MicroRNA-146a and Metabolic Disorder

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Abstract

MicroRNA-146a is one of the inflammation related microRNAs that constitutes an imperative part in the immune system. The present study was designed to evaluate whether microRNA-146a and its effect on the expression of target proteins (TRAF6 and IRAK1) are involved in metabolic disorders such as type II diabetes. In the current study, we investigated the expression levels of microRNA-146a in kidneys of twenty diabetic rats. The results showed a four-fold increase in microRNA-146a level in the kidneys of diabetic rats which was associated with a significant reduction in the expression of IRAK1 with no changes in TRAF6 expression levels. On the contrary, significant increase in NF-κB, TNFα, and IL6 and IL1β concentrations in kidney tissues of diabetic rats was observed. In conclusion, MicroRNA-146a can be used as a marker in case of diabetes.

Introduction

MicroRNAs belong to a class of small non-coding regulatory RNA containing about 22 nucleotides in length [1]. They act through binding to the 3′UTR (untranslated region) of target mRNA leading to transcriptional repression or degradation of the target mRNA at the post-transcriptional level. The expression of MicroRNA-146a, one of the inflammation-related MicroRNAs that play a crucial role in the immune system, was provoked through lipopolysaccharide and nuclear factor kappa B dependent (NF-κB) [2]. MicroRNA-146a causes negative feedback regulation of the innate immune response via targeting the pro-inflammatory adapter proteins tumor necrosis factor (TNF) receptor-associated factor 6 and interleukin-1 receptor associated kinase1[3]. Activation of these two target proteins (IL-1 receptor-associated kinase (IRAK1) and tumor necrosis factor receptor-associated factor 6 (TRAF6) (IRAK1 and TRAF6), motivates the NF-κB which encourages the transcription of the MicroRNA-146a gene that, sequentially, down-regulates two key adapter molecules, TRAF6 and IRAK1, to decrease NF-κB activity [4]. Diabetes mellitus (DM) is a chronic inflammatory disease, chronic hyperglycemia provides a case with high concentration of pro-inflammatory mediators inducing tissue inflammation and cell death [5]. It is believed that chronic hyperglycemia enhances glucose oxidation and enhanced glycation end products, activates protein kinase C, hexosamine and polyol pathway fluxes and finally increases pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1B [6].

Many of these hyperglycemia induced mechanisms converge to activate NF-κB, a pro-inflammatory master switch, which activates pro-inflammatory cytokines genes expression [7]. NF-κB has been reported to be a basic role in the development of diabetic nephropathy [8].

Diabetic nephropathy is characterized by glomerular and tubular hypertrophy, glomerulosclerosis and tubulointerestitial fibrosis [7]. Albeit numerous systems have explained the pathogenesis of diabetic nephropathy, it stays a standout amongst

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the most imperative reasons of mortality in diabetic patients [8]. In recent years, several studies suggested that MicroRNA-146a participates in immune responses, cell proliferation, cell death and inflammation through a NF-κB-dependent negative loop [9]. Some new studies have uncovered the association of MicroRNA-146a in the pathogenesis of diabetic complications [10].

The damaged MicroRNA-146a has shown expression linked subclinical inflammation and insulin resistance in diabetic patients [11]. It also participated in regulating the extracellular matrix protein production in the retinas, hearts and kidneys of diabetic rats [12]. Such observation was associated to the involvement of MicroRNA-146a in the pathogenesis of chronic renal inflammation. It is possible that MicroRNA-146a contributes in the pathogenesis of diabetic nephropathy.

Therefore, the aim of this study was to evaluate whether microRNA-146a and its effect on the expression of TRAF6 and IRAK1 were involved in metabolic disorders such as type II diabetes.

Material and Methods

Animals management

Twenty male adult albino rats aged 8 weeks and weighting 150-200 grams were used in the experimental investigation of this study. The animals were obtained from the Laboratory Animal Unit (LAU), Faculty of Veterinary Medicine, Zagazig University, and were housed under traditional laboratory circumstances through the experimental period at the Experimental Animals Research Unit (EARU). The animals were fed on a standard rat pellet diet and allowed free access to water. Rats were kept at constant environmental and nutritional condition throughout the periods of the experiment and received balanced ration. All animals were acclimatized for a minimum period of two weeks prior to the beginning of the study.

Experimental design

Rats were divided into two groups: Control group (n=10) and diabetic group (n=10), rats were fed on standard rat feed and water for two months.

Induction of diabetes

Overnight fasting adult albino rats (n=10) were rendered diabetic via a single Intra-peritoneal injection of nicotinamide (110 mg/kg) prepared in normal saline. After 15 min Intra-peritoneal injection of Streptozotocin (STZ) (Sigma −Aldrich, Chemical Cp. St. Louis, Mo, USA) 60 mg/kg dissolved in cold citrate buffer (pH 4.5) was carried out. The rats were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycemia [13].

Blood glucose levels were measured two consecutive days after STZ injection by making an injury at the tail tip, squeezing gently and using the glucose oxidase method. Rats were considered diabetic if the blood glucose level reached 200 mg/dl or more [14]. The control group rats (n = 10) were injected only with the vehicle (0.01 M citrate buffer, pH 4.5).

Sampling

Blood samples were collected from orbital venous plexus and were used for estimation of blood glucose, blood urea nitrogen and creatinine [15].

Table 1: Primers used in determination of IRAK1 and TRAF6 gene expression

Gene	Primers
*Irak*1	F: 5`-GCTGTGGACACCGAT-3`
	R: 5`-GCTACACCCATCCACA-3`
*Traf*6	F: 5`-CAGTCCCCTGCACATT-3`
	R: 5`-GAGGAGGCATCGCAT-3`
GAPDH	F: 5`-CTTCAACAGCAACTCCCATTC-3`
	R: 5`-GTAGCCATATTCATTGTCATACCAG-3`

Immediately after scarifying, kidneys were removed and wrapped in aluminum foil and put immediately in liquid nitrogen container, for real time-PCR analysis and for estimating the concentrations of TNF-α, IL-1β, IL-6 and NF-κB in kidney tissues.

Biochemical determinations

Serum glucose concentrations were assayed enzymatically using Glucose Assay Kit (ab65333). Blood Urea Nitrogen was assayed enzymatically by using Enzymatic Kit (Cat no-5602-01). Creatinine was assayed enzymatically using Enzymatic Kit (Cat no ab65340). The tissue level of rat TNF-α of experimental groups was measured by using Abcam's TNF-α (ab100785) Rat ELISA kit following the manufacture instruction. The tissue level of rat IL1β of experimental groups was measured by using RayBio® Rat IL-1 beta ELISA Kit (ELR-IL1beta-001C) following the manufacturer instructions. The tissue level of rat IL-6 of experimental groups was measured by using RayBio® Rat IL-6 ELISA Kit (ELR-IL6-CL) following the manufacturer instructions. The tissue level of NF-κB of the experimental groups was measured by using mybiosource Enzyme-linked Immunosorbent Assay Kit for Nuclear Factor Kappa B (NF-κB) (MBS2020410 96) following the manufacturer instructions.

Table 2: Primers used in determination of the gene expression of miRNA-146a

Gene	Primers
MicroRNA-146a	F: 5`-UGAGAACUGAAUUCCAUGGGUU-3`
	R: 5`-ACCUGUGAAGUUCAGUUCUUU-3`
miR-191a	F: 5`-CAACGGAAUCCCAAAAGCAGCUG-3`
	R: 5`-GCUGCACUUGGAUUUCGUUCCC-3`

Molecular analysis

Real-time quantitative RT-PCR (qRT-PCR) Analysis of IRAK1 and TRAF6 gene expression

To validate the magnitude of IRAK1 and TRAF6 genes with SYBR® green based data, real-time quantitative PCR analysis has been performed with tissue homogenate and specific synthesized primers (Table 1). Total RNA from tissue samples was extracted using Jena Bioscience total RNA purification kits (Cat-No. PP-210S) following the manufacturer guidelines. Reverse transcription of total RNA using Jena Bioscience SCRIPT RT-PCR Two-Step Kit (Cat.-No. PCR-506S) was carried out.

Real-time quantitative PCR (qPCR) Analysis of miRNA-146a in experimental groups

Extraction of miRNA-146a from the tissue using *mir*Vana miRNA Isolation Kit (Part Numbers AM1560, AM1561) was carried out. Reverse Transcription for Quantitative Real-Time PCR using the miScript PCR Starter Kit (Cat.-No. 218193) was performed. The final reaction mixture was placed in a Rotor-Gene 3000 real time thermal cycler and real time PCR program was carried out by initial denaturation at 95ºC for 15 min .to activate host *Taq* DNA polymerase followed by 40 cycles of 94ºC for 15 sec for DNA denaturation, annealing at 55ºC for 30 sec, extension at 70ºC for 30sec, and fluorescence data collection. The sequences of the primers are illustrated in Table (2).

Statistical analysis

Data were expressed as the mean±SE and were analyzed by Independent t test using PASW Statistics (computer program SPSS, Inc. version 22, IBM Corp. 2013, Armonk, NY). A level of $p \leq 0.05$ was considered statistically significant.

Results

Biochemical Analysis

Blood glucose

The blood glucose concentration of STZ-diabetic rats was significantly highly increased (364.65±17.69mg/dL) compared with the control one (71.15±2.62mg/dL) (Table 3).

Serum creatinine

Serum creatinine showed highly significant increase in the STZ-diabetic group (2.35±0.15mg/dl) compared with control one (0.51±0.02mg/dl) (Table 3).

Blood urea nitrogen

As shown in Table (3) BUN levels of STZ–diabetic rats were significantly high (49.37±2.02mg/dl) compared with the control one (19.67±0.59mg/dl).

Cytokine and NF-κB levels in kidney tissues

The level of the pro-inflammatory cytokines IL6, IL1β, TNFα and NF- κB in the STZ-diabetic rats were significantly increased (106.36±4.48, 49.35±2.06, 43.16±1.80 and 1.74±0.08 pg/mg), compared with the control group 66.42±2.40, 15.42±1.14, 14.93±0.82 and 0.56±0.02 pg/mg, respectively (Table 3).

Molecular Analysis

Relative quantification of IRAK1 and TRAF6 gene expression in renal tissues

Results in Figure (1A) showed that by the change in the CT number there was down regulation about the two thirds in the expression of IRAK1 in diabetic rats. Results in Figure (1B) showed that by the change in the CT number there was slightly change in the levels of gene expression of TRAF6.

Relative quantification of micro RNA 146a expression in renal tissues

Results in Figure (1C) showed that by the change in the CT number there was fourfold increase in the level of MicroRNA-146a in diabetic kidney compared with the control.

Table 3: Means ± S.E. of blood glucose, blood urea nitrogen, serum creatinine and the pro-inflammatory cytokine levels in kidney homogenate of STZ-diabetic rats

Parameters	Control	STZ-Diabetic group	t-value
Glucose (mg/dl)	71.15±2.62	364.65±17.69	-16.42[**]
Blood urea nitrogen (mg/dl)	19.67±0.59	49.37±2.02	-14.14[**]
Creatinine (mg/dl)	0.51±0.02	2.35±0.15	-13.21[**]
Interleukin-6 (pg/mg)	66.42±2.40	106.36±4.48	-7.85[**]
Interleukin-1β (pg/mg)	15.42±1.14	49.35±2.06	-14.41[**]
Tumor necrosis Factorα (pg/mg)	14.93±0.82	43.16±1.80	-14.27[**]
Nuclear factor -кappaB (pg/mg)	0.56±0.02	1.74±0.08	-13.74[**]

Each value represented the means ± S.E. of 10 samples, each group. ** Highly significant difference (p≤0.01)
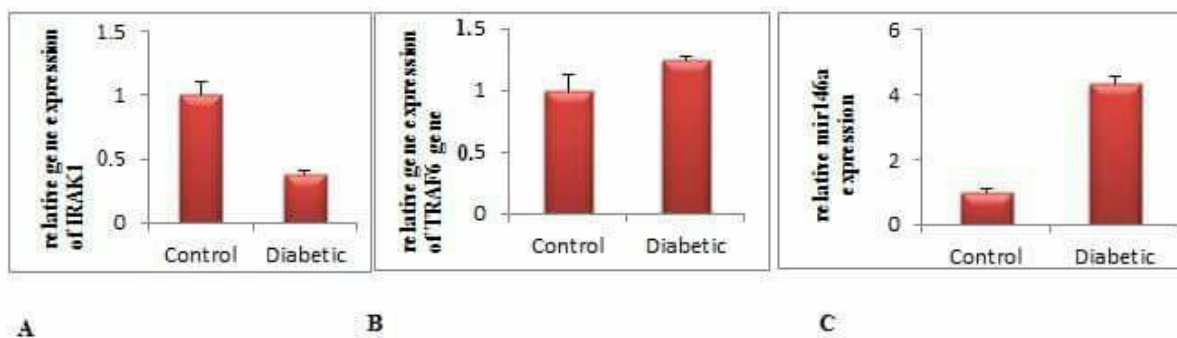

Figure 1: (A) relative gene expression of IRAK1 gene in renal tissues of diabetic and control rats (B) relative gene expression of TRAF6 gene in renal tissues of diabetic and control rats (C) relative gene expression of microRNA146a expression in renal diabetic and control rats

Discussion

Recently, chronic inflammations and oxidative stress had been recognized to play a crucial task in the commencement, promulgation, and development of metabolic syndromes [16-18]. Field studies revealed a solid correlation among pro-inflammatory biomarkers (such as IL-6, TNF-α) and perturbations in glucose homeostasis, obesity and atherosclerosis [19].

MicroRNAs were expressed not only in a tissue-specific mode, but also in cell-type specific manner that assume fundamental parts in numerous biological processes, embracing propagation, apoptosis, progress and discrimination [20]. Moreover, MicroRNA is related to inflammation, oxidative stress, damaged adipogenesis, insulin signaling, apoptosis and angiogenesis in relative to obesity. All of these routes play a role in the expansion of type II diabetes, atherosclerosis, and the linked with cardiovascular disorders [20].

In the current study we investigated the expression levels of MicroRNA-146a in kidneys of diabetic rats. Our results showed a fourfold increase in MicroRNA-146a level in the kidneys of diabetic rats which was associated with significant reduction in the expression of IRAK1 with no changes in TRAF6 expression levels.

On the contrary, significant increase in NF-κB, TNFα, IL6 and IL1β concentrations in kidney tissues of diabetic rats was observed. It was believed that the increased level of blood glucose activated protein kinase C, polyol and hexosamine pathway fluxes, enhanced the advanced glycation end products and induced oxidative/ nitrosative stress in different cells, including leukocytes and renal cells [21]. Also, it has been depicted that sustainably increased level of blood glucose enhances the blood concentration of angiotensin II [22]. All of these factors enhance activation of NF-κB to produce its effect on decreased pro-inflammatory cytokines [8].

Diabetes either of type I or type II DM is characterized by sustained hyperglycemia and chronic elevation of pro-inflammatory environment induced and perpetuated the inflammatory response [23]. However this condition was considered a low–grade inflammation, since the hyperglycemia-provoked oxidative stress upgraded inflammation via augmented endothelial cell impairment, microvascular permeability and amplified the production of pro-inflammatory cytokines, consisting of TNFα, IL6 and IL1β eventually decreased the insulin sensitivity and diabetic complications [23].

It's well-known that, pro-inflammatory cytokines in renal tissue chip create and progress the diabetic nephropathy [7]. Thomson *et al.* [24] added that there was a direct relationship between the kidney weight and the renal TNFα, IL6 and IL1β expression. These data coincide with the obtained results in our study in which TNF-α, IL-6 and IL-1β and NF-κB levels were significantly increased in the kidneys of diabetic rats. Moreover, the blood urea and creatinine levels were higher in diabetic rats than in the control rats. All of these results can be considered an indication of the renal damage that results from two-month uncontrolled diabetes.

NF-κB is a transcription factor which has the ability to incorporate signaling pathways to transcriptional regulation of many genes related to inflammation, immunity, apoptosis, cell proliferation and demarcation [25]. NF-κB regulates its activation partially by a negative feedback loop during sever inflammatory processes, in which, NF-κB activation up regulated MicroRNA-146a gene that, upon processing and maturation, down regulated IRAK1 and TRAF6 (the two key adaptor molecules downstream of cytokine and Toll-like receptors) to minimize the activation of NF-κB [9].

Although the anti-inflammatory effect of MicroRNA-146a gene has been reported by several studies, the above mentioned negative loop has not been shown by some investigations. For instance, a recent study has shown an overexpression of MicroRNA-146a and TRAF6 with attending decreased

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expression of IRAK1 in the peripheral mononuclear cells of patients with Sjogren syndrome [26].

Also, Liu *et al*. [27] reported that the up regulation of MicroRNA-146a was not accompanied with down regulation of IRAK1 and TRAF6 in helicobacter pylori induced inflammatory response in human gastric epithelial cells. The results showed highly significant increase in NF-κB associated with an increase in the expression of MicroRNA-146a. It has been suggested that the expression of MicroRNA-146a was directly controlled by NF-κB and mutation of NF-κB binding sites in the miR-146a promoter abolishes NF-κB mediated induction of MicroRNA-146a [28]. When the activation of NF-κB taken a vital occasion in the progression of diabetes complications, it was possible that a defect in the MicroRNA-146a mediated negative loop gave a situation for maintained inauguration of NF-κB and its objectives to promote cells in the direction of abnormalities.

In this regard, Balasubramanyam *et al*. [10] reported that the high expression of NF-κB in peripheral mononuclear cells of patients with diabetes was not accompanied by MicroRNA-146a gene overexpression resulting in elevated expression level of TRAF6 but not IRAK1. Also, the experiment showed a fourfold increase in MicroRNA-146a expression level, accompanied with a significant down regulation of IRAK1 but not in TRAF6. It may be possible that TRAF6 to be under other levels of control, or TRAF6 had different response to moderate or severe induction of MicroRNA-146a in different levels of cytokines stimulations [28]. Overall, the up regulation of MicroRNA-146a in renal inflammatory diseases has been previously reported [12].

Feng *et al*. [11] showed a reduction in the expression level of MicroRNA-146a in the kidneys of diabetic rats either type 1 or type II. At present, it is unknown whether MicroRNA-146a dysregulation is causal to diabetes or is resulted from it. However, its action may depend on tissue type, blood and tissue cytokines concentrations, timing and duration

of inflammation. Alipour *et al*. [29] introduced the initial data on overexpression of MicroRNA-146a in the diabetic kidney; however this up regulation was not followed by significant decrease in IRAK1 and TRAF6 expressions.

Conclusion

It is unknown whether MicroRNA-146a up regulation is causal to diabetes or is resulted from it. However, its action may depend on tissue type, blood and tissue cytokines concentrations, timing and duration of inflammation, so all this points need further study. Also MicroRNA-146a can be used as a marker in case of diabetes.

Conflict of interest

The authors declare no conflict of interest.

References

[1] Ambros, V. (2004): The functions of animal microRNAs. Nature, 431:350-355.

[2] Perry, M.M.; Moschos, S.A.; Williams, A.E.; Shepherd, N.J.; Larner-Svensson, H.M. and Lindsay, M.A. (2008): Rapid changes in microRNA-146a expression negatively regulate the IL-1beta induced inflammatory response in human lung alveolar epithelial cells. J Immunol, 180 (8): 5689-5698.

[3] Taganov, K.D.; Boldin, M.P.; Chang, K.J. and Baltimore, D. (2006): NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci USA, 103 (33): 12481-12486.

[4] Rusca, N. and Monticelli, S. (2011): MiR-146a in immunity and disease. Mol Biol Int, 2011, doi:10.4061/2011/437301.

[5] Luis-Rodríguez, D.; Martínez-Castelao, A.; Górriz, J.L.; de Álvaro, F. and Navarro-González, J.F. (2012): Pathophysiological role and therapeutic implications of inflammation in diabetic nephropathy. World J. Diabetes, 3 (1): 7-18.

[6] Zhou, X.; Wang, B.; Zhu, L. and Hao, S. (2012): A novel improved therapy strategy for diabetic nephropathy: Targeting AGEs. Organogenesis, 8: 18-21.

[7] Palsamy, P. and Subramanian, S. (2010): Ameliorative potential of resveratrol on proinflammatory cytokines, hyperglycemia mediated oxidative stress, and pancreatic β-cell dysfunction in streptozotocin-nicotinamide-induced diabetic rats. J Cell Physiol, 224(2): 423-432.

[8] Patel, S. and Santani, D. (2009): Role of NF- kappa B in the pathogenesis of diabetes and its associated complications. Pharmacol Rep, 61(4), 595-603.

[9] Ma, X.; Becker Buscaglia, L.E.; Barker, J.R. and Li, Y. (2011): MicroRNAs in NF-κB signaling. J Mol Cell Biol, 3(3):159-166.

[10] Balasubramanyam, M.; Aravind, S.; Gokulakrishnan, K.; Prabu, P.; Sathishkumar, C.; Ranjani, H. and Mohan, V. (2011): Impaired miR-146a expression links subclinical inflammation and insulin resistance in Type 2 diabetes. Mol Cell Biochem, 351(1-2), 197-205.

[11] Feng, B.; Chen, S.; McArthur, K.; Wu, Y.; Sen, S.; Ding, Q.; Feldman, R.D. and Chakrabarti, S. (2011): miR-146a-Mediated extracellular matrix protein production in chronic diabetes complications. Diabetes, 60(11): 2975-2984.

[12] Ichii, O.; Otsuka, S.; Sasaki, N.; Namiki, Y.; Hashimoto, Y. and Kon, Y. (2012): Altered expression of microRNA miR-146a correlates with the development of chronic renal inflammation. Kidney international, 81(3): 280-292.

[13] Akbarzadeh, A.; Norouzian, D.; Mehrabi, M.R.; Jamshidi, Sh.; Farhangi, A.; Allah Verdi, A.; Mofidian S.M.A. and Rad, B.L. (2007): Induction of diabetes by streptozotocin in rats. Indian J Clin Biochem, 22(2): 60-64.

[14] Badr, G.; Bashandy, S.; Ebaid, H.; Mohany, M. and Sayed, D. (2012): Vitamin C supplementation reconstitutes polyfunctional T cells in streptozotocin-induced diabetic rats. Eur J Nutr, 51(5): 623-633.

[15] Parasuraman, S.; Raveendran, R. and Kesavan, R. (2010): Blood sample collection in small laboratory animals. J pharmacol pharmacother, 1(2): 87-93.

[16] Conen, D.; Rexrode, K.M.; Creager, M.A.; Ridker, P.M. and Pradhan, A.D. (2009): Metabolic syndrome, inflammation and risk of symptomatic peripheral artery disease in women: A Prospective Study. Circulation, 120 (12): 1041-1047.

[17] Holvoet, P.; Lee, D.H.; Steffes, M.; Gross, M. and Jacobs, D.R. (2008): Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. JAMA, 299 (19): 2287-2293.

[18] Fonseca, V.; Desouza, C.; Asnani, S. and Jialal, I. (2004): Nontraditional risk factors for cardiovascular disease in diabetes. Endocr Rev, 25 (1): 153-175.

[19] Duncan, B.B.; Schmidt, M.I.; Pankow, J.S.; Ballantyne, C.M.; Couper, D.; Vigo, A.; Hoogeveen, R.; Folsom, A.R. and Heiss, G. (2003): Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes, 52 (7), 1799-1805.

[20] Bonauer, A.; Boon, R.A. and Dimmeler, S. (2010): Vascular microRNAs. Curr Drug Targets, 11(8): 943-949.

[21] Wu, C.C.; Sytwu, H.K. Lin, Y.F. (2012): Cytokines in diabetic nephropathy. Adv Clin Chem, 56: 55-74.

[22] Leung, K.K. and Leung, P.S. (2008): Effects of hyperglycemia on angiotensin II receptor type 1 expression and insulin secretion in an INS-1E pancreatic beta-cell line. J Pancreas (Online), 9 (3): 290-299.

[23] Ponugoti, B.; Dong, G. and Graves, D.T. (2012): Role of forkhead transcription factors in diabetes-induced oxidative stress.

Exp Diabetes Res, 2012, Doi:10.1155/2012/939751

[24] Thomson, S.C.; Deng, A.; Bao, D.; Satriano, J.;Blantz, R.C. and Vallon, V. (2001): Ornithine decarboxylase, kidney size, and the tubular hypothesis of glomerular hyperfiltration in experimental diabetes. J Clin Invest, 107 (2): 217-224.

[25] Sanz, A.B.; Sanchez-Niño, M.D.; Ramos, A.M.; Moreno, J.A.; Santamaria, B.; Ruiz-Ortega, M.; Egido, J. and Ortiz, A. (2010): NF-kappaB in renal inflammation. J Am Soc Nephrol, 21 (8), 1254-1262.

[26] Zilahi, E.; Tarr, T.; Papp, G.; Griger, Z.; Sipka, S. and Zeher, M. (2012): Increased microRNA-146a/b, TRAF6 gene and decreased IRAK1 gene expressions in the peripheral mononuclear cells of patients with Sjögren's syndrome. Immunol Lett, 141(2): 165-168.

[27] Liu, Z.; Xiao, B; Tang, B.; Li, B.; Li, N.; Zhu, E.; Guo, G.; Gu, J.; Zhuang, Y.; Liu, X.; Ding, H.; Zhao, X.; Guo, H.; Mao, X. and Zou, Q. (2010): Up-regulated microRNA-146a negatively modulate Helicobacter pylori-induced inflammatory response in human gastric epithelial cells. Microbes Infect, 12 (11): 854-863.

[28] Roggli, E.; Britan, A.; Gattesco, S.; Lin-Marq, N.; Abderrahmani, A.; Meda, P. and Regazzi, R. (2010): Involvement of microRNAs in the cytotoxic effects exerted by proinflammatory cytokines on pancreatic β-cells. Diabetes, 59 (4): 978-986.

[29] Alipour, M.R.; Khamaneh, A.M.; Yousefzadeh, N.; Mohammad-nejad, D. and Soufi, F.G. (2013): Upregulation of microRNA-146a was not accompanied by downregulation of pro-inflammatory markers in diabetic kidney. Mol Biol Rep, 40 (11): 6477-6483.

الملخص العربي

دارسة الحمض النووى الريبوزي الميكرو علي المستوي الجزيئي

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الحمض النووي الريبوزي الميكرو ينتمي الي فئة من الحمض النووي الريبوزي التنظيمي الصغيرالغيرمترجم يتكون تقريبا من ٢٢ من النيوكليوتيدات في الطول تعمل من خلال الارتباط بالمنطقه الغير مترجمه من الطرف ٣ في الحمض النووي الريبوزي الرسول المستهدف تؤدي إلى تثبيطه وتحلله في مرحلة ما بعد النسخ. الحمض النووي الريبوزي الميكرو ١٤٦a متعلق بالالتهاب ويلعب دور هام في نظام المناعه . حيث يعتمد تعبيره الجيني علي وجود NF-κβ. الذي يسبب ردود فعل سلبية من الاستجابة المناعية عبر استهداف البروتينات المستهدفه ومنها (IRAK1 and TRAF6)والذين بدورهم يؤدو الي تنشيط NF-κB وعند تنشيطه يؤدي الي زياده التعبير الجيني للحمض النووي الريبوزي الميكرو ١٤٦ والذي بدوره يؤدي الي تقليل التعبير الجيني لكل من (IRAK1 and TRAF6). تهدف الدراسه الحالية الي الكشف عن الحمض النووي الريبوزي الميكرو الذي يحمل رقم ١٤٦ a والبروتينات المستهدفه وعلاقتها ببعض عمليات اضطراب التمثيل الغذائي مثل مرض السكري. لقد استخدم في هذه التجربه عشرون من ذكور الجرذان كانت مقسمة إلى مجموعتين. المجموعه الاولى:جرذان ضابطة تغذت على عليقه طبيعيه والمياه لمدة شهرين.المجموعة الثانية:جرذان مصابه بداء السكري وتغذت علي عليقه طبيعيه والمياه لمده شهرين تم ذبح الفئران بعد انتهاء مدة التجربه وتم أخذ عينات الدم من أجل القياسات الكيميائية الحيوية وكذلك عينات الانسجة المختلفة لتحليلها بواسطة التكنولوجيا الجزيئية باستخدام تفاعل البلمره المتسلسل الكمي. وقد وجد ان مستوى الحمض النووي الريبوزي الميكرو ١٤٦a في كلى الجرذان المصابة بداء السكري قد زاد اربعه اضعاف مقارنه بالمجموعه الضابطه والذي كان يرافقه انخفاض كبير في التعبير عن IRAK1 ولا يوجد أي تغييرات في مستويات TRAF6 التعبيري وعلى العكس من ذلك، زيادة كبيرة في NF-κB، TNFα، IL1β IL6. لم يعرف ما اذا كان زياده معدل التعبير الجيني للحمض النووي الريبوزي الميكرو ١٤٦a سببا للسكري أو ناتج منه. ومع ذلك،ممكن ان تتوقف الزياده على نوع النسيج وعوامل الالتهاب الاوليه المتكونه في الانسجه او في الدم وتوقيت ومدة الاتهاب كل هذه النقاط تحتاج إلى مزيد من الدراسة. الحمض النووي الريبوزي الميكرو ١٤٦a يمكن ان يستخدم كعلامه علي وجود اضطراب في التمثيل الغذائي.