UNIVERSIDAD AUTÓNOMA DE GUERRERO
FACULTAD DE CIENCIAS QUÍMICO BIOLÓGICAS
FACULTAD DE MEDICINA
UNIDAD DE INVESTIGACIONES ESPECIALIZADAS EN MICROBIOLOGÍA
MAESTRÍA EN CIENCIAS BIOMÉDICAS

RNAs largos no codificantes (lncRNAs) y su relación con diabetes tipo 2

T E S I S

QUE PARA OBTENER EL GRADO DE

MAESTRÍA EN CIENCIAS BIOMÉDICAS

Presenta

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Directora

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Chilpancingo, Gro., enero de 2019

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ACTA DE APROBACIÓN DE TESIS

En la ciudad de Chilpancingo, Guerrero, siendo los 03 días del mes de diciembre de dos mil dieciocho se reunieron los miembros del Comité Tutoral designado por la Academia de Posgrado de la Maestría en Ciencias Biomédicas, para examinar la tesis titulada **"RNAs largos no codificantes (lncRNAs) y su relación con diabetes tipo 2"**, presentada por la alumna Vianet Argelia Tello Flores, para obtener el Grado de Maestría en Ciencias Biomédicas. Después del análisis correspondiente, los miembros del comité manifiestan su aprobación de la tesis, autorizan la impresión final de la misma y aceptan que, cuando se satisfagan los requisitos señalados en el Reglamento General de Estudios de Posgrado e Investigación Vigente, se proceda a la presentación del examen de grado.

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Este trabajo se realizó en el Laboratorio de Investigación en Epidemiología Clínica y Molecular, en la Facultad de Ciencias Químico Biológicas perteneciente a la Universidad Autónoma de Guerrero, en la ciudad de Chilpancingo, Guerrero, México.

Se realizaron dos estancias de investigación en el laboratorio de la Unidad de Investigación Médica en Bioquímica perteneciente al Hospital de Especialidades "Bernardo Sepúlveda" del Centro Médico Nacional Siglo XXI perteneciente al Instituto Mexicano del Seguro Social, en la Ciudad de México, México.

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Expression of circulating lncRNAs H19 and MALAT1 in serum and in serum exosomes in type 2 diabetes

Tello-Flores et al: Expression of H19 and MALAT1 lncRNAs in type 2 diabetes

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Key words: Type-2 diabetes, exosomes, H19, MALAT1, MIAT

Abstract

Type-2 diabetes (T2D) has been linked to different environmental, genetic and epigenetic factors. It has been described that the expression of H19, MALAT1 and MIAT is related to metabolic diseases such as metabolic syndrome, cardiovascular disease and T2D. Aim of the Study was to analyze the relationship between the expression of H19, MALAT1 and MIAT lncRNAs with T2D or biochemical alterations. A study was conducted in patients with T2D and in subjects without diabetes, without kinship among them, who residents of Mexico City and users of the services of the Mexican Institute of Social Security. Anthropometric measurements were made, blood concentrations of glucose, glycosylated hemoglobin, total cholesterol, triglycerides, high- and low-density lipoprotein cholesterol were measures. Total RNA was obtained from serum samples by the exoRNeasy Serum/Plasma Midi Kit and by the Trizol technique. The expression levels of H19, MALAT1 and MIAT were quantified by RT-qPCR. Using generalized linear models, the relationship between the expression levels of the lncRNAs and the study groups was evaluated. An average increase in the expression levels of MALAT1 in serum (β=0.79; p=0.04) was identified in T2D patients compared with controls. Additionally, a positive tendency was found in the expression levels of MALAT1 from exosomes with glycosylated hemoglobin levels. We identified a significant increase in the serum expression levels of MALAT1 in subject with T2D, and in exosomes a higher expression of MALAT1 associated with the increase of HbA1c. Findings that may have an important effect on the follow-up and prognosis of T2D.

Introduction

Diabetes is one of the most important pathologies worldwide, it is considered one of the main causes of morbidity and mortality among the adult population. The International Diabetes Federation for the year 2015 estimated 415 million cases (uncertainty interval: 340-536 million) of diabetes among adults aged 20-79 years. For 2040, it was estimated that 642 million (uncertainty interval: 521-829 million) people aged 20-79 will have diabetes (1). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves heart and blood vessels. Although the relationship between environmental factors, diet and level of physical activity with type 2 diabetes (T2D) and their comorbidities is known, it is a challenge to identify the genetic and epigenetic factors related to these pathologies (2). The transport of genetic material contained within extracellular vesicles (EVs), which include apoptotic bodies, microvesicles, and exosomes, have emerged as important players in intercellular communication in normal physiology and pathological conditions. Indeed, EVs function as the carriers of small bioactive molecules, such as peptides, proteins, lipids, and nucleic acids, that act as regulators in the recipient cells in a paracrine or endocrine manner (3).

Exosomes are small EVs and encompassed by a bilayer of phospholipid molecules, with a size that varies between 40 to 100 nm in diameter, it have been identified as containing proteins, small peptide chains, lipids, DNA fragments, mRNAs, miRNAs and long non-coding RNAs (lncRNAs) (4-6). Exosomes can be detected in a variety of biological fluids including serum, plasma, urine, saliva, cerebrospinal fluid and milk. At present, several investigations have focused on studying the relationship between exosomes with the pathophysiology of complex human diseases. In particular, it has been shown that in T2D exosomes secreted from skeletal muscle, adipose tissue and hepatocytes can transfer both functional proteins and RNA species that regulate the metabolic function of both remote tissues and of adjacent cells. Patients with different types of diabetes and different complications of diabetes showed diverse patterns of exosomal biomolecules, suggesting that exosomes may contribute to tissue- or stage-specific pathogenic mechanisms of diabetes and its complications (6).

Furthermore, the role of lncRNAs in regulating the expression of genes in health and disease has been evidenced. The lncRNAs are single-stranded nucleotide sequences with a length greater than 200 bp, and correspond to an important class of RNAs involved in the expression of genes, regulating various biological functions. It has been described that lncRNAs act as activators or

repressors of transcription, enhancers of gene expression through the recruitment of transcriptional complexes. Also as molecular scaffolds for the recruitment of chromatin-modifying complexes, interacting with pre-mRNAs or mRNAs, inhibiting splicing or translation, or as molecular sponges by binding to miRNAs. Based on genomic localization, lncRNAs are classified as intergenic, intronic, exonic, antisense, divergent and enhancer lncRNAs (7-9).

Accumulating evidences has demonstrated that mutations and dysregulations of lncRNAs are associated with the development and progression of various diseases including various types of cancer, diabetes, metabolic syndrome, obesity, Alzheimer, cardiovascular diseases, among others (10-13). In such a way, lncRNAs provide a therapeutic opportunity in the control and monitoring of metabolic diseases, such as the control of glucose metabolism in T2D. As well as, a better understanding of the participation of the lncRNAs in the regulation of glucose metabolism and diabetic complications. Recently, the decrease in H19 in skeletal muscle has been identified in diabetic subjects and in mice with insulin resistance (14). On the other hand, the levels of expression of MALAT1 have been associated in processes related to inflammation and hypoxia within the context of diabetes, with retinopathy and diabetic nephropathy, as well as in dysregulation of glucose homeostasis (15, 16). Likewise, the lncRNA MIAT has been associated with microvascular dysfunction of retinopathy and nephropathy, this lncRNA sequesters the miR-150-5p inhibiting the expression of vascular endothelial growth factor (VEGF). The decrease in the expression of MIAT increases the expression of VEGF, promoting angiogenesis linked to diabetic retinopathy (17, 18).

In metabolic diseases, extensive exploration has not been carried out regarding the expression of lncRNAs and their relationship with these diseases. There are few antecedents and these have been performed mainly in cell lines and experimental murine models. Principally, trying to elucidate the relationship between these lncRNAs with T2D, as well as with their metabolic and systemic complications. In Mexico, this research is a pioneer in providing information on the expression of lncRNAs and the transport of these through EVs in patients with T2D. The purpose of the study was to analyze the relationship between the levels of expression of H19, MALAT1 and MIAT lncRNAs in serum and in exosomes with T2D and the presence of biochemical alterations.

Materials and Methods

Patients. Samples from patients with a diagnosis of less than 5 years of T2D and subjects without T2D as controls were analyzed. The participants were residents of Mexico City and users of the medical services of the Mexican Institute of Social Security (IMSS) whose serum samples were stored at -70 ºC. The research was approved by the National Ethical Committee of the Mexican Institute of Social Security and by the Ethics Committee of the Autonomous University of Guerrero. All patients signed the informed consent.

Anthropometric and biochemical measurements. The participants were evaluated by the clinical laboratory after an overnight fast of 12 hours. Each one was measured stature with a portable stadiometer, waist circumference (WC) at the midpoint between the lower rib and the hip, and the weight with a digital scale. Based on these measurements the body mass index (BMI) in kg/m^2 was calculated. Likewise, blood pressure was measured using a mercurial sphygmomanometer, obtaining two readings and calculating the average for each of the patients. Blood measurements of total cholesterol (c-total), triglycerides, high lipoprotein cholesterol (HDL-c) and low-density (LDL-c) and glycosylated hemoglobin (HbA1c) were performed.

Isolation of exosomes and RNA extraction. Samples were thawed on ice and subsequently centrifuged at 3,000 g for 15 minutes, the exosomes and RNA contained within these were isolated using the exoRNeasy Serum/Plasma Midi Kit (cat# 77044, QIAGEN, Hilden, Germany), following the manufacturer's instructions. Briefly, serum/plasma was mixed 1 to 1 with 2X binding buffer XBP, the mixture was then passed through the exoEasy membrane affinity column to bind the EVs to the membrane and centrifuged at 500 g for 1 min. The flow-through was discarded and wash buffer XWP was added to the column to wash off non-specifically retained material. After another centrifugation and discarding of the flow-through, the vesicles were lysed by adding of 700 µL QIAzol to the spin column, and the lysate was collected by centrifugation at 3,000 g for 5 min. Subsequently, 90 µL of chloroform was added and centrifuged at 12,000 g by 15 min at 4 °C, the aqueous phase was recovered and mixed with ethanol. 700 µL of the sample-ethanol mixture was added to a RNeasy MinElute spin column and centrifuged. The column was washed once with buffer RWT, and then twice with buffer RPE followed by elution of RNA in water.

Extraction of serum RNA. From samples of frozen serum at -80 ° C, total RNA was extracted with TRizol™ reagent (Thermo Fisher Scientific, Waltham, MA, USA), in order to precipitate the highest number of proteins, the samples were centrifuged at 6,200 g at 4 °C per 15 min. Subsequently, 500 µL of serum sample was placed in a 2 mL tube, adding 1.5 mL of trizol reagent and incubating at room temperature for 5 min, then adding 40 µL of chloroform, mixing and incubating for 15 min at room temperature, and centrifuging at 12,000 g for 25 min at 4 °C. The aqueous phase (containing the total RNA) was transferred to a new tube and adding 1 mL of 100% isopropanol and incubating at room temperature for 10 min. Again, it was centrifuged at 21,100 g for 15 min at 4 °C and then the supernatant was carefully removed, avoiding the release of the RNA pellet. To the pellet, 1.5 mL of 75% ethanol was added to resuspend the RNA, again centrifuged at 21,100 g, for 10 min at 4 °C, then it was allowed to dry inside the extraction hood at room temperature. The RNA was resuspended with 50 µL of DEPC water and stored at -80 °C until use.

Obtaining cDNA (from serum or exosomes). Prior to the synthesis of the cDNA, the DNase I RNase free kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to eliminate the gDNA and avoid interference in the results, briefly: In a tube of 0.2 mL free of RNases, 1 µg of RNA, 1 µL of 10X reaction buffer with MgCl2, 1 µL DNase I RNase-free, and 10 µL of DEPC water were added. It was incubated at 37 °C for 30 min, then 1 µL of 50 mM EDTA was added, incubating at 65 °C for 10 min. Subsequently, 10 µL was taken for cDNA synthesis using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions, briefly, the master mix 2X RT was prepared on ice, adding for each reaction 2 µL of 10X RT buffer, 0.8 µL of 25X dNTP, 2 µL of RT 10X primers, 1 µL of reverse transcriptase (RT), 1 µL of RNase inhibitor and 3.2 µL of nuclease-free water. Subsequently, 10 µL of master mix 2X RT was placed in each reaction well, adding 10 µL of RNA (2 µg), the plate was covered and centrifuged for a few seconds, keeping the plate on ice until it was processed. The reaction volume was adjusted to 20 mL, the amplification was performed in the MAXYGEN II Thermal Cycler (Axygen Scientific, Union City, CA, USA).

Expression of long non-coding RNAs. Expression of lncRNAs was quantified using TaqMan Gene Expression Assays (Thermo Fisher Scientific, Waltham, MA, USA), H19 (cat# Hs00399294_g1), MALAT1 (cat# Hs00273907_s1) and MIAT (cat# Hs00402814_m1) by RT-qPCR using the

7900HT detection system (Applied Biosystems, Foster City, CA, USA). The reaction conditions were: 50 °C for 2 min for 1 cycle; 95 °C for 2 min, 95 °C for 10 s and 60 °C for 30 s for 40 cycles. Gene expression data was normalized to the expression levels of GAPDH housekeeping gene (cat# Hs02786624_g1) and analyzed using the ΔΔCq method (19).

Statistical analysis. The data are presented as means ± standard deviation, or medians and percentiles ($p25^{th}$ - $p75^{th}$), and in frequencies for qualitative variables. Comparisons between groups were performed by t-test, or Mann-Whitney test for non-parametric data, and Chi-square test by qualitative data. Correlations were evaluated using the Pearson correlation coefficient. Generalized linear models were analyzed to evaluate the association between lncRNAs with T2D or with biochemical alterations. Two-tailed statistical tests were conducted with a significance level of 5 % using STATA v. 13 software (Stata Corp, College Station, TX, USA).

Results

Using the exoRNeasy Serum/Plasma Midi Kit extraction method, exosomes, total RNA and cDNA were obtained from 46 serum samples, amplifying 36 for the lncRNA H19 (16 T2D and 20 without T2D) and 39 for the lncRNA MALAT1 (19 T2D and 20 without T2D). From total serum, total RNA and cDNA were extracted from 120 samples, of which 83 amplified for lncRNA H19 (40 T2D and 43 without T2D) and 99 for lncRNA MALAT1 (48 T2D and 51 without T2D). We did not find expression of lncRNA MIAT, neither in serum samples nor in exosomes.

In our study, there was greater participation of women compared to men, however no significant differences were identified. Patients with T2D had higher body mass index (BMI), waist circumference, glucose, triglycerides and a decreased HDL-c levels, compared with the group without T2D (Table 1). No significant differences were identified between the medians of the lncRNAs H19 and MALAT1 with the study groups (T2D vs without T2D), both of the lncRNAs that were extracted directly from serum, and those obtained from serum exosomes. However, greater expression of these lncRNAs was found in diabetic patients compared to controls, mainly those obtained in serum exosomes (Figure 1). On the other hand, in order to evaluate the effect of obesity, as an important inflammatory process related to the expression of lncRNAs, we analyzed the correlation coefficients between the expression of MALAT1 and H19 with BMI and WC. No significant correlation was found between these lncRNAs with BMI and WC (Table 2).

However, when performing association analysis using generalized linear models adjusted for BMI, triglycerides and HDL-c, we identified an average increase of 0.79 in the expression of MALAT1 of serum ($p = 0.04$) in diabetic patients compared with controls, regardless of BMI, triglycerides and HDL-c. While we found an average increase of 0.27 in the expression of MALAT1 of exosomes ($p = 0.03$) with the increase in blood levels of glycosylated hemoglobin in patients with T2D (Table 3).

Discussion

The T2D is complex and multifactorial disease; lifestyle, exposure to environmental factors and genetic predisposition play an important role in the development of the disease. Despite research efforts to identify biological targets and signaling pathways related to these factors, the molecular mechanisms by which environmental influences affect the pathogenesis of T2D in susceptible individuals remain largely unknown. On the other hand, an increase in serum levels of extracellular vesicles (EVs) has been identified in individuals with obesity, metabolic syndrome, insulin resistence, or diabetes (20). Additionally, it has been demonstrated that in T2D the secreted exosomes of skeletal muscle, visceral adipose tissue and hepatocytes, can transfer both functional proteins and RNAs that regulate the metabolic function of adjacent cells and distant tissues (5, 20, 21). In animal models it has been identified that when EVs derived from adipose tissue of obese mice are transferred to mice without obesity, they favor an inflammatory phenotype and insulin resistance, demonstrated by elevated blood levels of TNF-α and IL-6 (22). The role of ncRNAs in the pathogenesis of T2D has only recently become recognized, yet a growing list of lncRNAs involved in glucose homeostasis is emerging (12, 15). Recent evidence indicates that the expression of several lncRNAs varies in diabetic animal models and in clinical studies of diabetic patients, indicating that lncRNA can serve as new biomarkers for the prognosis of T2D (23).

In our study we identified a tendency to increase the expression of H19 and MALAT1 in T2D compared to controls, and when analyzing the association of lncRNAs with diabetes or biochemical alterations, we identified significant increase in the average level of MALAT1 expression in patients with T2D compared to controls, as well as an increase in this lncRNA from exosomes with HbA1c levels in T2D, both associations were independent of BMI (as an indicator of subclinical inflammation), serum concentrations of triglycerides and HDL-c. We did not find significant association between H19 with the groups or biochemical alterations, this could be due

to the fact that the T2D patients are mostly in adequate control, which is demonstrated by the blood levels of HbAc1 within the parameters of a diabetic controlled person (median = 5.6 %), possibly due to attachment to treatment or the few years they had the disease. On the other hand, expression of MIAT lncRNA in serum or in exosomes was not identified.

LncRNA-H19 is highly conserved imprinted gene which is expressed only in maternal allele. H19 was firstly found to play important roles in embryonal development and growth control. Both maternal and paternal H19 alleles are expressed at first stage of embryonal development (6-8 weeks gestation). However, only maternal chromosomes express H19 after 10 weeks of gestation. H19 is predominantly located in the cytoplasm, but a smaller fraction is found in the nucleus, this lncRNA has been associated with decreased expression in diabetic patients (23, 24). For its part, it has been identified that the levels of expression of MALAT1 have been associated with retinopathy and diabetic nephropathy, through the promotion of angiogenesis in these organs, as well as in dysregulation of glucose homeostasis (24, 25).

In the study conducted by Gao et al., in skeletal muscle of diabetic subjects and mice with insulin resistance, they identified that H19 is reduced approximately five times in this tissue compared to its controls (14). Under normal physiological conditions H19 binds to miR-let-7, preventing the inhibition of the expression of its target genes, *INSR* (insulin receptor) and *LPL* (lipoprotein lipase) genes. In T2D it has been reported that downregulation of H19 increased the bioavailability of let-7 without affecting its level, leading to decreased expression of *INSR* and *LPL* (9, 14). The study of Bitarafan et al., reported that the H19 lncRNA was over-expressed in coronary artery disease patients in comparison with the controls, although without statistical significance. The research mentioned results similar to two other studies and concluded that H19 has a relevant role in hypoxic endothelium and hence in the vascular physio-pathology even though the function of it is still largely unknown (26)

On the other hand, the MALAT1 lncRNA can act as a transcriptional regulator of numerous genes, including some genes involved in metastasis and cell migration in cancer, for which it has been implicated in cell cycle regulation (24, 27). In the study by Liu et al., they identified the overexpression of MALAT1 with microvascular dysfunction in diabetic animals, and its reduction with a significant relief of this alteration, for which they propose MALAT1, as a therapeutic target of microvascular dysfunction (24). In another study conducted by Puthanveetil et al., where human umbilical vein endothelial cells were exposed to different concentrations of glucose, they reported

high levels of MALAT1 after 12 h of incubation at high glucose concentrations. In this same study they confirm that this lncRNA is a direct regulator of inflammatory ligand, SAA3 (amyloid serum A3), increasing the expression of interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) (16). In the study conducted by Sathishkumar et al., they reported overexpression of MIAT in patients with T2D ($\beta = 6.6$ $p = 0.002$), in comparison with the control group. Unlike our study, Sathishkumar et al., performed the extraction of RNA from peripheral blood mononuclear cells (28), so we could infer that this lncRNA is not secreted free in the blood circulation, nor is it found within exosomes secreted to circulation in both study groups.

The main limitations of our study are the small sample size, the few years of evolution of T2D, and the use of serum samples stored at -70 º C. Previous studies have identified that the use of frozen serum samples for measuring the expression of various types of RNAs, can yield unreliable results, because in the thawing of the serum, the RNA is rapidly denatured. Another factor is the storage time, since prolonged freezing times decrease the stability and integrity of these molecules. Previous studies have established that in certain diseases such as diabetes, RNase levels are increased in blood circulation. From what has been suggested, the time between obtaining venous blood and RNA extraction should not be longer than 24 h (29-30).

Conclusions

In summary, we identified a significant increase in the serum levels of MALAT1 expression in subjects with T2D, and in exosomes a higher expression of MALAT1 associated with the increase of HbA1c. Future studies with a larger sample size, patients classified in strata for different years of evolution of T2D, and the use of recently obtained serum samples are required, to clarify the effect of T2D on the expression of lncRNAs.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

VATF, AVS and JACP in sample collection, performed experiments collected the data. DHS, OdelMH, MRR, MC and AAS, contributed reagents/materials/analysis tools, and provided necessary logistical support and critical comments on the manuscript. EFA, VATF and AVS designed the study, analyzed the data, drafted the manuscript and edited the manuscript for intellectual content.

Ethics approval and consent to participate

The research was approved by the National Ethical Committee of the Mexican Institute of Social Security and by the Ethics Committee of the Autonomous University of Guerrero.

Patient consent for publication

Written informed consent obtained from all patients.

Competing interests

The authors declare that they have no conflicts of interests.

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Table 1. General characteristics of the study groups

Characteristic	Total n=120	T2D n=60	Without T2D n=60	p value
Age (years)	52 (47-55)	52 (47-55)	51 (45-55)	0.76[a]
Years of diagnosis		2.8 ± 1.7	-	
Gender, n (%)				
Male	38 (31.7)	19 (31.7)	19 (31.7)	1.0[b]
Female	82 (68.3)	41 (68.3)	41 (68.3)	
BMI (kg/m^2)	29.6 (25.1-31.5)	29.6 (26.6-32.4)	27.4 (24.4-30.7)	0.01[b]
WC (cm)	93 ± 13	97 ± 14	89 ± 10	<0.001[c]
BP systolic (mmHg)	113 ± 14	113 ± 16	113 ± 13	0.97[a]
BP diastolic (mmHg)	78 ± 11	80 ± 12	76 ± 10	0.07[a]
Glucose (mg/dL)	94.5 (87-119.5)	120 (102-158)	87.5 (83-92)	<0.001[a]
Cholesterol (mg/dL)	201 ± 45	200 ± 41	202 ± 48	0.87[b]
Triglycerides (mg/dL)	168 (125.5-229)	188 (140-257)	142 (101-196.5)	<0.001[a]
HDL-c (mg/dL)	45.5 (39-55)	41 (35.5-49)	50.5 (41.5-57)	<0.001[a]
LDL-c (mg/dL)	140 ± 36	140 ± 34	142 ± 38	0.73[c]
HbA1c (%)		5.6 (4.9-7.2)	-	

The data are shown as means ± standard deviation, or medians and percentiles (p25th - p75th). T2D: Type-2 diabetes; BMI: Body mass index; WC: Waist circumference; BP: Blood pressures; HDL-c: High-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; HbA1c: Glycosylated hemoglobin. [a] Mann-Whitney test; [b] Chi-square test; [c] t-test.

Table 2. Correlation between lncRNAs with body mass index and waist circumference

	Suero			
	MALAT1		H19	
	T2D	Without T2D	T2D	Without T2D
	r (p value)	r (p value)	r (p value)	r (p value)
BMI	0.15 (0.32)	-0.18 (0.20)	0.23 (0.16)	-0.05 (0.77)
WC	0.19 (0.19)	-0.29 (0.05)	0.19 (0.24)	0.02 (0.92)
	Exosomes			
	MALAT1		H19	
	T2D	Without T2D	T2D	Without T2D
	r (p value)	r (p value)	r (p value)	r (p value)
BMI	-0.43 (0.06)	0.08 (0.74)	0.27 (0.31)	-0.25 (0.29)
WC	-0.40 (0.09)	0.29 (0.25)	-0.43 (0.10)	-0.15 (0.54)

T2D: Type-2 diabetes; BMI: Body mass index; WC: Waist circumference

Table 3. Association between the levels of expression of H19 and MALAT1 with type-2 diabetes and HbA1c

	Serum						Exosomes					
	H19			MALAT1			H19			MALAT1		
	β	95% CI	p	β	95% CI	p	β	95% CI	p	β	95% CI	p
T2D	0.21	-0.9, 1.3	0.70	0.79	0.03, 1.6	**0.04**	0.92	-0.82, 2.7	0.29	0.64	-0.4, 1.7	0.22
BMI	0.01	-0.05, 0.07	0.75	-0.01	-0.06, 0.04	0.67	-0.04	-0.11, 0.02	0.20	-0.008	-0.04, 0.03	0.68
TG	0.001	-0.003, 0.01	0.64	-0.01	-0.01, 0.001	0.13	0.002	-0.01, 0.01	0.53	-0.001	-0.01, 0.003	0.60
HDL-c	0.02	-0.01 - 0.05	0.19	-0.01	-0.03, 0.02	0.52	-0.01	-0.1, 0.08	0.82	-0.02	-0.07, 0.03	0.50
HbA1c	-0.15	-0.4, 0.1	0.25	0.60	-0.1, 0.3	0.53	0.24	-0.3, 0.7	0.32	0.27	0.04, 0.52	**0.03**

T2D: Type-2 diabetes; BMI: Body mass index; HDL-c: High-density lipoprotein cholesterol; HbA1c: Glycosylated hemoglobin; β: Regression coefficients calculated by generalized linear models; CI: Confidence interval.

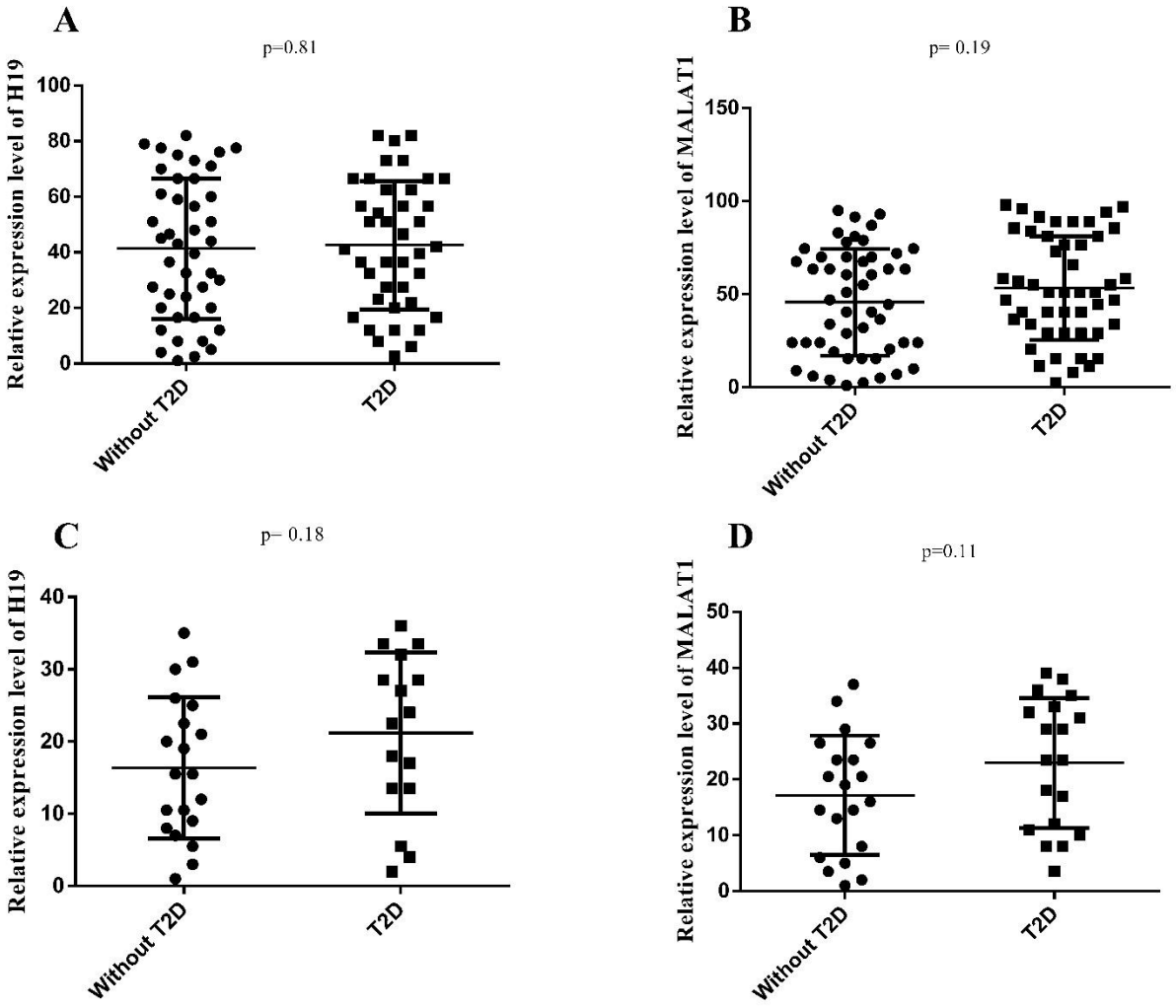

Figure 1: Expression levels of the H19 and MALAT1 lncRNAs in serum (A and B) and in exosomes (C and D). P-value obtained by the Mann-Whitney test.