level of expression in target genes (Schmittgen & Livak, 2008) (Table 2). In relative gene quantification, changes in the level of relative expression (fold change) > 1 indicates an increase in expression; conversely, if the change in expression level < 1, the expression declines. Number 1 value is the quantity agreed upon for determining target genes (Agilent Technologies, 2012).

Table 1: *Primer Sequences*

Gene	Forward	Reverse
LY86-AS1	TGGAGAGCAAGAACTATAGGAGGA	TTGACCAGACTACAGACATAGCAC

Table 2: *Concentration of Expression of Target Gene Determination*

SAMPLE	SAMPLE CT	lncRNA (ng/ul)	lncRNA (pg/ul)
1	16.44681404	0.1678505038	167.85
2	18.52386191	0.9749434374	974.94
3	18.2774493	1.1243256959	1124.33
4	19.31042397	0.6185157474	618.52
5	18.22665943	1.1578529558	1157.85
6	17.84384165	1.4449032938	1444.90
7	17.4187288	1.8477833703	1847.78
8	18.89959539	0.7844658166	784.47
9	18.11060646	1.2382617189	1238.26
10	20.45618953	0.3187645135	318.76

Cq : 16.446814044444697
Dye : SYBR Green I
Position : 1
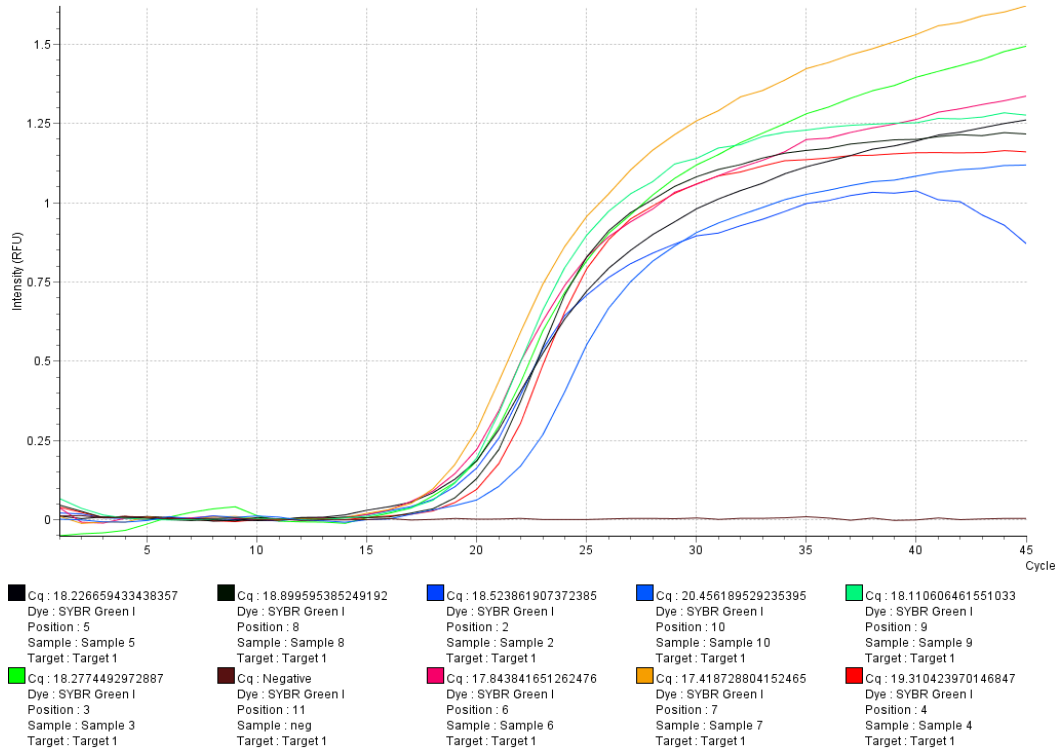Sample : Sample 1
Target : Target 1
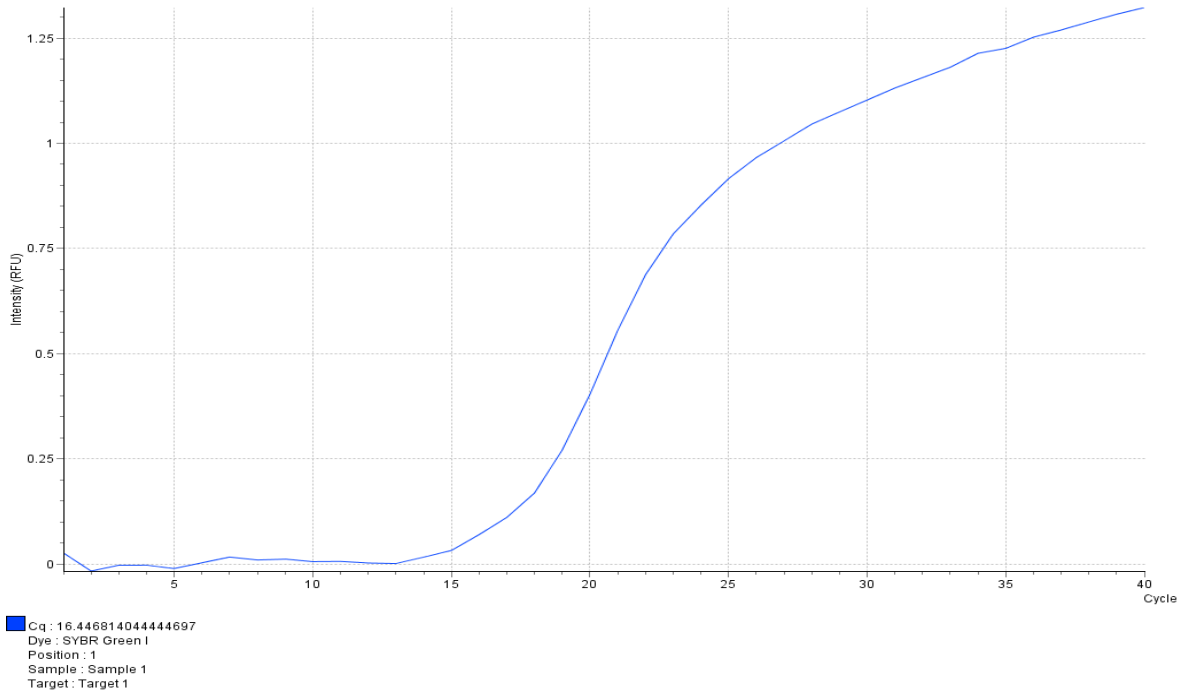
Figure 1: *Amplification Chart (Fluorescence Intensity to Cycle Number Curve)* (Lappin et al., 2012)

Figure 1 shows that the Real-time PCR amplification curve consists of three phases, namely the exponential phase, the linear phase, and the sloping phase (plateau). The three phases will be related to the quantification technique using Real-time PCR amplification results curve. This quantification technique is commonly called Quantitative PCR (qPCR). The exponential phase is quite important in the process of determining the threshold line because the threshold is generally made in exponential phase. The threshold created in the exponential phase produces more representative data than if the threshold created in another phase. Threshold lines are set to determine the value of Ct (Ct value). In the exponential phase, amplification products increase exponentially because the number of available reagents is still excessive. When the number of reagents begins to decrease, the curve will enter a linear phase. The linear phase is the phase when the number of amplification products increases linearly. The sloping phase begins when the number of reagents decreases so that the amount of amplification product does not change significantly (Yuan et al., 2006).

3.1 Discussion

The length of non-coding RNA is part of the noncoding RNA member recently associated with various human diseases (Abhishek et al., 2019). LncRNA produces a

multifaceted controlling network through connections with transcription can influence transcription (Pastori et al., 2012). Many studies conducted to investigate the impact of lncRNA in various pathological conditions (Abhishek et al., 2019). LncRNA contributes to endocrine function and disease (Sun and Kraus, 2013). Recent research on genetic has focused mainly on lncRNA expression in T2DM (Abhishek et al., 2019). There have been many studies examining the pathogenesis that causes type 2 diabetes. It's characterized by impaired insulin resistance (Kong et al., 2017). However, the main causes of T2DM cannot be fully understood to date. The genetic area can describe part of the heritability of diabetes (Lyssenko and Laakso, 2013). LncRNA is one of genetic factors involved in the pathogenesis in diabetes mellitus type 2 (Giroud and Scheideler, 2017).

Based on the results seen in Table 2, it shows that the level of gene expression varies in each sample. The highest value of lncRNA concentration is at the number 7 sample while the lowest value of lncRNA concentration is at sample number 1. This is also related to Figure 1 (Amplification Graph), the more it is higher than the target gene, if the expression value > 1, then it shows an increase in gene expression, which means that if gene expression increases, the sample is said to have decreased blood sugar, and vice versa. If gene expression decreases, then the sample is stated to have a blood sugar increase. According to Saedi et al (2018), the sample of T2DM sufferers was stated to have significantly reduced the level of lncRNAs LY86-AS1 expression, which means that with a decrease in lncRNA LY86-AS1 expression, it would likely factor of the pathogenesis of T2DM. A distinctive feature of T2DM is the reduced sensitivity of insulin in target tissue. Fasting blood sugar (FBS) measurement is a measurement of blood sugar during fasting, where the expression of LY86-AS1 shows a significant inverse correlation with FBS. This is also supported by previous research that with different gene expression, at the level of expression of LINC00523 and LINC00994 of T2DM in the Iran group, it still shows an inverse correlation between FBS with LINC00523 and LINC00994 expression (Mansoori et al., 2018).

Reduced mass and function of β cells are the cause of reduced insulin secretion (Robertson, 2004). Chronic hyperglycemia results damage of insulin gene expression attended by decreased expression or increased DNA result in two pancreatic transcription factor genes: PDX-1 and MafA which further suppress biosynthesis and insulin secretion. The change in transcription factors explains the suppression of biosynthesis and insulin secretion, which is caused by glucose toxicity in β-cells (Kaneto and Matsuoka, 2015). Oral Glucose Tolerance Test

is a test conducted cases of unclear hyperglycemia. This test can be signified in obese patients with a family history of diabetes mellitus, neurologic with unclear reasons. On the other hand, HbA1C is well thought of dependable test for the diagnosis of T2DM. However, there is still a shortage of markers with high specificity for detection of T2DM (Abhishek et al., 2019). Decreased lncRNA LY86-AS1 expression might be signal ofcharacter T2DM. LncRNA LY86-AS1 well thought of potential diagnostic for type 2 diabetes mellitus (Saedi et al., 2018).

4. Conclusion

The existence of technological advances to detect molecular T2DM disease with RNA long non-coding, it was concluded that the possibility of using LY86-AS1 lncRNA can detect diagnostic markers of T2DM sufferers for the Munggu community in particular. The limitation of this research is the lack of research funding from the government so that the samples used are not many.

5. Supporting Institution

Ministry of Research and Technology Republic of Indonesia (Kementrian Riset dan Teknologi Indonesia)

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