ORIGINAL ARTICLE

ASSOCIATION ANALYSIS OF PIOGLITAZONE EFFECTIVENESS IN TREATMENT OF NAFLD PATIENTS WITH OBESITY AND PPARG RS1801282 (PRO12ALA) GENOTYPE

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ABSTRACT

The aim: To study the association between the effectiveness of treatment with pioglitazone non-alcoholic fatty liver disease (NAFLD) in patients with obesity and PPARG rs1801282 (Pro12Ala)-polymorphism in Ukrainians.

Materials and methods: 123 patients with NAFLD in combination with obesity 1, 2, 3 classes were included in comprehensive weight loss program (5 visits, 12-weeks). The case group was treated with pioglitazone 15 mg / day, while the control group received only program. Ultrasound (US) steatometry and genetic testing rs1801282 polymorphism in PPARG gene were performed.

Results: Pioglitazone, PPARG rs1801282 genotype, CAP before treatment, previous weight loss attempts, and duration of obesity were associated with the change in controlled attenuation parameter (CAP) during treatment. There was a significant association between the target CAP reduction achievement and pioglitazone treatment (adjusted odds ratio 0.23, 95% CI 0.07–0.73; $p = 0.01$) with the CC genotype of PPARG gene (adjusted odds ratio 92.9, 95% CI 7.4–1159; $p < 0.001$) compared to patients with the CG genotype.

Conclusions: Pioglitazone and PPARG rs1801282 polymorphism could influence on dynamics of CAP reduction during treatment.

KEY WORDS: controlled attenuation parameter (CAP), steatometry, previous weight loss attempts, duration of obesity

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INTRODUCTION

All over the world, prevalence of both obesity and non-alcoholic fatty disease liver (NAFLD) is alarming.

Routine simple screening for steatosis with ultrasound in patients with obesity or metabolic syndrome can enhance diagnosis of NAFLD [1,2] but it does not affect treatment outcomes because of poor primary motivation and low long-term adherence [3].

Existing medication for NAFLD has shortcomings in its efficacy and focuses basically on treatment of progressive non-alcoholic steatohepatitis (NASH) or high risk groups for progression – only after liver biopsy [4,1]. Although pioglitazone (Peroxisome proliferator activated receptor gamma (PPARG) agonist) has been considered the most effective for the treatment of NASH [5], the lack of efficiency is probably due to the pleiotropic action of PPARG gene. The most common among the mutations in PPARG gene is Pro12Ala polymorphism. The presence of polymorphism is associated with a reduced transcriptional activity of the PPARG [6]. It is well known that PPARG inhibit activation of hepatic stellate cells thus improving fibrosis. Activation of PPARG leads to increase in 2-3 fold of adiponectin level and improves insulin tolerance by decreasing the level of ceramides [7]. Consequently, treatment with low doses of pioglitazone also can decrease «lypotoxicity» as well as elimination of subclinical inflammation in extrahepatic organs [8].

Better adherence rates to comprehensive lifestyle changes have plausibly better long-term outcomes [9]. Moreover, there are many factors predisposing to drop-out besides non-modified [10]. The number of previous weight loss attempts and the duration of obesity might predict adherence since it might reveal the level of motivation to lifestyle changes [11].

Overall synopsis of obesity management consists of communication, medical evaluation and treatment. Now among patients with obesity and NAFLD, there is a long-standing dilemma of adherence because of absence of awareness of NAFLD progression among patients and perception of themselves as «healthy obese» [12] rather than «metabolically ill». Obviously, the prescription of pioglitazone without biopsy proven NASH demonstrates the need to optimize management tactics. Usage of the targeted motivational self-reported questionnaire to assess the adherence in treatment of patients suffering from obesity with concomitant NAFLD might promote

self-monitoring [13]. Lifestyle via diet and physical activity contribute to NAFLD, though it – should be modified. The macronutrient composition should be adjusted as in Mediterranean diet therefore lowcarbohydrate diet is likely to be beneficial [14], unfortunately correct research in not feasible [15]. Futhermore, sociocultural long-term dietary pattern similar to traditional Mediterranean diet is not suitable for all population in the world [16].

Considering the dramatic forecast of increase in prevalence of advanced fibrosis and cardiovascular disease, diabetes mellitus (TD) 2 type all over the world [17], stronger measures for the cases of deliberate non-compliance should be required. In this context, the influence of pioglitazone and PPARG Pro12Ala genotype on the effectiveness treatment NAFLD patients with obesity might help to optimize treatment approach.

THE AIM

The aim was to study the association between the effectiveness of treatment with pioglitazone NAFLD in patients with obesity and PPARG rs1801282 (Pro12Ala)-polymorphism in Ukrainians.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethic Committee (Ref. № 122/ 29.05.2019). The prospective interventional study included 123 Ukrainians who were consulted in 2016 – 2020 [10, 12]. Each patient signed an informed consent to be included in the study. Inclusion criteria: patients diagnosed with obesity using BMI 30-44.99 kg / m2 in combination with NAFLD (ALT ≤ 2.5 ULN), determined by ultrasound steatometry (controlled attenuation parameter (CAP) ≥ 2.2 dB/cm), age – 18-60 years. Exclusion criteria: hepatitis of different etiologies, TD type 1 and TD type 2, heart failure (NYHA class 2-4), history of bladder cancer in patients and first line of inheritance, other severe comorbidities.

By randomization, patients were divided into two groups. The primary outcome was the reduction of CAP during treatment higher than median value (≥0.33 dB/cm). All patients were offered a comprehensive weight loss program consisting of 5 doctor visits over a 12-week follow-up. The program included a modification of the patient's lifestyle: a decrease in the patient's energy diet by 500 kcal from physiological daily energy expenditure and his moderate physical activity of 150-200 min per week (walking at a speed of 5-6 km / h). In addition, self-report adherence and weight loss history with the help of questionnaires were conducted. All patients underwent genetic testing for the presence rs 1801282 polymorphism in the PPARG gene, standard anthropometric measurements, laboratory screening and ultrasound steatometry before and after 12 weeks of treatment.

Clinical genetic stage of the research involved buccal epithelium sampling and genotype testing. DNA was extracted using AmpliPrime DNA-sorb-AM test system (Next-Bio LLC, Russia). Amplification of PCR sequences were carried out by ROTOR GENE 6000 analyzer (Corbett, Australia), using the SNP-EXPRESS-SHOT diagnostic kit by Liteh (Russia). PCR primers from the kit supplied by forward: 5'-GCCAATTCAAGCCCAGTC-3' and reverse: 5'-CGTCCCCAATAGCCGTATC-3'.

Ultrasound steatometry was used to diagnose NAFLD with a Soneus P7 instrument (Ultrasign, Ukraine). The score of steatosis was determined by the results of ultrasound steatometry on a scale of CAP, which corresponded to the morphological scale of NAS (NAFLD activity score): light (S1 –2.20-2.29 dB/cm), moderate (S2 – 2.30-2.90 dB/cm), heavy (S3 – more than 2.90 dB/cm) [4].

After 12 weeks of treatment the dynamics of ultrasound were studied (reduction of liver size, improvement of echostructure, decrease in CAP during treatment).

Statistical processing of the obtained results was made by using the Statistical Package EZR v. 1.35 (R statistical software version 3.4.3, R Foundation for Statistical Computing, Vienna, Austria) [19]. Shapiro-Wilk test was performed to check the normality of the distribution of the quantative variables. The arithmetical average and standard deviation (±SD) were calculated case of the normal distribution of variables. The median (Me) and the interquartile range (Q_I–Q_{III}) were calculated for non-normal distributed value. Since some variables did not follow normal distribution, non-parametric methods were used for analysis of the data. All statistical tests were based on the two-tailed probability. The null hypothesis was rejected at the significance level of $p < 0.05$. Hardy-Weinberg equilibrium (HWE) online calculator was obtained from https://wpcalc.com/en/equilibrium-hardy-weinberg. Allele frequencies were determined by gene counting. A χ^2 test was used to test the HWE and to compare qualitative variables. The multivariate logistic regression analysis was used to evaluate the effect of pioglitazone treatment, PPARG rs1801282 polymorphism, BMI, sex, age, CAP before treatment, adherence level at baseline, previous weight loss attempts, obesity duration on target reduction of CAP. The receiver operating characteristic (ROC) curve analysis was used to evaluate the logistic regression models. So the power of the values predicted by the model to discriminate between negative and positive cases was quantified by the area under the ROC curve (AUC). The degree of association of the score of steatosis was determined by calculating the odds ratio (OR) and its confidence interval (CI).

RESULTS

The clinical characteristics of the study groups are shown in Table I. There were no significant differences between these two groups in BMI, sex, age, adherence at baseline, CAP after treatment ($p > 0.05$). The pioglitazone group included 61 individuals with an the average CAP of 2.61 (2.47–2.77) dB/cm, which was much higher. The control group consisted of 62 subjects with 2.47 (2.28–2.69) dB/cm

Table I. Clinical characteristics of the patients with NAFLD and obesity

Parameter	Me (QI–QIII)		p
	Pioglitazone group (n=61)	Control group (n=62)	
BMI, kg/m^2	33.4 (31.2-36.0)	32.6 (31.4-35.5)	0.53
Sex, male/female	29/32	35/27	0.42
Age, years	42 (35–53.25)	42 (34–50)	0.45
Adherence at baseline ,%	70 (65–80)	70 (65–80)	0.78
CAP before treatment, dB/cm	2.61 (2.47–2.77)	2.47 (2.28–2.69)	0.02
CAP after treatment, dB/cm	2.23 (2.1–2.33)	2.23 (2.15–2.38)	0.44
Dynamics of CAP, dB/cm	0.39 (0.29–0.485)	0.25 (0.16–0.34)	<0.001

Table II. Results of questionnaire on weight loss history in two groups

Parameter		Pioglitazone group (n= 61), n(%)	Control group (n=62),n(%)	p
Number of previous weight loss attempts, n	0	13 (21.2)	10 (16.1)	0.005
	1-2	25 (41.0)	9 (14.5)	
	3-4	7 (11.5)	18 (29.0)	
	5-6	7 (11.5)	10 (16.1)	
	≥7	9 (14.8)	15 (24.3)	
Duration of obesity, years	0-4	9 (14.8)	11 (17.7)	0.46
	5-9	14 (23.0)	11 (17.7)	
	10-14	18 (29.5)	12 (19.4)	
	15-19	13 (21.2)	21 (33.9)	
	≥20	7 (11.5)	7 (11.3)	

Table III. The distribution of alleles and genotypes for PPARG rs1801282(C>G) polymorphism in two groups

Variant	Pioglitazone group (n=61)		Control group (n=62)		p_{HWE}	p
	n	%	n	%		
Genotypes						
CC	50	81.97	51	82.26	–	0.824
CG	10	16.39	9	14.52		
GG	1	1.64	2	3.23		
Alleles						
C	110	90.16	111	89,52	0.0876	0.967
G	12	9.84	13	10,48		

as an average CAP. Thus, there was significant difference in CAP before treatment (p=0.02). At the same time, significant difference of dynamics of CAP reduction during treatment were observed (p<0.001).

The results of questionnaire on weight loss history are summarized in Table II. There was significant difference in previous weight loss attempts (p=0.005), since 38 (62.8%) subjects of pioglitazone group had during life 0-2 previous weight loss attempts, but 43 (69.4%) individuals from control group – > 2 attempts.

The distribution of alleles and genotypes in two groups is presented in Table III. Alleles distribution matched with HWE expectation (p_{HWE}=0.088). Both the distribution of genotypes and alleles had no significant differences (p_g=0.824; pa =0,967).

To manage patients with NAFLD in combination with obesity we used hypothesis that predicted risk of inefficacy of treatment – may provide differential effective and early treatment. The logistic regression model was used mainly to estimate the effect of pioglitazone and rs1801282 polymorphism in PPARG gene on dynamics of CAP reduction. The analysis of the association between pioglitazone effectiveness and PPARG rs1801282-polymorphism with dynamics of CAP reduction was presented in Table IV.

As seen from the Table IV, in an unadjusted model the OR was 0.27 that was approximately 4 times higher in pioglitazone group than in control ones (p=0,001). There was no association (p>0.05) between rs1801282 polymorphism in PPARG

Table IV. Logistic regression models of the effect of pioglitazone and rs1801282-polymorphism PPARG gene on dynamics of CAP reduction. Response variable: dynamics of CAP reduction < 0.33 dB/cm during treatment

Constant/variables		Estimate β1±SE	Pr	OR (95%CI)
One-factor model: Pioglitazone and PPARG genotype				
Pioglitazone 15 mg	No		Reference	
	Yes	–1.33±0.38	0.001	0.27 (0.13–0.56)
PPARG genotype	CG		Reference	
	CC	0.87±0.52	0.09	–
	GG	1.23±1.31	0.35	–
Five-factor model: Pioglitazone+ PPARG genotype + CAP before treatment + Number of previous weight loss attempts + Duration of obesity				
Pioglitazone	No		Reference	
	Yes	–1.46±0.58	0.01	0.23 (0.07–0.73)
PPARG genotype	CG		Reference	
	CC	4.53±1.78	<0.001	92.9 (7.4–1159)
	GG	2.82±1.96	0.15	–
CAP before treatment (for 0,1 dB\cm)		–0.71±0.18	<0.001	0.49 (0.34–0.70)
Number of previous weight loss attempts (for 2)		0.79±0.25	0.002	2,51 (1.76–3.58)
Duration of obesity (for 5 years)		0.57±0.16	<0.001	1.77 (1.29–2.44)

gene and target reduction of the CAP. Thus, pioglitazone can be useful, so genotypes alone do not affect the achievement of the successful results. Another obtained results indicate that neither sex, age, nor adherence were confounding factors.

In this study we have identified 5 factors owing to the association analysis with adjustment for several covariates from Table I. In the pioglitazone-adjusted model, these OR for pioglitazone were almost unchanged – 0.23 compared to one-factor model.

There was a significant association (p<0.001) between the target CAP reduction achievement with the CC genotype of PPARG gene (OR 92.9, 95% CI 7.4–1159), compared to patients with the CG genotype, when it was adjusted. There was no difference in the risk of treatment failure for patients with the GG genotype of the PPARG gene polymorphism (p=0.15).

Area under the curve of operational characteristics is AUC = 0.92 (95% CI 0.85 – 0.96).

DISCUSSION

Findings from the present study demonstrate that in participants undergoing comprehensive lifestyle program were some significant differences between groups before treatment. In control group, there were 69.4% of subjects with more than 2 weight loss attempts in general. Therefore, the difference in CAP before treatment in pioglitazone group may be owing of the less attempts as a motivation marker [19]. Finally, during the study (Table I; Table II) we identified significant difference in the dynamics of CAP reduction (p<0.001) in case group 0.39 (0.29–0.485) dB/cm and 0.25 (0.16–0.34) dB/cm in control group. It must be mentioned that low doses of pioglitazone were well tolerated by patients without adverse effects during treatment.

As seen in Table III and Table IV, the GG genotype was not a significant confounding factor because of small sample (3 subjects), thus cannot interpreted adequately. The approach is based on minimal sample for comparing groups.

The study developed scientifically designed, personalized prediction system to management of patients based on additional revealing Pro12Ala polymorphism in PPARG gene and assessing the requirement of pioglitazone use. All the data presented above shed some light on the demand for use of pioglitazone bypassing an attempt to lose a patient`s motivation, duration of obesity thus can help to encourage patient`s adherence.

We are aware that our study has some limitations. All data obtained about the influence of PPARG Pro12Ala polymorphism on weight-associated parameters could be controversial in different populations as well as can have shortcomings in dietary interventions. Evaluation of the score steatosis using ultrasound steatometry has not been recommended by EASL-EASD-EASO Clinical Practice Guidelines [1] yet, because of not having been compared to the gold standard. Studies examining patients' adherence to lifestyle change are not also validated, so they do not provide an accurate assessment of the implementation of the recommendations. Summing up, further studies with extended samples are required to validate these results [20].

CONCLUSIONS

In conclusion, our research identified 5 covariables that were associated with the change of CAP during treatment:

pioglitazone, rs1801282 polymorphism in PPARG gene, CAP before treatment, previous weight loss attempts, duration of obesity. There was a significant association between pioglitazone treatment and the PPARG gene CG genotype with dynamics of steatosis reduction.

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The Authors declare no conflict of interest.

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