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Obesity in induced female rats enhances cardiac fibrosis signaling pathway via Lipofuscin receptors

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Abstract

Background:

Today, obesity is a growing disease that is associated with a variety of cardiovascular risk factors. One of the causes of obesity with high-fat is the accumulation of Lipofuscin in cells .receptor of Lipofuscin is (retinoic acid receptor) A2E that is related to the enhancement of several signaling pathways including aging, autophagy and ultimately cell death. in this study assessment of the role of Lipofuscin receptors and PKR receptors in fibrosis induction.

Methods:

Induction of obesity female rats was done with a high-fat diet. The heart of each rat was removed. heart sections were transferred to glass slides, stained with hematoxylin-eosin (H&E) solution for morphological evaluation under a light microscope. The Protein was isolated from the heart tissue by lysis buffer. then protein content of PKR1, RARα, Smad2, TGF-β was assessed by western blotting.

Results:

After proving obesity in female rats. the heart of the rats after induction of obesity with a high-fat diet increased connective tissue compared to the control group in histopathology. Western blotting showed an increase in the protein level of TGF-β, RARα and SMAD2 in heart of obesity rat compare with the control group. but results showed a decrease in the protein level of PKR1in heart of obesity rat compare with the control group.

Conclusion:

Induction of obesity with a high-fat diet can stimulate cell fibrosis pathways in cardiac tissue through stimulation of lipofuscin receptors via Smad and TGF-β signaling pathways.

Keywords: High-Fat Diet, Fibrosis, Lipofuscin, Cardiac.

Intoduction

Today, obesity is a growing disease that is associated with a variety of cardiovascular risk factors. Obesity is a restructuring of cardiac function that contributes to cardiac dysfunction[1]. Direct intracellular signaling causes cardiac dysfunction. One of the causes of obesity with high-fat induction is the accumulation of lipofuscin in cells[2]. Although it is non-toxic it can accumulate in the cell by reduces the useful cell space and thereby decreases cellular function. This is especially important in nerve cells, heart muscle and other organs[3]. acid receptor A2E receptors enhance several signaling pathways including aging, autophagy and ultimately cell death[4]. lipofuscin is an aging biomarker .lipofuscin act via retinoic acid receptors with causing a number of processes including changes in intracellular cytoskeleton, alteration, and accumulation of intracellular proteins, or changes in the level of protease balance[5]. On the other hand, in addition to the activity of retinoic acid receptors, other cellular receptors will also be linked to obesity and its effect on the cardiovascular system[6]. PKR1 signaling through AKT activity enhances the De novo vasculogenesis pathway. PKR1 plays a major role in protecting cardiomyocytes [7]. The issue is the crosstalk path of these two receptors and their activity, which may overlap, but the overlap between these two pathways is unknown to date[8]. It could be the recent attention given to these two receptors as a factor in the disorders caused by obesity. The role of lipofuscin in aging has been established over the last decade[9]. the key point in this study is the role of lipofuscin and PKR receptors in fibrosis induction of fibrosis signaling pathway.

Induction of obesity female rats by using high-fat diet

In this study, rats (160 g, 8 weeks old) were isolated from the animal house of Tabriz University of Medical Sciences and kept at 22 ° C for one week to maintain stable environmental conditions. Rats were divided into two groups randomly. In the control group, rats were fed on 11% fat, 28% protein and 61% carbohydrate. The rats were checked weekly and at the end after 8 weeks the necessary assessments were made.

The rats were anesthetized by injecting a combination of ketamine (75 mg / kg) and xylazine (3 mg / kg) intraperitoneally.After opening the chests, heart tissue was isolated and immediately put into a cool and oxygenated solution.

Pathological Evaluation

The heart of each rat was removed, fixed by fully immersion in 10% buffered formaldehyde solution (37%, Merck, Germany) for 1 week, embedded in paraffin, and cut into 4-µm-thick sections using a microtome. All sections were carefully transferred to glass slides, stained with hematoxylin-eosin (H&E) solution for morphological evaluation under a light microscope. The severity of heart injury in all experimental groups was calculated by using a semi-quantitative scoring system based on following pathological changes.

Evaluating the protein content of PKR1,RARa,Smad2,TGF2 by western blotting

Protein was isolated from heart tissue by lysis buffer (NaCl, Tris-HCl, NP-40 supplemented with inhibitor) for 30 min. To collect the soluble protein samples were centrifuged at 11,000 rpm for 20 min and supernatant harvested and stored at -20 °C until use. , 50 µg of protein lysate was loaded in each lane of 10% sulfate-polyacrylamide gel electrophoresis (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and transferred to nitrocellulose membranes (Pierce). The membranes were blocked for 2 h in a non-fat dried milk solution (5% in Tris-buffered saline) containing in 0.5% Tween 20, and then incubated with proprietary primary antibodies for 2h at 4°C. Specific protein expressions were revealed by enhanced chemiluminescent reagents (Thermo Scientific, Beijing, China) and detected using X-ray film. GAPDH were

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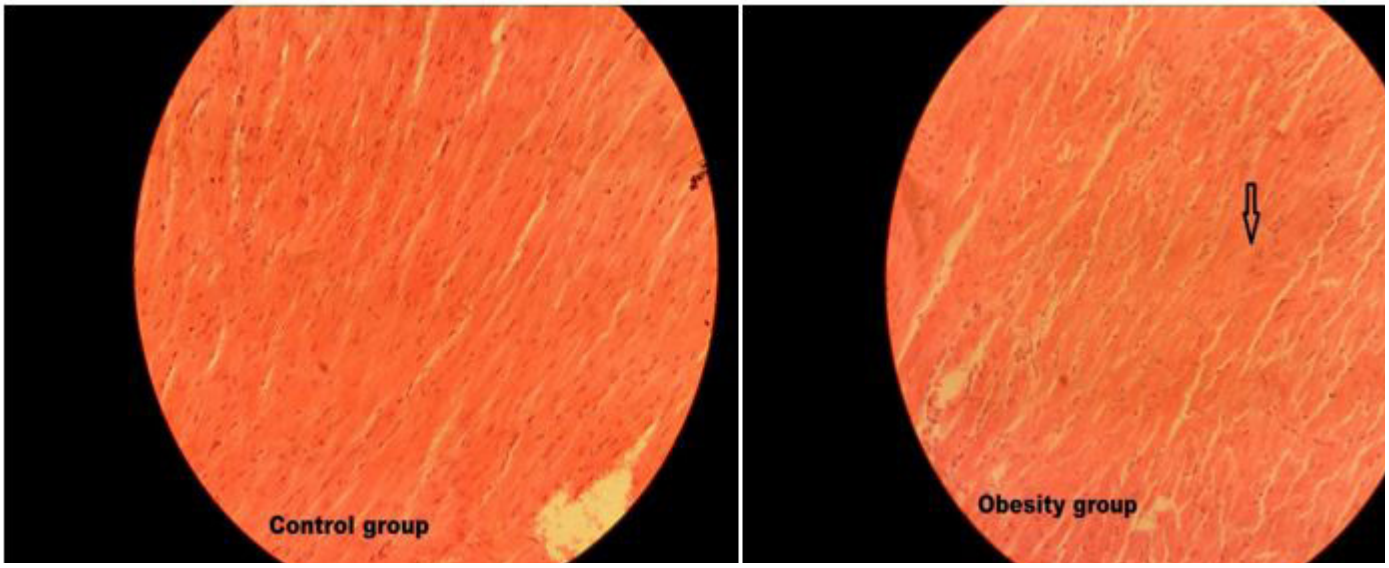
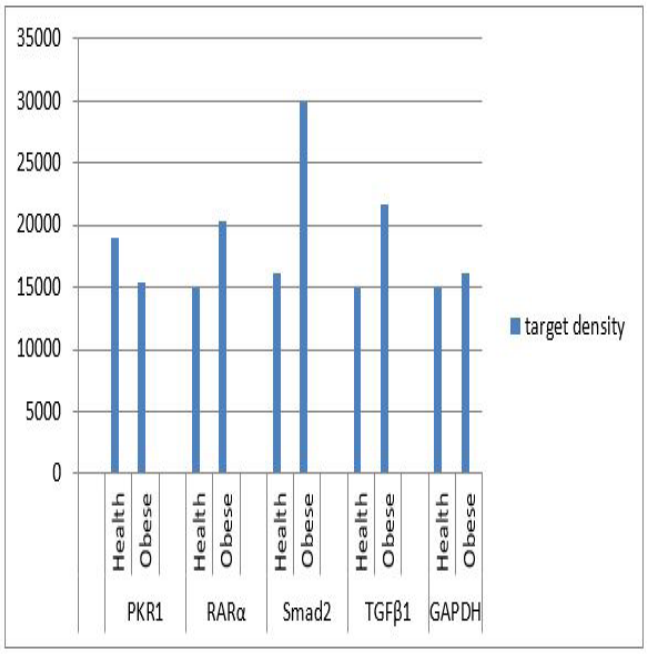


***Figure 1**: Histopatological changes of the female rat heart with hematoxylin eosin staining.*



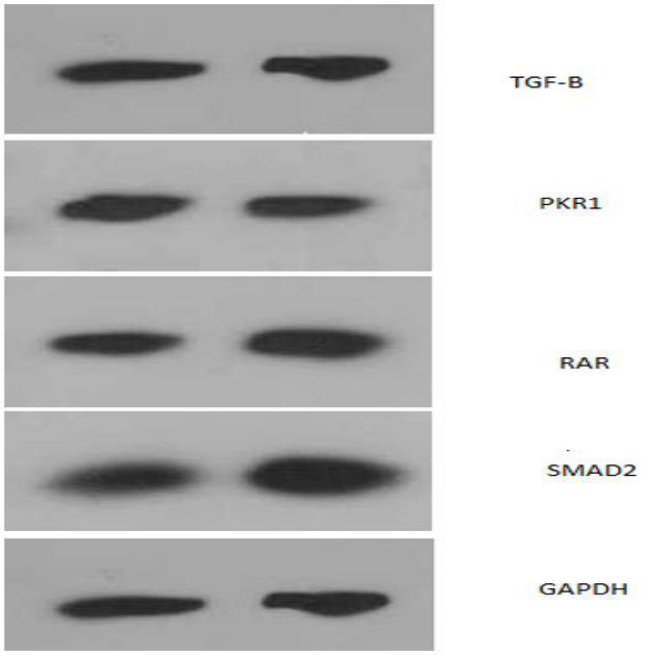


***Figure 2**: Level of TGF-B,PKR1,RAR and SMAD2 detected by Western blotting.*

Showed that the TGF-B,RAR and SMAD2 content were increased after Induction of obesity with hight fat diete.as you see PKR1 content was decreased after induction of obesity with hifgt fat diete.
used as loading controls. The data were presented as fold-increases relative to the control.expression genes(PKR1,RARa,Smad2,TGF2).

Results

Body Weight Estimation

Body weight of control and rats Treatment was measured weekly, mean weight gain percentage The body was also used in both groups at the end of the sixth week The following formula was calculated[10] Pathological Evaluation.

Histopathological changes

As shown in Figure 1, the heart of the rats after induction of obesity with a high-fat diet increased connective tissue compared to the control group.

Changing Level of protein expression TGF-B, PKR1, RAR and SMAD2 in heart rat

Western blotting showed an increase in the protein level of TGF-B, RAR and SMAD2 in heart of obesity rat compare with the control group (Fig.2). but we were decreased in the protein level of PKR1in heart of obesity rat compare with the control group as you see in Fig .2.

Discussion

Heart failure is a growing disease that by increasing cardiac output. Obesity can play an important role in remodeling and causing fibrosis it can due to decreased cardiomyocyte function [11]. Heart fibrosis is involved in heart failure that cause by fibroblasts[12]. These cells are known as myofibroblasts in cardiac [12].In this study, after induction of high-fat obesity in female rats, cardiac tissue was evaluated, results were shown that RARα protein expression was increased in comparison to the control group. Another study confirmed the role of RARα in hyperglycemia-induced by apoptosis[13]. Decreased expression of RARα will activate the apoptotic signaling pathway in the cardiovascular system. the expression of TGF-β, Smad protein was increased after the induction of obesity in heart tissue[14]. TGF-β is a key factor in the activation of myofibroblasts that facilitates the production of extracellular matrix. TGF induces phosphorylation transcription factors such as smad2 and smad4 in heart failure [15]. Previous studies have shown that cardiac vascular fibrosis is marked by elevated expression of growth factor (TGF) -β and plasminogen

activator inhibitor (PAI) -1 [16]. The association between the hyperactive TGF-β cascade and cardiac fibrosis is well established. TGF-β fibrogenic actions are primarily mediated through effects related to Smad signaling, although Smad-independent actions are also involved[17]. Increased TGF-β expression in myocardial has been consistently reported in experimental models of obesity and associated with cardiac fibrosis[18]. Another result of this study was the alteration of PKR1 protein expression level after induction of obesity with a high-fat diet. Signaling through Prokineticin and its receptor, PKR1, is required for cardiomyocyte survival during hypoxic stress[19]. Binding of Prokineticin to PKR1 causes proliferation, migration, and angiogenesis in endothelial cells. Expression of PK and PKR1 is increased during cardiac remodeling after myocardial infarction[20]. Low levels of PKR1 protein transcripts in heart samples from humans with end-stage heart failure indicate the importance of PKR1 signaling in the heart. PKR1 signaling through Akt protects cardiomyocytes against apoptosis. In addition, it can cause endogenesis under injury conditions[8, 19].

In overall, induction of obesity in high-fat female rats induced the expression of RAR receptors, which increased protein expression in the fibrosis pathway, including TGF-β and Smad. Considering the RARα receptor as the receptor for lipofuscin aggregates induced by adipocyte adhesion, this receptor will act as a fibrosis signaling pathway activator in the cardiac tissue of obese female rats.

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Competing interests

The authors declare that they have no competing interests.

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