European Journal of Obstetrics & Gynecology and Reproductive Biology 222 (2018) 166–170

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

Diurnal rhythm of follicle-stimulating hormone is associated with nonalcoholic fatty liver disease in a Chinese elderly population

Xiaoming Li[a], Long Jing[b,1], Fang Lin[a], Huan Huang[a], Zhizhong Chen[b], Yan Chen[a], Lina Wang[a], Xing Lin[a], Tailin Guo[a], Jin Yang[a], Jingming Ruan[a], Kaiyang Lin[a], Chunjing Li[a], Zhebing You[a], Linlin He[a], Jiankang Chen[a], Zhuzhou Li[a], Pengli Zhu[a,*], Gang Chen[c,*]

[a] Department of Geriatric Medicine, Fujian Provincial Hospital, Fujian Provincial Institute of Clinical Geriatrics, Fujian Provincial Key Laboratory of Geriatric Diseases, Fujian Medical University, Fuzhou, 350001, China
[b] Department of Pathology, Fujian Provincial Hospital, Fujian Medical University, Fuzhou, 350001, China
[c] Department of Endocrinology, Fujian Provincial Hospital Key Laboratory of Endocrinology, Fujian Medical University, Fuzhou, 350001, China

ARTICLE INFO

Article history:
Received 31 January 2017
Received in revised form 14 January 2018
Accepted 29 January 2018
Available online xxx

Keywords:
Nonalcoholic fatty liver disease (NAFLD)
Follicle-stimulating hormone (FSH)
Diurnal rhythm

ABSTRACT

Objective: Previous studies have found that impairment of the circadian clock appears to contribute to the development of nonalcoholic fatty liver disease (NAFLD) and the circulating follicle-stimulating hormone (FSH) level showed a diurnal cycle. A recent study reported that a lower FSH level was associated with NAFLD. However, the effects of the diurnal rhythm of FSH on NAFLD have not been reported. The aim of this study was to evaluate whether the diurnal rhythm of FSH was associated with NAFLD in an elderly population.

Study design: We performed a cross-sectional study among 71 elderly patients between August 2015 and November 2015 at Fujian Provincial Hospital. Anthropometrics and tests for laboratory were performed for each patient. FSH was determined by radioimmunoassay. The FSH receptor (FSHR) expression was identified in liver and ovary tissue by immunohistochemical staining. NAFLD was diagnosed by sonographic features.

Results: Of the 71 patients, 33 (42.9%) had NAFLD on their ultrasound. There were no significant differences between subjects with NAFLD and those without NAFLD in terms of age, sex, body mass index, waist-to-hip ratio, fasting plasma glucose, postload plasma glucose, liver enzyme, triglycerides, total cholesterol, high-density lipoprotein-cholesterol and low-density lipoprotein-cholesterol. Both the serum FSH levels of 8AM and 0AM showed no differences between the groups. The proportion of the 'normal' diurnal rhythm of FSH was higher among the patients with NAFLD (78.1% vs. 52.6%, $P = .027$). After adjusting for all potential confounders, the fully adjusted odds ratios (OR) of diurnal rhythm of FSH for NAFLD was 3.86 (95%CI: 1.01, 14.81, $P = .049$). Immunohistochemical staining showed that the FSHR protein was detected in human ovarian and hepatic tissues.

Conclusions: These results suggest that the 'normal' diurnal rhythm of FSH was independently associated with NAFLD in an elderly population. This study provides a novel insight into the diurnal rhythm of FSH in the pathogenesis of NAFLD.

Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to a wide spectrum of chronic liver diseases, ranging from fatty liver alone to nonalcoholic steatohepatitis with or without inflammation and fibrosis [1]. The prevalence of NAFLD is between approximately 10–30% of the general population in various countries and is considered to be increasing [2,3]. NAFLD is linked to increased morbidity and mortality rates, independent of traditional risk factors [4,5]. Therefore, elucidating the mechanism involved in the temporal regulation of NAFLD is of great interest. The pathogenesis of NAFLD is multifactorial [6]. Obesity, malnutrition and physical inactivity are recognized as such key-effectors [7]. Recent studies have demonstrated the relationship between circadian clock

* Corresponding authors.
E-mail addresses: zpl7755@126.com (P. Zhu), chengangfj@163.com, Gang.Chen@joslin.harvard.edu (G. Chen).
[1] Contributed equally to the study.

https://doi.org/10.1016/j.ejogrb.2018.01.034
0301-2115/

function and the development of metabolic diseases, such as type 2 diabetes, metabolic disease and NAFLD [8–12]. Thus, impairment of the circadian clock appears to contribute to the development of NAFLD.

Follicle-stimulating hormone (FSH), which is also called gonadotropin because it stimulates the gonads, is produced by the anterior pituitary gland of the brain [13]. A previous study found that FSH shows a diurnal cycle with the highest mean level at 8:00 h and the lowest mean level between 24:00 and 4:00 h [14]. Circulating FSH levels increase sharply and persistently with aging in both males and females because of the loss of negative feedback from inhibin [15]. FSH was first thought to affect only the reproductive system. However, further studies found that FSH affects non-reproductive organs and tissues, including osteoclasts, [16] and that it increases cholangiocyte proliferation in mice [17]. Recent studies also reported that lower FSH is significantly associated with diabetes [18] and NAFLD [19] in postmenopausal women. Another two studies proved that FSH is implicated in lipid biosynthesis [20] and may interact with its receptors in hepatocytes and reduce LDLR levels [21], suggesting that FSH maybe involve in the pathogenesis of NAFLD.

However, the effects of the diurnal rhythm of FSH on NAFLD have not been reported. Therefore, we investigated whether the diurnal rhythm of FSH is associated with NAFLD in an elderly population.

Materials and methods

Study design and patient population

We performed a cross-sectional study between August 2015 and November 2015 at Fujian Provincial Hospital. The subjects were the patients who visited the hospital for treatment. In total, 102 patients without other chronic liver diseases were initially screened and 31 of them were excluded for the following reasons: incomplete data (n = 20), diseases or medication usage that may affect sex hormone levels (such as pituitary adenoma, spironolactone, antiparkinsonian drugs and antipsychotic drugs) (n = 11). Seventy-one subjects were included in the final analyses.

This study was approved by the Ethics Committee of Fujian Provincial Hospital. Written informed consent was obtained from all patients prior to study inclusion.

Anthropometric and biochemical measurements

Weight and height were measured while the participants were clothed in a light gown. Waist circumference was measured midway between the lowest rib margin and the iliac crest in a standing position. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). The waist-to-hip ratio (WHR) was calculated from these values. Blood pressure was measured during the first 72 h after admission while the participants were in the supine position, using a standard mercury sphygmomanometer according to a standard protocol. The mean of multiple recordings was used for the final analysis.

Venous blood samples were collected at 0 and 120 min following a 75-g oral glucose challenge for non-diabetics. Fasting blood glucose (FBG), postload plasma glucose (PPG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c), triglycerides (TG) and liver enzymes were measured by enzymatic analysis.

Twenty milliliters of heparinized venous blood was obtained at 8:00 and 24:00 on the same day for each patient. The blood was immediately centrifuged and the plasma was stored at −20 °C until analyzed. FSH was determined by radioimmunoassay. To obtain the circadian rhythm of the FSH, FSH (8AM)/FSH (0AM) > 1 was defined as normal circadian rhythm.

All biochemical values were measured within the first 24 h after admission, using a minimum of an 8 h overnight fasting state.

Ultrasonographic examination

Hepatic ultrasonography scanning was performed in all patients by an experienced radiologist, who was blinded to participants' details. Hepatic steatosis was diagnosed based on characteristic sonographic features, i.e., poor visualization of intrahepatic vessel borders and diaphragm, evidence of ultrasound beam attenuation and diffuse hyper-echogenicity of the liver relative to the kidneys [22].

Collection of human liver and ovarian samples

In this study, we collected a few additional human liver tissues from the elderly patients with hepatic tumor during hepatectomy surgery. The samples were taken at least 1 cm from the tumor margin and were diagnosed as hepatic steatosis by pathologists. The liver tissues were fixed in formalin immediately.

We also collected human ovary tissues from young female patients who received adnexectomy due to cervical cancer. The ovarian samples were acquired during adnexectomy surgery and were immediately fixed in formalin. All of the ovary tissues were diagnosed as normal by pathologists.

The acquisition of human liver and ovary tissues was approved by the Ethics Committee of Fujian Provincial Hospital, Fujian Medical University, Fuzhou, China.

Tissue immunohistochemistry analysis

Human liver tissue samples were sectioned at 4-μm intervals, blocked in 1%BSA, and incubated with an FSHR primary antibody (1:200; Abcam; rurong Biological Technology Co., LTD, fuzhou, China) at 4 °C overnight. Tissue sections were then washed with PBS and incubated with a secondary biotinylated antibody (Dako Cytomation LSAB Plus System-HRP, Glostrup, Denmark) at room temperature for 30 min, followed by Dako ABC for 20 min and developed with 3-3 diaminobenzidine (Dako Cytomation Liquid DAB Plus Substrate Chromogen System). Tissues were counterstained using Harris' hematoxylin (Abcam) prior to dehydration and cover slipping. Immunohistochemical observations were taken using a BX-51 light microscope (Olympus, Tokyo, Japan).

Definitions and diagnostic criteria

Hypertension was defined using the published definition in the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [23]. The diagnoses of diabetes mellitus (DM) were based on criteria from the American Diabetes Association 2010 [24]. Mets were defined according to a joint interim statement [25].

Statistical analysis

Data were expressed as the means ± SD, frequencies, or medians with 25th and 75th percentiles. Skewed variables (ALT, AST, TG, FSH (8AM) and FSH (0AM)) were logarithmically transformed to improve normality prior to analysis. Chi-squared and Student's *t*-tests were used to compare whether there were significant differences in the frequency distributions and the means between participants who were stratified by the presence or absence of diagnosed ultrasound. To further detect the differences in the FSH diurnal rhythm between non-NAFLD and