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ASSESSMENT OF COMPLEX TREATMENT INFLUENCE ON SYSTEMIC INFLAMMATION IN OVERWEIGHT TYPE 2 DIABETES PATIENTS

OCENA WPŁYWU LECZENIA SKOJARZONEGO NA SYSTEMOWY STAN ZAPALNY U OSÓB Z CUKRZYCĄ TYPU 2 I NADWAGĄ

Olena V. Said, Valentyna I. Velychko, Halyna O. Danylchuk
ODESSA NATIONAL MEDICAL UNIVERSITY, ODESSA, UKRAINE

ABSTRACT

Introduction: Long-term systemic inflammation may cause type 2 diabetes. Medications used to treat type 2 diabetes don't target inflammation therefore it's necessary to study how hypoglycemics can improve patient prognosis through modification of systemic inflammation.

The aim: Our goal was to assess influence of liraglutide in complex therapy on proinflammatory cytokine levels in overweight patients with type 2 diabetes and compare it with metformin.

Meterials and methods: The study included 80 overweight patients with type 2 diabetes. We studied clinical parameters as well as antropometric: height, weight, BMI, abdomen circumference; hsCRP, proinflammatory cytokine (TNF-α, IL-1β, IL-6) levels. Patients were treated according to an individualized treatment plan which included eating habit modification and dosed physical exercise. First and second groups were comparison groups. Patients in the first group received metformin as primary treatment up to 2500 mg/day (n=20). Patients in the second group received liraglutide up to 1.8 mg/day (n=30). Patients in the third (main) group received a combination of metformin (up to 2500 mg/day) and liraglutide up to 1.8 mg/day (n=30).

Results and conclusions: Main group achieved a decrease in BMI from 28,48±2,1 kg/m^2 to 23,9±1,8 kg/m^2 ($p<0,05$), whereas such decrease in the liraglutide monotherapy group was from 28,59±2,5 kg/m^2 to 25,87±2,3 kg/m^2 ($p<0,05$) and from 28,65±3,2 kg/m^2 to 27,46±2,8 kg/m^2 ($p>0,05$) in the metformin monotherapy group. Liraglutide was more efficient in lowering inflammatory cytokine concentrations with TNF-α and IL-6 being more sensitive to its effects. Main group saw a decrease in TNF-α levels from 10,14±0,6 to 7,49±0,33 pg/ml ($p<0,001$) and IL-6 levels from 11,12±0,7 to 7,84±0,62 pg/ml ($p<0,001$).

KEY WORDS: diabetes, overweight, inflammatory cytokines, liraglutide, body mass index

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INTRODUCTION

Prolonged low-grade systemic inflammatory process may lead to the development of clinical presentation of type 2 diabetes mellitus (T2DM) [1, 2]. Such systemic and subclinical inflammatory process can be characterized by elevated concentrations of circulating proinflammatory cytokines (PC), including C-reactive protein (CRP), or high-sensitivity CRP (hs-CRP), interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) [3]. Different mechanisms with which these cytokines can lead to the development of diabetes were identified. For example, these cytokines can directly inhibit insulin receptor signaling by activating c-Jun amino-terminal kinase and an inhibitor of nuclear factor kappa-beta kinase, leading to serine phosphorylation of insulin receptor substrate-1. Additionally these cytokines have been found to stimulate fatty acid synthesis in liver and production of even more acute phase proteins by the liver as well as stimulating an increasing number of inflammatory cells in fatty tissue and pancreatic beta-cells [4]. Therefore, PC do not only influence insulin resistance but can also lead to beta-cell apoptosis and insufficiency leading to the development of T2DM [5]. These cytokines are part of a cytokine family which is produced by adipose tissue, so called, adipokines, and may be a link between overweight, obesity and T2DM [6].

As most of antidiabetic drugs available for T2DM do not specifically target lowering the intensity of inflammatory processes, more investigations are needed to discover how hypoglycemics may improve patient prognosis by modifying the process of systemic inflammation.

The connection between obesity, inflammation and T2DM was first demonstrated when TNF-α was found to be excessively expressed in obese rat adipose tissue and neutralizing of TNF-α was associated with an increase in peripheral sensitivity to insulin in these animals [7]. Additionally, TNF-α was found to be associated with obesity and insulin resistance in various clinical populations.

Interleukin-6 serum levels were also associated with insulin resistance and DM [8, 9].

TNF-α and IL-6 are important regulating cytokines for CRP which is considered to be the most accepted serum inflammatory marker. Numerous prospective studies have proven that elevated CRP levels were a predictor of T2DM development. As a main acute phase response mediator, hs-CRP may be responsible for the summation of TNF-α and IL-6 effects [10].

Since there is a strong association between chronic systemic inflammation and DM development, it is plausible that treatment with anti-inflammatory agents would lower added risks for T2DM patients. However there are very few hypoglycemics that are used in treatment of T2DM that specifically influence inflammatory processes although different interventions that target DM prevention have a proven anti-inflammatory effect. Among such treatment strategies are sodium salicylates, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, angiotensin-converting enzyme inhibitors, peroxisome proliferators-activated receptor gamma (PPAR- γ) agonists and lifestyle modification aimed to lower body weight [11, 12].

These observations open further investigatory possibilities as to treatment strategies that would specifically target systemic inflammation to treat insulin resistance in patients with T2DM.

THE AIM

Goal of this investigation was to assess the influence of liraglutide as a part of complex treatment plan on serum proinflammatory cytokine levels in overweight patients with type 2 diabetes mellitus and compare this influence with metformin effects.

MATERIALS AND METHODS

Overweight patients with T2DM that had all of the inclusion and none of the exclusion criteria below were included in the study.

Inclusion criteria*:* adults aged 35 and up that have signed an informed consent form; diagnosed moderately severe T2DM; BMI from 25 to 29,9 kg/m^2; HbA1c level from 6.5 to 10%.

Exclusion criteria: absence of signed informed consent; diagnosed T1DM; ongoing insulin treatment or indications for such; presence of acute pathology that requires emergency medical assistance; NYHA III-IV congestive heart failure; diabetic foot syndrome; any decompensated chronic condition that could potentially influence study results or patient's ability to take part in the study; chronic liver failure; chronic kidney failure; history of acute or recurrent pancreatitis; presence of malignant tumors.

Diagnosis verification was conducted according to diagnostic protocols.

An in depth study of clinical and anthropometric parameters was conducted: patients' weight, height and abdominal circumference (AC) were measured, body mass index (BMI) was calculated; serum levels of hs-CRP and proinflammatory cytokines (TNF-α, IL-1β, IL-6) were assessed.

The study included 80 overweight patients with T2DM – subjects aged from 41 to 78 years, including 43 males and 37 females. Mean age was (57,81±9,83) years for the cohort; (56,90±8,56) years for male subjects and (58,86±11,1) years for female subjects.

All subjects received an individualized treatment regimen. Depending on the method of treatment the subjects were divided into three groups. All of the groups were equivalent according to their age, gender and number of patients. First and second groups were comparison groups. Subjects in the first group (g 1.1) received metformin up to 2500 mg/day as a basic hypoglycemic agent as well as lifestyle modification and differentiated dosed controlled physical exercise (n=20). Mean BMI among the subjects of this group was 28,65±2,3 kg/m^2, and AC – 104,0±1,2 cm. Subjects in the second group (g 1.2) received liraglutide treatment up to 1.8 mg/day as well as lifestyle modification and differentiated dosed controlled physical exercise (n=30). This group included treatment naïve patients with T2DM and patients who had a history of poor metformin tolerance. Mean BMI for the subjects in this group was 28,59±3,1 kg/m^2, and AC – 107,7±0,9 cm. Subjects in the third (primary treatment) group (g1.3) received a combination of metformin up to 2500 mg/day and liraglutide up to 1.8 mg/day as well as lifestyle modification and differentiated dosed controlled physical exercise (n=30). Mean BMI for the subjects in this group was 28,48±2,8 kg/m^2, and AC 104,2±1,3 cm.

Lifestyle modification included recommendations on smoking cessation and alcohol consumption limitation as well as food intake behavior modification.

All subjects with a smoking habit received a recommendation to quit smoking and were regularly monitored for current smoking status. It was recommended that patients consumed alcohol (if any) in moderation (no more than 30 ml of ethanol equivalent per day for male subjects and 15 ml for female subjects).

Food intake behavior modification and diet therapy included a hypocaloric diet with caloric deficit up to 500 ccal/day and frequent meals (3 main meals and 2 snacks a day). Subjects were encouraged to apply portion control to their meals. General carbohydrate intake was controlled and subjects were especially educated to critically lower intake of simple carbohydrates. Also, it was recommended that patients decrease consumption of starchy fruits and vegetables. Patients were encouraged to choose products that contained high levels of fiber and had a low glycemic index. It was recommended that subjects choose whole grain products and it was stressed that carbonated sugary beverages, sugar and highly processed foods needed to be excluded from daily consumption.

Patients were instructed to substitute products rich in saturated fats or trans-fats with products that contain unsaturated fats (such as liquid oils, etc). Among meat products or meat substitutes, patients were advised to choose lean products.

Subjects were recommended to consume no more than 2.3 g/day of salt.

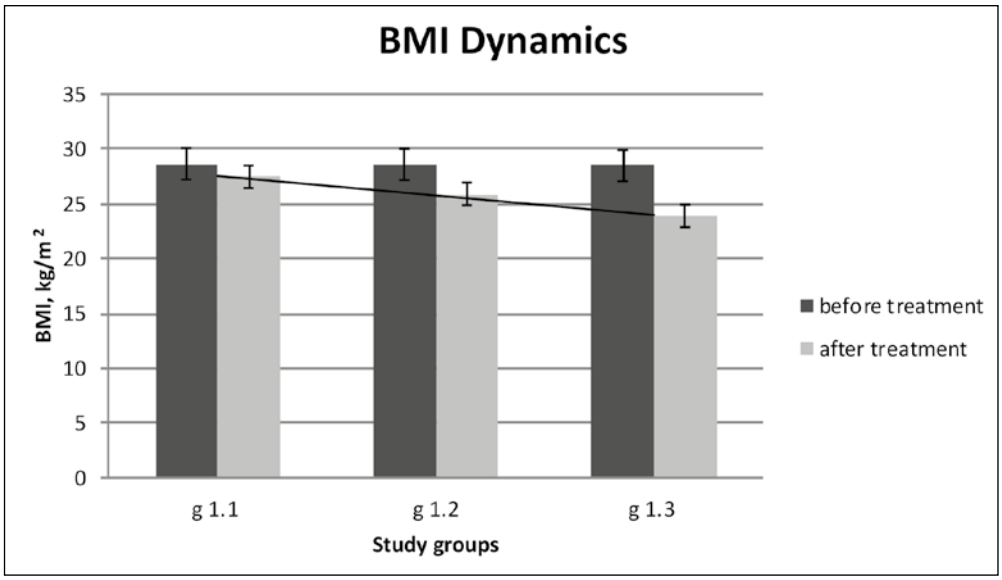

Fig. 1. BMI dynamics in overweight patients with T2DM receiving complex treatment.

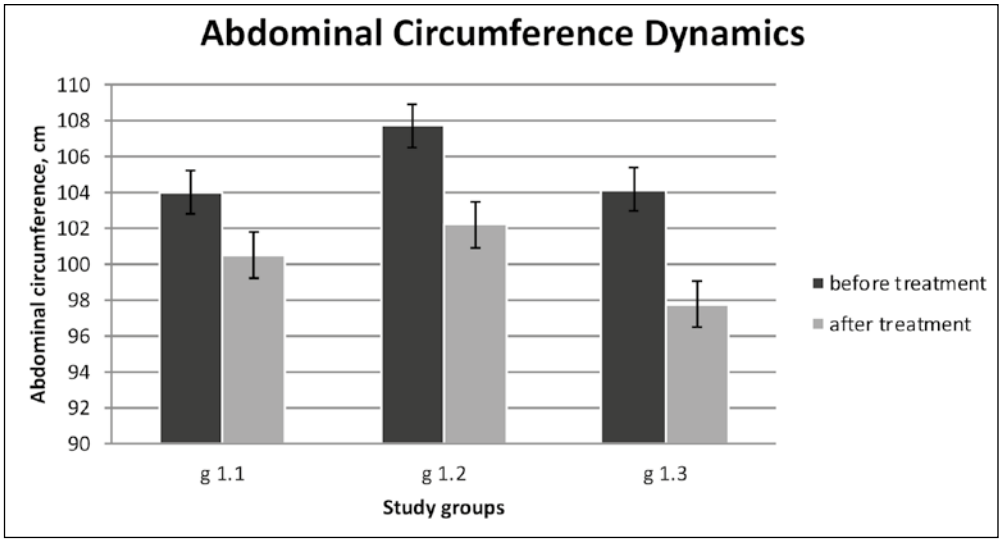

Fig. 2. Abdominal circumference dynamics in overweight patients with T2DM receiving complex treatment.

To increase motivation and compliance, patients were asked a series of questions in conversation that allowed finding out and excluding of harmful food intake behaviors such as "emotional eating", eating in front of a TV, computer, etc.

Differentiated controlled dosed physical exercise included moderate walking that was recommended to all subjects depending on their starting level of physical activity. If the subjects' starting daily step count measured by a pedometer was < 3000 steps/day, they were recommended a gradual increase by increments of 500 steps/day per week until they reached 6500 steps/day. Upon reaching 6500 steps/day with good tolerance the subjects were encouraged to increase their daily step count up to 10000-12000 steps/day in increments of 1000 steps/day per week. Additionally all subjects were encouraged to perform moderate physical exercise (swimming, biking, jogging, calisthenics) according to their tolerance for 30 minutes daily no less than 5 days a week.

Patients with T2DM were treated according to the treatment protocol of MOH of Ukraine № 1118 issued on 21.12.2012 and European Association for the Study of Diabetes Guidelines.

Statistical calculations and processing of the collected data was performed using Statistica 7.0 (StatSoft Inc., USA) and Microsoft Excel 2010.

RESULTS AND DISCUSSION

While assessing the efficacy of treatment in overweight patients with T2DM it is necessary to stress that subjects who received liraglutide had achieved a decrease in BMI from 28,48±2,1 kg/m^2 to 23,9±1,8 kg/m^2 ($p<0,05$) in the main treatment group and from 28,59±2,5 kg/m^2 to 25,87±2,3 kg/m^2 ($p<0,05$) in the liraglutide monotherapy group (graph 1). In the metformin monotherapy group patients did not achieve a statistically significant decrease in BMI however the tendency towards lower BMI was

Table I. Proinflammatory cytokine levels dynamics in study groups, M±σ

Parameter	g 1.1		g 1.2		g 1.3	
	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
hs-CRP, mg/l	10,12±1,2	9,02±1,14	9,46±0,97	7,34±0,75*	9,31±0,93	6,98±0,82**
TNF-α, pg/ml	10,59±0,72	9,39±0,64**	10,07±0,83	7,68±0,49**	10,14±0,6	7,49±0,33**
IL-1β, pg/ml	7,32±0,38	6,56±0,49	7,08±0,51	5,43±0,45*	7,03±0,46	5,08±0,43**
IL-6, pg/ml	11,88±0,65	10,26±0,9**	11,01±0,92	8,12±0,84**	11,12±0,7	7,84±0,62**

Notes: * - p_{b-a}<0,01, **- p_{b-a} <0,001, b- before treatment, a- after a course of treatment, M- mean, σ-standard deviation

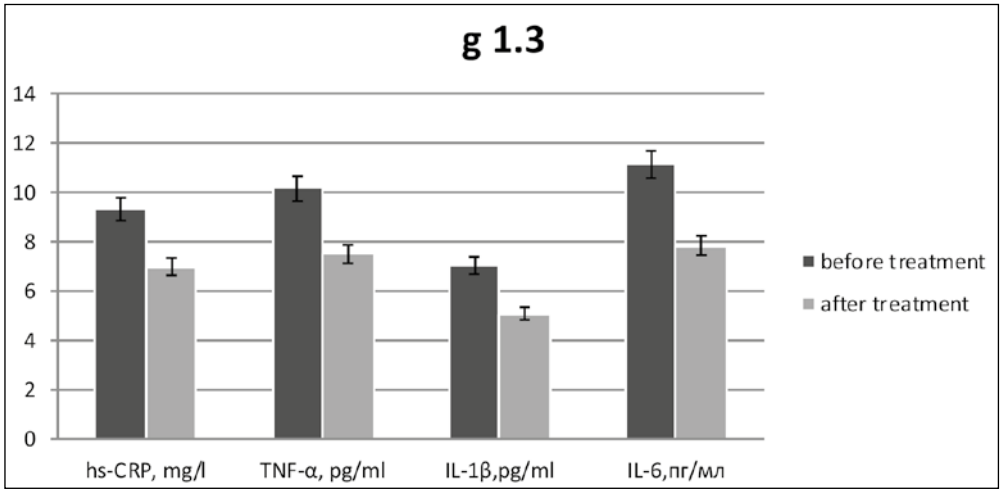

Fig. 3. Proinflammatory cytokine levels dynamics in overweight patients with T2DM receiving a combination of liraglutide and metformin.

present: from 28,65±3,2 kg/m^2 pre treatment to 27,46±2,8 kg/m^2 post treatment (p>0,05). Adding liraglutide into the treatment complex is an effective way in lowering body weight (RR=0,41; RRR=0,47; NNT=3,6; CI 95% 3,2-4,3).

According to the data seen in graph 1, patients of groups g1.2 and g1.3 achieved a significant decrease in body weight and BMI in 3 months of treatment and regular physical exercise (p<0,05). Group g1.1 subjects achieved a decrease in BMI that wasn't statistically significant (p>0,05). However subjects in this group saw a mean decrease of (3,35±1.37) kg in weight, while some of the patients achieved a 6.2 kg and 7.1 kg reduction in weight. This tendency significantly lowered future risks linked to overweight in these patients.

The AC dynamics is featured in graph 2. Subjects in the first group demonstrated a decrease of AC by 3,5±1,2 cm ((3,37±1,5)% (p<0.05), whereas subjects in the second and third groups demonstrated a comparable AC decrease of 5,5±1,3 cm ((5,13±1,12)%) and 6,4± 1,8 cm ((6,14±1,52)%) respectively (p<0,01, p<0,01). There was a significantly greater AC decrease in groups where subjects received liraglutide (p<0,05).

Dynamics of PC in subjects of different groups in response to different hypoglycemic agents is featured in table I. The data presented in the table shows that there was a significant decrease of PC concentrations across all groups but to different degrees. Serum hs-CRP and IL-1β levels demonstrated a statistically significant decrease only in groups of subjects which received a combined liraglutide and metformin treatment and liraglutide monotherapy treatment. TNF-α and IL-6 levels decreased following a course of treatment in all subject groups. Most significant decrease in these parameters was seen in subjects of the third and second groups (p<0,001, p<0,001).

Subjects receiving a combination of liraglutide and metformin demonstrated a statistically significant marked decrease in all of the studied PC parameters (p<0,001) (graph 3). In subjects of the main treatment group serum levels of hs-CRP and IL-1β were decreased by (25,02±2,2)% and (27,7±2,8)% respectively, whereas TNF-**α** and IL-6 decreased by (26,13±2,1)% and (29,5±1,9)% respectively.

Introducing liraglutide monotherapy into the treatment regimen also lead to a significant decrease of PC parameters (graph 4). A statistically significant decrease in TNF-**α** and IL-6 levels by (23,7±1,8)% and (26,25±2,1)% respectively (p<0,001), occured in the group, practically comparable to the subjects of the main treatment group which proves liraglutide's anti-inflammatory effect. Additionally the levels of hs-CRP and IL-1β were reduced by (22,4±3,6)% and (23,3±2,9)% respectively (p<0,01), but this was a much less pronounced decrease unlike the subjects in the main treatment group which received a combination of liraglutide and metformin.

Metformin monotherapy treatment group saw a moderate decrease in all of the PC parameters (graph 5), however a statistically significant decrease was only noted in TNF-**α** and IL-6 levels, which were reduced by (11,33±1,2)% and (13,6±1,8)% respectively (p<0,001). This decrease was practically twice less pronouced that in the main treatment group and liraglutide monotherapy group.

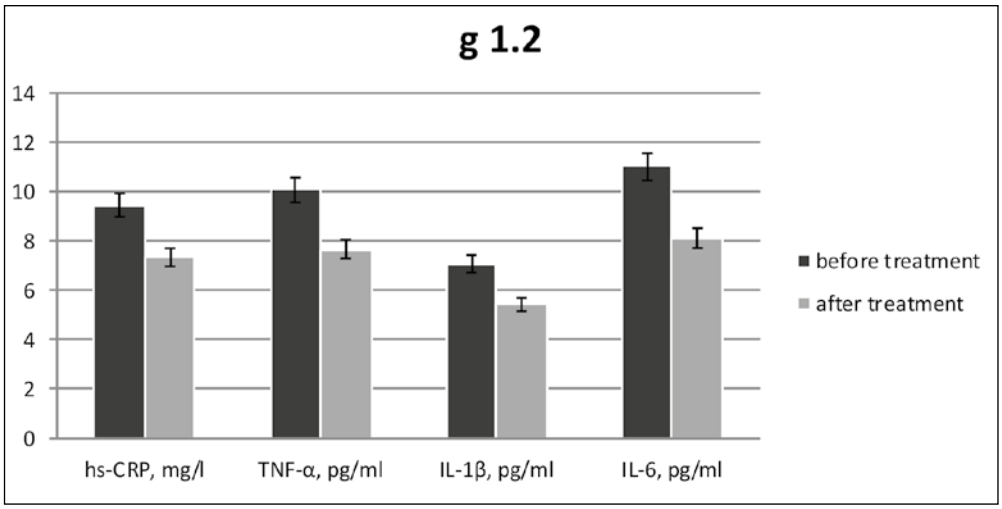

Fig. 4. Proinflammatory cytokine levels dynamics in overweight patients with T2DM receiving liraglutide monotherapy.

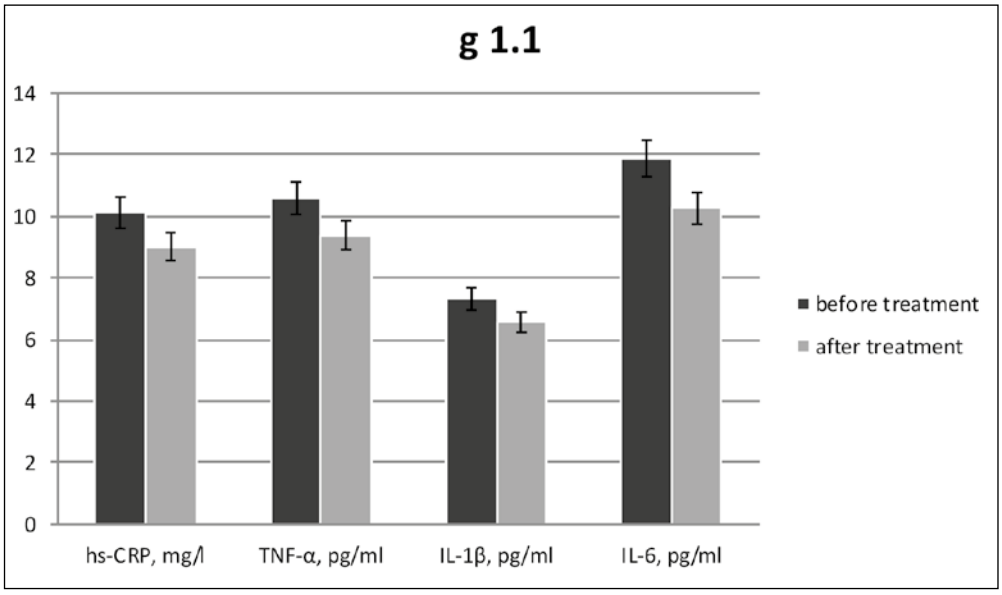

Fig. 5. Proinflammatory cytokine levels dynamics in overweight patients with T2DM receiving metformin monotherapy.

Therefore, liraglutide caused a more significant decrease in serum PC concentrations in overweight patients with T2DM in comparison to metformin. Liraglutide's influence was most pronounced on the levels of TNF-**α** and IL-6 among the studied PC, and less pronounced but still significant influence was noted on the levels of hs-CRP and IL-1β, which determines a more significant anti-inflammatory effect of liraglutide in comparison to metformin.

Including liraglutide as a part of complex treatment strategy of T2DM in overweight patients may potentially have a more pronounced anti-inflammatory effect, which is an etiologically beneficial therapeutic approach in the conditions of chronic systemic inflammatory process due to overweight and T2DM.

In terms of further research, it seems relevant to study the influence of liraglutide as part of complex treatment of T2DM in overweight patients on proinflammatory cytokine levels in the follow-up period and long term effects as well as conducting prospective studies as to the number of cardiovascular events in this patient population. Additionally, it seems relevant to conduct research of potential influence of genetic variations of overweight patients with T2DM on the efficacy of such treatment strategy.

CONCLUSIONS

1. Individualized complex therapy, which includes differentiated dosed controlled physical exercise and food intake behavior modification, is the most beneficial for patients with T2DM, especially those overweight.
2. Adding liraglutide as part of complex therapy for T2DM in overweight patients is a more effective way to manage weight loss. A decrease in BMI was seen in the main treatment group from 28,48±2,1 kg/m^2 to 23,9±1,8 kg/m^2 ($p<0,05$). A similar decrease was seen in AC in the main treatment group.
3. More sensitive PC parameters to liraglutide treatment were found to be levels of TNF-**α** and IL-6 among those studied. Subjects in the main treatment group saw a decrease in TNF-**α** levels from 10,14±0,6 to 7,49±0,33 pg/ml ($p<0,001$) and IL-6 from 11,12±0,7 to 7,84±0,62 pg/ml ($p<0,001$), therefore these two PC parameters can be used in practice for treatment efficacy assessment.

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Authors' contributions:
According to the order of the Authorship.

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Olena Said
19 Malinowski st., apt. 23, 65059, Odessa, Ukraine
tel: +380979232148
e-mail: danaodessa@hotmail.com

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