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Role of Micro RNAs and its Associated Genes in Prediction of Obesity and Diabetes Mellitus (Type II)

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Abstract

Obesity and type 2 diabetes mellitus are considered as the most risk criteria in metabolic syndrome. Twenty four 3 months old male albino rats, weighting (120±15) gm were purchased from animal house at the Faculty of Veterinary Medicine, Zagazig University, Egypt. After acclimatization at 23-25°C, 12 h light-dark cycle and free availability of water and diet, rats were allocated into 2 main groups. The first group (G1) was a control group (8 rats) that was kept on a standard diet with free access to drinking water. The second group (G2) was consisted of 16 rats that were kept on high fat high fructose diet (HFHF), which then was subdivided into 2 equal subgroups, G2.1 and G2.2. Subgroup G2.1 was kept for 7 wk (Obese animals). Subgroup G2.2 was kept for 12 wk (Type II diabetic animals). Feeding HFHF diet resulted in marked increase in body weight in both obese and diabetic groups, then started to decrease and stopped between 7th and 8th wk till the end of the experiment. Moreover, HFHF diet decreased the expression levels of insulin hormone gene which was reflected on the increase in the expression levels of fibroblast growth factor 21 (FGF-21), interleukin 1 beta (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) genes. The highest level of IL-1β was recorded in the diabetic animals (G2.2). While the maximum FGF-21 gene detection in obese group (G2.1). On the other hand, IL-6 and TNF-α equally increased in both groups. Hitopathologically, hepatic tissues reveled fatty change with pancreatic atrophy in both HFHF diet treated groups. The results showed that, feeding HFHF diet resulted in increasing the expression levels of regulatory miRNA 146a and miRNA 126 genes. It could be concluded that the expression levels of miRNA 146a, miRNA 126, FGF 21 and some cytokines may be a good early indicator for obesity and diabetes mellitus type II, having a major role in the mechanism of incidence and may have a great role in the treatment and follow-up the disease.

Keywords: Micro RNAs, Associated genes, Obesity, Diabetes Mellitus (Type II).

Introduction

MicroRNAs (miRNAs) are a large family of small (≈ 22 base) regulatory non-coding RNAs that can bind to 3' untranslated region (UTR) of target sequence producing either inhibitory or enhancing effect that regulates transcriptional levels of the genes [1]. miRNAs have emerged to play important roles in many processes either physiological or pathophysiological such as organogenesis and embryonic development. They also have a role in diabetes, arrhythmia, cardiac hypertrophy, ischemic heart disease, tumor genesis, and viral hepatitis [2]. It has been also reported that miRNAs can increase expression of its target genes [3].

Recently many researchers directed their work toward studying the role of miRNA in regulating the metabolic functions in the body through controlling the expression levels of metabolic genes.

One of the main regulatory miRNA is miRNA-146a as regard its role in the immune system [4]. Its expression in inflammatory cells is induced by lipopolysaccharide and nuclear factor-κB-dependent. MiRNA-146a causes negative feedback regulation of the innate immune response via targeting the pro-inflammatory adapter proteins as Tumor necrosis receptor-associated factor 6 (TRAF6) and interleukin-1 receptor associated kinas 1

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(IRAK 1) [5]. MicroRNA-146a is involved in the pathogenesis of autoimmune diseases and several types of cancer [3]. In addition, miRNA-146a can be involved in the pathology of inflammatory degenerative diseases in human, such as prion disease [6], Alzheimer's disease [7, 8] and epilepsy [9]. In ischemic stroke the increased microRNA-126 in endothelial cells can promote angiogenesis, and attenuate vascular dysfunction in diabetics [10].

A degenerative disorder is a medical condition that causes deterioration of organs or tissues over time and many of them are related to aging, or worsen during the aging process. Three main categories are present under degenerative disorders including cardiovascular, neoplastic, and nervous system disorders. Coronary diseases, myocardial diseases and hypertension are the most common cardiovascular diseases whereas tumors and cancer are the main neoplastic and Parkinson's and Alzheimer's are the main diseases affecting nervous system [10].

Metabolic syndrome (MetS) is considered as a sequence of linked biochemical, clinical, physiological and metabolic factors that have a risk of causing type 2 diabetes mellitus, hypertension, and cardiovascular disease (CVD), resulting in increasing mortality rates [11]. It does not have a single reason but may be caused by many contributing genetic and environmental factors [12]. Elevating the hazards of MetS may be created through increasing the hereditary incidence of hypertension, type II diabetes and insulin resistance (IR) [13]. Moreover, another main fundamental factor that increases the risk of MetS is aging and environmental hazards. The dangerousness of metabolic syndrome comes from its too many complications including CVD, type II diabetes, pancreatic dysfunction, organ cancer and non-alcoholic fatty liver disease [14].

Chronic hyperglycemia increases the concentration of pro-inflammatory mediators and resulted in induction of micro- and macro-angiopathy, inflammation of the tissues ending by cell death which makes diabetes mellitus as chronic inflammatory disease [15].

Obesity can be considered as an inflammatory disorder. TNF-α has been found in obese individuals and animals and its levels are directly proportional to insulin resistance. Pathogenic role of inflammatory molecules was also proposed, like TNF-α, in the development of diabetes and insulin resistance [16]. In lean animals, TNF- α can induces insulin resistance. It is evident also that some pro-inflammatory cytokines can initiate intracellular pathways as the Nuclear Factor for Jun kinase (JNK), IkB kinase-b (IKKb) and Kappa light chain in B-cells (NF- κB) which can inhibit signaling insulin pathway [17, 18].

The aim of this study was to assess the role of some miRNAs and other inflammatory biomarkers in prediction of the onset of obesity and type 2 diabetes mellitus in susceptible individuals. Moreover, the role of miRNA and their adaptor proteins in regulating the mechanisms of some metabolic disorders as obesity and diabetes at the molecular levels was also studied.

Materials and Methods

Animal management and grouping

Three months old twenty four male albino rats, weighting (120±15) gm were purchased from animal house at the Faculty of Veterinary Medicine, Zagazig University, Egypt. The rats were kept 2 weeks 23-25°C with 12 h light-dark cycle and free availability of water and diet to be acclimatized before beginning the experiment. Rats were then randomly divided into 2 main groups, group G1 was a control group (8 rats) kept on a standard diet with free access to drinking water. The second group G2 (16 rats) was subdivided into two subgroups G2.1 and G2.2. Subgroup G2.1 (Obese group) was consisted of 8 rats and fed on high fat high fructose diet (HFHF) for 7 wk. Subgroup G2.2 (Type II diabetic group) included 8 rats that were kept for 12 wk on the same HFHF diet according to De Castro *et al.* [5]. The experimental procedures were conducted according to the guidelines of Institutional Animal Care and Use Committee of Zagazig University (ZU-IACUC) under the approval number of ZU-IACUC/2/F/24/2018.

Formulation of HFHF diet

The formulated diet was consisted of lard (60%) and fructose (17%) added to the standard rat chow, while, sucrose 10% was added to water supplied to the rats. The caloric intake of HFHF diet was calculated; Lard contains 8000 kcal /kg, Chow contains 3000 kcal/kg, and fructose contains 3000 Kcal/Kg.

The experiment lasted 12 wk. This model was planned to mimic nearly the cafeteria diets (CAF) which essentially contain high caloric value [5]. The body weight and blood glucose were taken once weekly for 12 wk [9].

Sampling

At the end of the experimental period, rats were subjected to night fasting and then decapitated by cervical dislocation. Blood were collected for serum preparation, hepatic and pancreatic tissues were also collected, washed in normal saline and divided into two parts; the first one was taken and kept in liquid nitrogen for determination of gene expression of miRNA and its receptor proteins. The other part was taken and kept in 10% formalin for histopathological investigation.

Biochemical determination

The separated serum were used for determination of serum glucose, total lipids, triacylglycerol, total cholesterol, low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), very low density lipoprotein cholesterol (VLDL-c), using specific Kits specialized for each parameter according to El-Gayar *et al.* [6], Friedewald *et al.* [8], Zoungas *et al.* [19], Bucolo and David, [4], and Young, [20], respectively. Serum insulin was determined by ELISA rat insulin kit according to the method of Wang *et al.* [21].

Molecular investigation

Total RNA was extracted from hepatic tissues using easy-RED TM Total RNA Extraction Kit for animal tissue (Intron Biotechnology cat.No.17063) according to the manufacturer's instructions. The obtained RNAs were quantified and checked for the purity using NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). Pure samples with purity more than 1.8 were used for cDNA synthesis using TOPscript™ cDNA synthesis Kit following the manufacturer's guidelines. One microliter of cDNA was used for semi-quantitative real-time PCR using TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (Intron Biotechnology cat. No. RT500S). The designed primers for the desired genes were synthesized by Bio Basic Inc., Canada. The cycling program was performed in Stratagene Mx3005P qPCR System with the following conditions: initial denaturation at 95°C for 15 min, followed by 50 cycles of denaturation at 95°C for 15 sec, annealing at various temperature according to each gene (55 °C for GAPDA, 56 °C for FGF21, 58°C for each of Insulin, IL-1β and TNF-α, 60°C for each of miRNA 146a and miRNA 126 and 62°C for IL-6) and extension at 72°C for 30 sec). The primer sequences for FGF21 were F: 5′AGATCAGGGAGGATGGAACA-3′ and R: 5′-TCAAA-GTGAGGCGATCCATA -3' according to Zhang *et al.* [22]. Insulin gene primer sequences were F: 5'-AGCAAGCAGGTCATTGTTCC-3' and R: 5'-TTGCGGGTCCTCCACTTC-3' according to Assaei *et al.* [2]. However, the primer sequences for TNF-α were F: 5′-GCCACCACGC-TCTTCTG--3'and R: 5′-GCAGCCTTGTCCCTTGA according to Li *et al.* [23]. IL-1β gene primer sequences were F: 5'-TTGTGGCTGTGGAGAAGC-TG-3' and R: 5' CCAGTTGTTGACCAAAGGCTTTT-3' according to Singh *et al.* [24]. IL-6 specific primer sequences were F: 5'-TCTCTCCGCAAGAGACTTCCA-3' and R: 5'-ATACTGGTCTGTTGTG-GGTGG-3' according to Singh *et al.* [24]. miRNA 146 a specific primer sequences were F: 5'-UGAGAACUGAAUUC-CAUGGGUU-3' and R: 5'-ACCUGUGAA-GUUCAGUUCUUU -3' according to Yamasaki *et al.* [25].Primer sequences of F: 5'-CATTATTACTTTTGGTACGCG-3' and R: 5'-GTCGTATCCAGTGCGTGTC-GTG-3' were used for miRNA 126 gene according to Huang *et al.* [10]. On the other hand, primer sequences of F: 5'-TGCA-CCACCAACTGCTTAG-3'and R: 5'-GATGCAGGGATGATGTTC-3' were used for GAPDH gene according to Schmittgen and Zakrajsek [26]. Efficiency of amplification was measured by the slope of a standard curve.

The transcription levels of target genes were normalized to those of GAPDH gene which used as reference gene. The relative expression was expressed as fold change by $2^{-\Delta\Delta CT}$ relative to control [16].

Statistical analysis

Statistical analysis was done using SPSS 16 (SPSS, Chicago, Ill) program for windows. Data were expressed as mean ± SEM and was performed using one way ANOVA to analyze the variance followed by post hoc test. The criterion for statistical significance was $P< 0.05$.

Histopathological examination

Hepatic and pancreatic tissues were collected and then washed in normal saline. After that the tissues were fixed in 10% buffered neutral formalin solution. The tissue was dehydrated in gradual ethanol (70-100%), cleared in xylene and then embedded in paraffin. The paraffin sections were prepared as five-micron thickness and then stained with hematoxylin and eosin (HE) dyes as a routine [27]. Then, the sections were examined microscopically.

Results

Feeding HFHF diet resulted in marked increase in body weight in both obese and diabetic group from 1st till 7th and 8th week then started to decrease till 12th week (Table 1). The results illustrated in Table 2 showed that, feeding HFHF diet resulted in marked increase in serum glucose, total lipids, triacylglycerol (TAG), total cholesterol, LDL-c and VLDL-c. Their respective values in control versus obese versus diabetic groups were (73.3±0.88 Vs 152.67±1.7 Vs 339.67±11), (461.3±16 Vs 694.0±38.2 Vs 968±34.0), (127.7±4.9 Vs 233.3±5.8 Vs 463.0±19.5), (195.7±3.7 Vs 244.7±9.2 Vs 314.7±16.7), (119.33±3.3 Vs 156.3±2.6 Vs 211.33±18) and (24.5±1.98 Vs 36.67±1.1 Vs 58.6±3.9). On the other hand, a significant decrease in the serum levels of both insulin and HDL-c. Their respective values were respectively, 3.20±0.20 and 39.0±4.04 in obese, 1.26±0.088 and 22.67±2.33 in diabetic group, compared to 5.0±0.057 and 54.0±4.5 in control group.

Table (1): Effect of HFHF diet feeding on body weight of adult male rats.

Group	Control	Obese	Diabetic
1st wk	125.33 ± 4.9^{a}	125.67 ± 3.0^{a}	126.12 ± 5.0^{a}
2nd wk	133.3 ± 3.88^{b}	142.67 ± 4.7^{a}	143.67 ± 11^{a}
4th wk	145.0 ± 6.057^{b}	166 ± 5.2^{a}	168 ± 9.88^{a}
6th wk	161.3 ± 6.5^{b}	194.0 ± 8.2^{a}	196 ± 4.0^{a}
7th wk	168.3 ± 6.5^{b}	211.0 ± 8.6^{a}	212 ± 4.0^{a}
8th wk	175.7 ± 5.7^{b}		210.7 ± 6.7^{a}
10th wk	191.33 ± 8.3^{b}		201.33 ± 8.6^{a}
12th wk	204.0 ± 8.5^{a}		188.67 ± 4.33^{b}

Means ± SE in the same row and carrying different superscripts are significantly different at $p<0.05$.

Table (2): Effect of HFHF diet feeding on biochemical parameters of adult male rats.

Group	Control	Obese	Diabetic
Blood Glucose (mg/dl)	73.3 ± 0.88^{d}	152.67 ± 1.7^{c}	339.67 ± 11^{a}
Serum insulin(µIU/ml)	5.0 ± 0.057^{a}	3.20 ± 0.20^{b}	1.26 ± 0.088^{d}
Total Lipids (mg/dl)	461.3 ± 16^{c}	694.0 ± 38.2^{b}	968 ± 34.0^{a}
TAG(mg/dl)	127.7 ± 4.9^{c}	233.3 ± 5.8^{b}	463.0 ± 19.5^{a}
Total cholesterol(mg/dl)	195.7 ± 3.7^{c}	244.7 ± 9.2^{b}	314.7 ± 16.7^{a}
LDL-c(mg/dl)	119.33 ± 3.3^{c}	156.3 ± 2.6^{b}	211.33 ± 18^{a}
HDL-C(mg/dl)	54.0 ± 4.5^{a}	39.0 ± 4.04^{b}	22.67 ± 2.33^{c}
VLDL-c(mg/dl)	24.5 ± 1.98^{c}	36.67 ± 1.1^{b}	58.6 ± 3.9^{a}

Means ± SE in the same row and carrying different superscripts are significantly different at $p<0.05$.

Molecular investigation

The results illustrated in Figure 1A showed that, feeding HFHF diet resulted in increasing the expression levels of regulatory miRNA 146a and miRNA 126 genes.

The results showed in Figure 1B clarified that, feeding HFHF diet resulted in decreasing the expression levels of insulin hormone gene which was reflected on the increase in the expression levels of FGF-21, IL-1β, IL-6 and TNF-α genes. IL-1β was higher in the diabetic group. IL-6 and TNF-α were increased in both groups. However, FGF-21 gene was higher in obese group.

Figure 1A

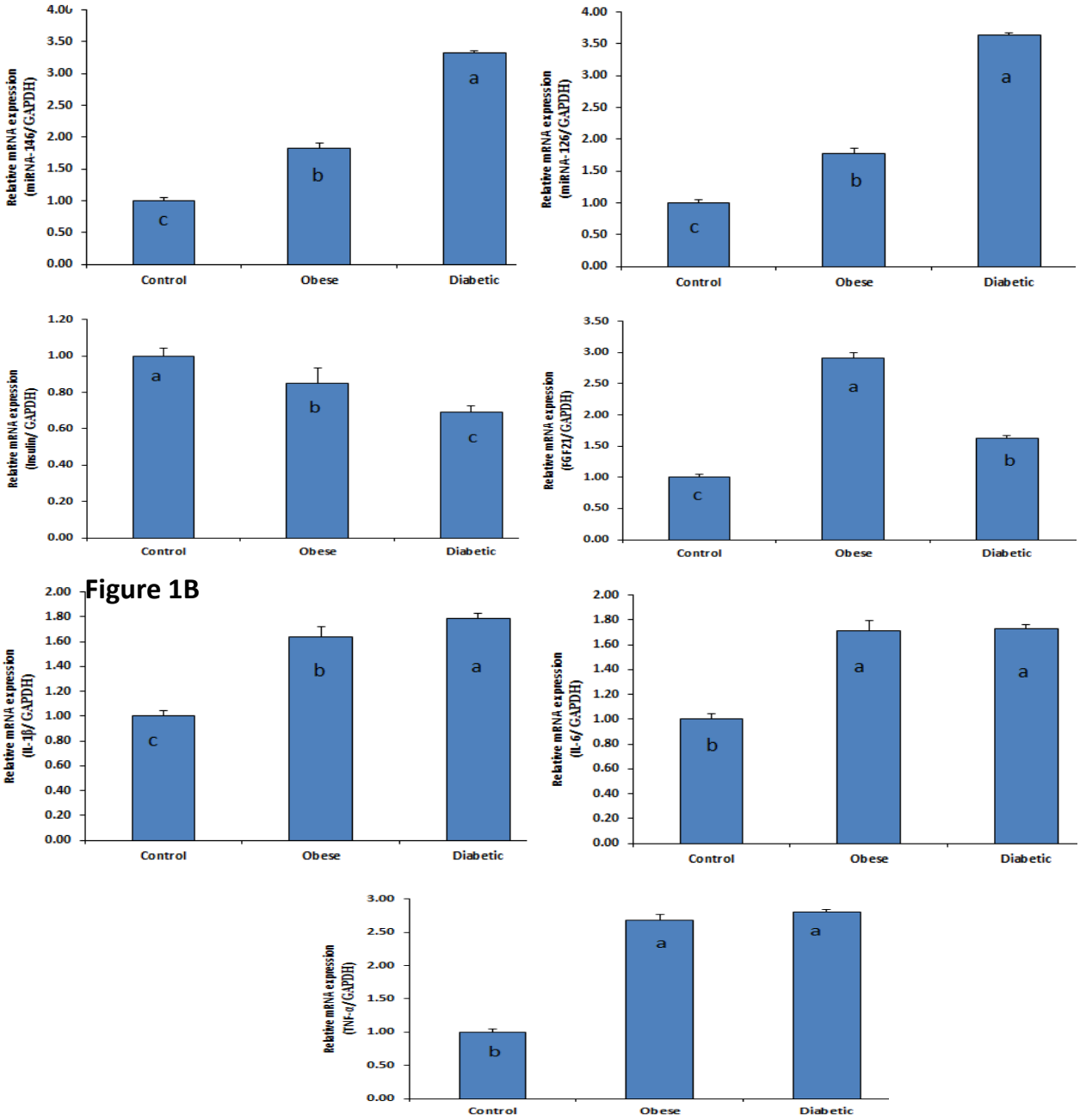

Figure 1B

Figure 1: A) the effect of HFHF diet feeding on the expression levels of miRNA-146a, miRNA-126, Insulin, Fibroblast Growth Factor 21 (FGF 21), IL-1β, IL-6 and TNF-α genes after 7 weeks (obese group) and 12 weeks (diabetic group). B) the effect of HFHF diet feeding on the expression levels of Insulin, Fibroblast Growth Factor 21 (FGF 21), IL-1β, IL-6 and TNF-α genes after 7 weeks (obese group) and 12 weeks (diabetic group).

Histopathological examination

In the control group (G1), liver tissues appeared as central vein of normal size which is surrounded by rows and cords of normal hepatocytes with central nuclei and abundant eosinophilic cytoplasm (Figure 2A). In the obese group (G2), liver tissues showed moderate fatty change of the hepatocytes with dilated congested central vein at the upper right corner (Figure 2B). However, in diabetic group (G3), liver tissue showed diffuse marked fatty change of the hepatocytes with clear cytoplasm and central vein at the upper right corner (Figure 2C). The control group (G1) showed normal sized islets of Langerhans surrounded by pancreatic acini (Figure 3A). Atrophy of islets Langerhans surrounded by normal pancreatic acini was detected in obese group and pancreatic group (Figure 3 B and C).

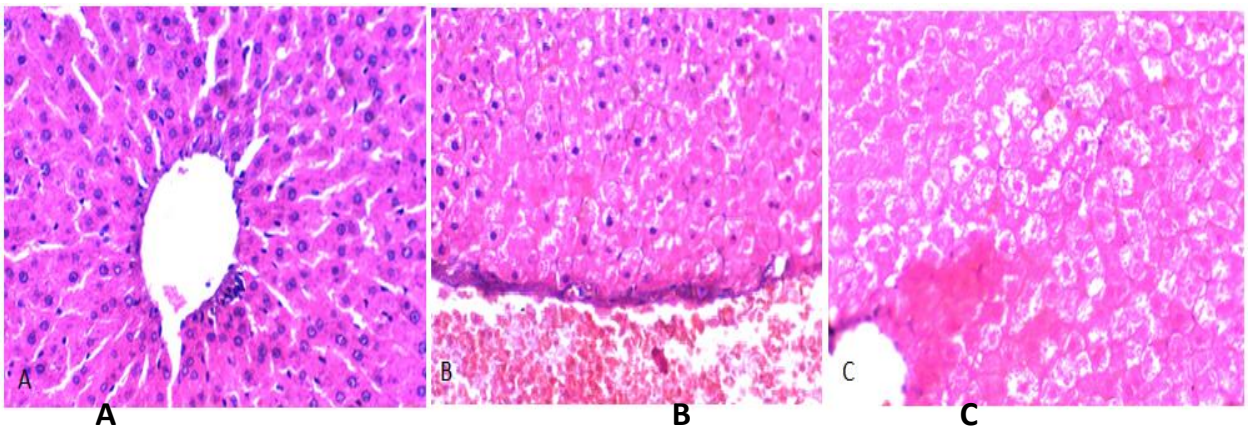

Figure 2: Histopathological findings of HFHF diet on hepatic tissues of adult male rats. A): from control group liver tissues was of normal sized central vein surrounded by rows and cords of normal hepatocytes with central nuclei and abundant eosinephilic cytoplasm B): from obese group liver tissues of obese rat exposed to (lard +fructose+ sucrose) for 7 weeks showed moderate fatty change of the hepatocytes with dilated congested central vein at the upper right corner C): from diabetic group liver tissue of diabetic rat exposed to (lard +fructose+ sucrose) for 12 weeks showed diffuse marked fatty change of the hepatocytes with clear cytoplasm and central vein at the upper right corner (H&EX400).

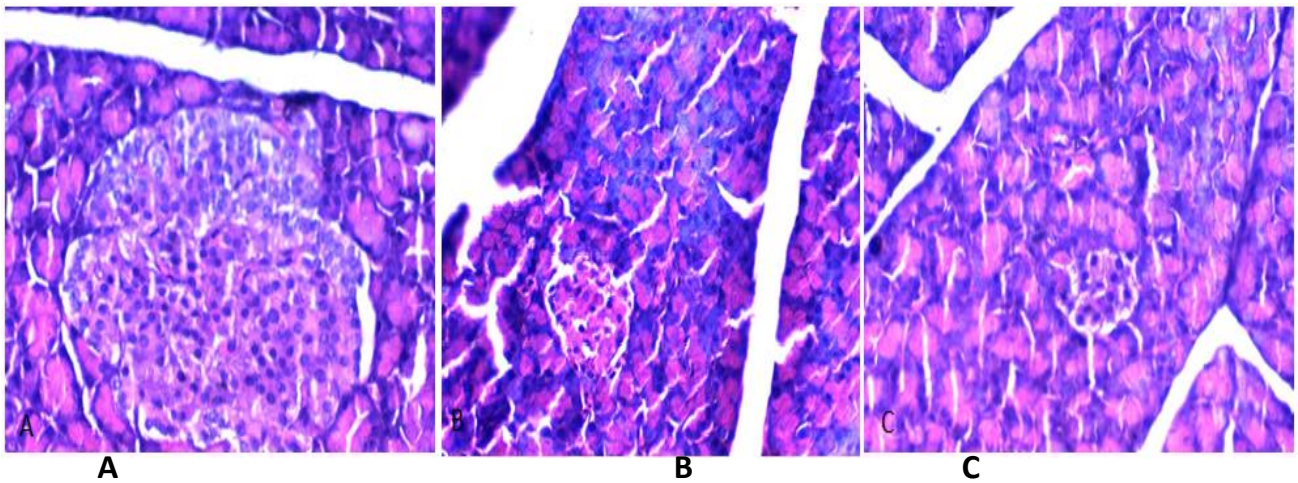

Figure 3: Histopathological findings of HFHF diet feeding on pancreatic tissues of adult male rats A): from control group showed normal sized islets of langerhans surrounded by pancreatic acini B): from obese group pancreatic tissue of obese rats exposed to (lard +fructose +sucrose) for 7 weeks showing atrophy of islets langerhans surrounded by pancreatic acini. C): from diabetic group rats exposed to (lard +fructose +sucrose) for 12 weeks showed marked atrophy of islets langerhans surrounded by normal pancreatic acini (H & E X400).

Discussion

There is no doubt that, metabolic and degenerative disorders now represent a great threat to life especially that is associated with new lifestyle and fast food that result in obesity and diabetes mellitus especially type II. Finding a new markers for early diagnosis and detection of metabolic syndromes, obesity and diabetes is an important point interested many scientists, also standing on the exact mechanism and role of these new markers in the mechanism of obesity, fat disorders, lipodystrophy and predisposing factors and mechanisms of type II diabetes is a branching point in reaching a new techniques facing all that problems. In the light of the above mentioned points, The current study were directed toward studying the expression pattern of some genes involved in metabolic pathway for obesity and type II diabetes in case of high fat high fructose (HFHF) diet feeding for induction of obesity in 7 wk or even induction of type II diabetes mellitus when taken for 12 wk.; at the same time serum glucose, insulin and lipid profile pattern was determined with that treatment.

According to De castro *et al*. [5], our results confirmed that, feeding HFHF diet increased the body weight till the 7th week and then the body weight started to decrease which may reflects the induction of Type II diabetes mellitus by HFHF diet feeding for 12 weeks. At the same line, the results of serum glucose and insulin levels and other lipid profile illustrated the body profile in obesity and diabetes mellitus that represented in high levels of serum glucose, total lipids, TAG, total cholesterol, LDL-c and VLDL-c with a decrease in serum insulin levels and HDL-c which reflects a state of a lipid disorder may be predisposing to many metabolic disorders, hypertension, CHD and so many degenerative disorders.

Many reports have addressed the effect of HFHF diet feeding on the glucose homeostasis but till now there is no exact data for the detailed effect on the molecular mechanism of the insulin resistant state and metabolic disorder state that produced by HFHF diet feeding (5). So, this study dealt with that problem and the obtained results revealed that HFHF diet feeding resulted in a significant decrease in the expression level on insulin hormone gene with a significant increase in the expression levels of Fibroblast Growth Factor 21 (FGF 21), IL-1β, IL-6 and TNF-α genes.

Previous reports stated that, HFHF diet resulted in an increase in the expression levels of insulin hormone together with a decrease in the insulin receptor gene expression which creates a state of insulin resistance and type II diabetes mellitus [28]. This point is inconsistent with our result that revealed a decrease in the expression that may be attributed to β-cell destruction that follow the insulin resistance state as suggested by Balakumar *et al.* [29].

Fibroblast growth factor 21 (FGF21) Fisher *et al.* [7] is an important mediator of lipid metabolism and fatty acid oxidation. It has a major role in controlling the fatty acid oxidation and so, weight reduction. It is clearly evident that the increased tissue expression and serum levels of FGF21 can be associated with obesity state. In genetically obese ob/ob mice [3] and db/db mice [30] the levels are increased.

The increase in the expression levels of FGF 21 was more in obese group than that for diabetic group which reflects the role of FGF 21 in the obesity state [7]. demonstrated that, exogenous FGF21 protein is associated with weight reduction, but observation of the expression level increases with a weight gain is refers to a state of FGF21 resistance with a decrease in the ability of FGF 21 to initiates the signaling of regulatory genes involved in fat loss through the ras-raf-MAPK cascade, with a reduction in the ability to induce the expected changes in immediate early genes Egr1 and cFos expression.

The pro- and anti-inflammatory cytokines IL-1β, IL-6 and TNF-α genes were also increased in their mRNA levels by HFHF diet feeding when compared with control group. This increase runs in the same pattern in both obese and diabetic groups except for IL-1β that has more increase in diabetic group than that observed in obese group which reflects the chronic inflammatory condition approved in case of type II diabetes mellitus according to

De Castro *et al.* [5] than that observed in case of obesity state. High-fructose diet in rats has been reported to activate the inflammatory factors such nuclear factor kappa B (NFκB) and TNFα in the liver, but not in the adipose tissue, showing its tissue-specific effects [31]. Consumption of HFHF diet for 8 and 12 weeks caused up-regulation TNFα, IL-1β, and IL-6. In obesity, macrophages infiltrate adipose tissue and begin to induce pro-inflammatory cytokines, such as IL-1β, TNFα, and IL-6, which contribute to insulin resistance. High-fructose diet in rats has been reported to activate the inflammatory factors including NF-κB and TNFα in the liver. [32, 33].

Type 2 diabetes mellitus (T2DM) is a major public health problem all over the world. Identifying new diagnostic markers is so important to identify the individuals who are at risk to develop T2DM to achieve healthy lifestyle [30].

Circulating miRNAs can be utilized as non-invasive biomarkers in many diseases. At the levels of diabetes, miRNAs has a major role in regulating the mechanism of insulin secretion and levels. There was a significant increase in the expression levels of both miRNA 146a and miRNA 126 genes. This increase in the expression levels was in response to decrease in the insulin levels observed in both obese and diabetic groups as supposed by Zhang *et al.* [30], and so this increase in the expression levels was more in diabetic group than obese group. Several studies indicated the association between miRNA 146a, miRNA 126 and diabetes [34- 35].

Conclusion

From all the above obtained results, it can be concluded that, Feeding HFHF diet disrupts the normal metabolic state in the body and resulted in marked increase in the body weight, serum glucose, and lipid profile which is predispose to obesity and diabetic syndrome. The expression levels of miRNA 146a, miRNA 126, FGF 21 and some cytokines may be good early indicators for obesity and diabetes mellitus type II, having a major role in the mechanism of incidence and may have a great role in the treatment and follow-up the disease.

Conflict of interest

The authors have no conflict of interest to declare

References

[1] Alexandrov, P.N.; Dua, P. and Lukiw, W.J. (2014): Up-regulation of miRNA-146a in progressive, age-related inflammatory neurodegenerative disorders of the human CNS. Front Neurol, 5: 1-5.

[2] Assaei, R.; Mokarram, P.; Dastghaib, S.; Darbandi, S.; Darbandi, M.; Zal F.; Akmali, M. and Omrani, G.H. (2016): Hypoglycemic effect of aquatic extract of stevia in pancreas of diabetic rats: PPARγ-dependent regulation or antioxidant potential. Avicenna J Med Biotechnol, 8: 65-74.

[3] Badman, M.K.; Kennedy, A.R.; Adams, A.C.; Pissios, P. and Maratos-Flier E. (2009): A Very Low Carbohydrate Ketogenic Diet Improves Glucose Tolerance in ob/ob Mice Independent of Weight Loss. Am J Physiol Endocrinol Metab, 297:5: 1197-1204.

[4] Bucolo, G. and David, H. (1973): Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem, 19: 476-82.

[5] De Castro, UG.; Silva, M.E.; de Lima, WG.; Campagnole-Santos, MJ. and Alzamora AC. (2013): Age-dependent effect of high-fructose and high-fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. Lipids Health Dis, 12:136.

[6] El-Gayar, K.E.; Ibrahim, M.A.; Mohamed, S.H.; Zakaria, Z. and Alhameed A.S.A. (2012): Application of extracted peroxidase enzyme from turnip roots (BRASSICA NAPUS) in clinical diagnostic kit. Int J Cur Res Rev, 4: 17-22.

[7] Fisher, FM.; Chui, P.C.; Antonellis, P.J.; Bina, H.A.; Kharitonenkov, A.; Flier, J.S. and Flier, E.M. (2010): Obesity Is a Fibroblast Growth Factor 21(FGF21)-Resistant State. Diabetes, 59: 2781-2789.

[8] Friedewald, W.T.; Levy, R.I. and Fredrickson, D.S. (1972): Estimation of the

concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, 18: 499-502.

[9] Gancheva, S.; Zhelyazkova-Savova, M.; Galunska, B. and Chervenkov, T. (2015): Experimental models of metabolic syndrome in rats. Scr Sci Med, 47: 14-21.

[10] Huang, C.; Xiao, X.; Chintagari, N.R.; Breshears, M.; Wang, Y. and Liu, L. (2014): MicroRNA and mRNA expression profiling in rat acute respiratory distress syndrome. BMC Med Genomics, 7: 46.

[11] Kaur, J.A. (2014): Comprehensive Review on Metabolic Syndrome. Cardiol Res Pract, 1: 1-21.

[12] Rampal, S.; Mahadeva, S.; Guallar, E.; Bulgiba, A.; Mohamed, R.; Rahmat, R.; ... and Rampal, L. (2012): Ethnic differences in the prevalence of metabolic syndrome: results from a multi-ethnic population-based survey in Malaysia. PLoS One, 7: e46365.

[13] Ranasinghe, P.; Cooray, D.N.; Jayawardena, R. and Katulanda, P. (2015): The influence of family history of hypertension on disease prevalence and associated metabolic risk factors among Sri Lankan adults. BMC Public Health, 15:576.

[14] Panchal, SK. and Brown, L. (2011): Rodent models for metabolic syndrome research. J Biomed Biotechnol, 351982: 1-12.

[15] Li, X.; Meng, Y.; Wu, P.; Zhang, Z. and Yang, X. (2007): Angiotensin II and Aldosterone stimulating NF-κB and AP-1 activation in hepatic fibrosis of rat. Regul pept, 1381: 15-25.

[16] Livak, K.J. and Schmittgen, T.D. (2001): Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods, 25: 402-408.

[17] Penttilä, I.M.; Voutilainen, E.; Laitinen P. and Juutilainen, P. (1981): Comparison of different analytical and precipitation methods for direct estimation of serum high-density lipoprotein cholesterol. Scand J Clin Lab Invest, 41: 353-360.

[18] Pers, Y.M. and Jorgensen, C. (2013): MicroRNA in bio therapeutic potential of microRNAs in rheumatic diseases, Nat Rev Rheumatol, 9: 76- 78.

[19] Zoungas, S.; Chalmers, J.; Neal, B.; Billot, L.; Li, Q.; Hirakawa, Y.; Arima H.; Monaghan, H.; Joshi, R.; Colagiuri, S. and Cooper, M.E. (2014): Follow-up of blood-pressure lowering and glucose control in type 2 diabetes. N Engl J Med, 371: 1392-1406.

[20] Young, D.S. (2002): Effects of Disease on Clinical Laboratory Tests, 4th Edition, Vol. 1 and 2. D.S. Young and R.B. Friedman, eds. Washington, DC: AACC Press, 2001.

[21] Wang, D.; Zhu, W.; Li, J.; An, C. and Wang, Z. (2013): Serum concentrations of fibroblast growth factors 19 and 21 in women with gestational diabetes mellitus: association with insulin resistance, adiponectin, and polycystic ovary syndrome history. PloS One, 8: 81190.

[22] Zhang, X.; Yeung, D.C.; Karpisek, M.; Stejskal, D.; Zhou, Z.G.; Liu, F.; … and Xu, A. (2008): Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes, 57: 1246–1253

[23] Li, L.; Shang, Q.; Lv, C.; Wang, C. and Su, B. (2015): Circulating miR-126 is a potential biomarker to predict the onset of type 2 diabetes mellitus in susceptible individuals. Biochem Biophys Res Commun, 463: 60-63.

[24] Singh, H.; Samani, D.; Nema, V.; Ghate, M.V and Gangakhedkar, R. R. (2018): IL-1RN and IL-1β Polymorphism and ARV-Associated Hepatotoxicity; Mediators Inflamm, 4398150: 1-13.

[25] Yamasaki, K.; Nakasa, T.; Miyaki, S.; Ishikawa, M.; Deie M.; Adachi N;… and Ochi, M. (2009): "Expression of MicroRNA-146a in osteoarthritis

cartilage". Arthritis Rheum, 60: 1035–١٠41.

[26] Schmittgen, T.D. and Zakrajsek, B.A. (2000): Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR. J Biochem Biophys Methods, 46 69-81.

[27] Bancroft, J.D. and Gamble, M. (2008): Theory and Practice of Histological Techniques. 6th edition, Churchill Livingstone, Elsevier, China; chapter 6 53-63.

[28] Lorenzo, C.; Wagenknecht, L.E.; D'Agostino, R.B.; Rewers, M.J.; Karter, A.J. and Haffner, S.M. (2010): Insulin resistance, beta-cell dysfunction, and conversion to type 2 diabetes in a multiethnic population: the insulin resistance atherosclerosis study. Diabetes Care, 33: 67–72.

[29] Balakumar, M.; Raji, L.; Prabhu, D.; Sathishkumar, C.; Prabu, P.; Mohan, V. and Balasubramanyam, M. (2016): High-fructose diet is as detrimental as high-fat diet in the induction of insulin resistance and diabetes mediated by hepatic/pancreatic endoplasmic reticulum (ER) stress. Mol Cell Biochem, 423: 93-104.

[30] Zhang, T.; Lv, C.; Li, L.; Chen, S.; Liu, S.; Wang, C. and Su, B. (2013): Plasma miR-126 is a potential biomarker for early prediction of type 2 diabetes mellitus in susceptible individuals, Biomed Res Int, 761617: 1-6.

[31] Velickovi, V.; Djordjevic, A.; Vasiljevi, A.; Bursa, B.; Milutinovic, B. and Mati, G. (2013): Tissue-specific regulation of inflammation by macrophage migration inhibitory factor and glucocorticoids in fructose-fed Wistar rats. Br J Nutr, 110: 456–465.

[32] Schneider, K.S. and Chan, J.Y. (2013): Emerging role of Nrf2 in adipocytes and adipose biology. Adv Nutr, 4: 62–66.

[33] Enos, R.T.; Velazquez, K.T.; McClellan, J.L.; Cranford, T.L.; Walla, M.D. and Murphy, E.A. (2014): Reducing the dietary omega6: omega-3 utilizing a-linolenic acid; Not a sufficient therapy for attenuating high-fat-diet-induced obesity development nor related detrimental metabolic and adipose tissue inflammatory outcomes. PLoS One, 9: e94897.

[34] Zampetaki, A.; Kiechl, S.; Drozdov, I.; Willeit, P.; Mayr, U.; Prokopi, M.; …. and Mayr, M., (2010): Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAsin type 2 diabetes, Circ. Res. 107: 810- 817.

[35] Zhao, C.; Dong, J.; Jiang, T.; Shi, Z.; Yu, B.; Zhu, Y.; … and Sha, J. (2011): Early second-trimester serum miRNA profiling predicts gestational diabetes mellitus, PLoS One, 6: 23925.

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الملخص العربي

دور الحمض النووى الريبوزى الميكروى والجينات المرتبطة به في التنبؤ بالبدانة والسكري (النوع الثاني)

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تعتبر السمنة والنوع الثاني من داء السكري من أكثر معايير الخطر في متلازمة التمثيل الغذائي. تم شراء أربعة وعشرون ذكرا من الجرزان الالبينو (وزن مرجح ٣ أشهر (١٢٠ ± ١٥) جم في بداية التجربة. تم الاحتفاظ بالجرذان في ظروف مناسبة (درجة حرارة ٢٣-٢٥ درجة مئوية مع ١٢ ساعة دورة مظلمة وخالية من المياه والحمية) ثم تقسيمهم عشوائياً إلى مجموعتين رئيسيتين بعد تأقلمهم لمدة أسبوعين، المجموعة الأولى كانت مجموعة مراقبة (٨ جرذان) محفوظة على نظام غذائي معياري مع إتاحة الوصول إلى مياه الشرب. المجموعة الثانية (١٦ جرذ) تم تغذيتها على نسبة عالية من الدهون عالية الفركتوز (HFHF) و هذه المجموعة تم تقسيمها الى ٨ جرذان تم الاحتفاظ بها لمدة ٧ أسابيع (G2.1) و التى تعتبر مجموعة ممثلة للسمنة و تم الاحتفاظ بالجرزان الثمانية الأخرى لمدة ١٢ أسبوعًا (G2.2) و التى تعتبر مجموعة ممثلة لمرضى السكري من النوع الثاني . أدت تغذية الجرزان على علائق عالية الدهون و الفركتوز الى زيادة ملحوظة في وزن الجسم في كل من مجموعة السمنة والسكري ،بدأت هذه الزيادة فى الانخفاض ثم توقفت بين الأسبوعين السابع والثامن.أدت هذه التغذية أيضا الى انخفاض مستويات التعبير عن جين هرمون الأنسولين الذي انعكس على زيادة مستويات التعبير من جينات FGF-21 ، IL-1β ، IL-6و .TNF-α. كانت الزيادة في IL-1β أكثر في المجموعة المصابة بالسكري ، بينما كانت الزيادة في IL-6 و-TNF αمتساوية فى المجموعتين, أوضحت النتائج زيادة فى FGF-21 و التى كانت اكثر فى المجموعة المصابة بالسمنة. كما أظهرت التحاليل الباثولوجيه حدوث تغيرات ملحوظه فى كل من انسجة الكبد والبنكرياس نتيجة المعاملة بهذه العليقة. مما سبق قد تكون مستويات التعبير من miRNA 146a و miRNA 126 و FGF 21 وبعض السيتوكينات مؤشرات مبكرة جيدة للسمنة وداء السكري من النوع الثاني ، ولها دور رئيسي في آلية الإصابة وقد يكون لها دور كبير في علاج ومتابعة المرض