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The Relationships Among Peripheral myelin protein 22, Ghrelin, Cholecystokinin level and Severity of Diabetic Gastroparesis

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ABSTRACT

Gastroparesis occurs in up to 30-50% of patients with chronic complications of diabetes and is associated with significant impairments in both quality of life and diabetic control. The ethical clearance of this study had been approved by bioethics unit of Ivano-Frankivsk National Medical University. The severity of diabetic gastroparesis (DG) was assessed by Gastroparesis Cardinal Symptom Index (GCSI) questionnaire and Gastric emptying rate (GER) with ^{13}C-octanoic breath test (^{13}C-OBT). Level of peripheral myelin protein 22 (PMP 22), ghrelin, cholecystokinin (CCK), were assayed by ELISA. The relationships was analysis by correlation-spearman rho test, p<0,05. According to the questionnaire results, physical examination data as well as additional laboratory and instrumental investigations, mild DG was found in 16.7% patients with type 1 diabetes, moderate DG was observed in 15.5% patients and the signs of severe gastroparesis were present in 8.9% patients only. There was very strong correlation among PMP 22, ghrelin, CCK level and the severity gastroparesis in patients with type 1 diabetes.

Keyword: peripheral myelin protein 22, ghrelin, cholecystokinin, diabetic gastroparesis

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INTRODUCTION

Nowadays the majority of etiopathogenetical factors of gastric dysfunction is unclear due to autonomic neuropathy in patients with diabetes. The mechanism of neurohumoral control of gastric emptying rate (GER) in diabetics has not been investigated with total clarity yet. Regulation of gastric emptying involves a complex interaction of neural and hormonal factors in response to feedback signals from nutrient receptors in the small intestine which modulate the motor activity of the stomach. The gastrointestinal peptides released in response to nutrient meals, are believed to have an important role in the control of GER in patients with diabetes. A variety of gastroduodenal motor abnormalities have been described in patients with DG, including reduced antral and duodenal motility, increased pyloric motility, and a reduction in propagated antropyloroduodenal contractions [2].

Diabetic peripheral somatic neuropathy (DPSN) is associated with impaired neuron myelination, nerve conduction and muscle function. PMP 22 is glycoprotein with proposed roles in peripheral nerve myelin formation [1,7, 16]. The pathogenic mechanisms of reduced myelination in patients with DG are poorly understood. PMP 22 is the main component of the myelin sheath being produced primarily by Schwann cells; the indicator of its serum level can be considered as the marker of myelin damage. Thus, the determination of PMP 22 in patients with DM is an early diagnostic criterion for the progression of DPSN symptoms.

Gastrointestinal tract is a rich source of regulatory peptides should be no surprise because gut hormones are major players in regulation of metabolism. Gut peptides regulate feeding behavior, digestion, absorption, and transport of nutrients; assimilation, partitioning, and use of energy [9,11].

Ghrelin is an endogenous ligand for the growth hormone secretagogue receptor (GHS-R) that expressed in the vagal afferent neurones of the nodose ganglion and migrates caudally.Immunohistochemistry studies have also demonstrated the presence of GHS-R in the myenteric plexus of the stomach and colon in both rats and human sand in guinea pig ileum [13,17,19].

Delayed gastric emptying is not always clinically apparent, the range of gastrointestinal symptoms may include early satiety, nausea, vomiting, regurgitation, fullness, bloating and decompensation of carbohydrate metabolism. DG is lead to intractable vomiting and an inability to feed, disabling condition with no consistently effective treatment and carries a poor prognosis.

AIM AND OBJECTIVE

Aim of the study is to determine the relationships among peripheral myelin protein 22, ghrelin, cholecystokinin level and severity of gastro paresis in patients with diabetes.

MATERIALS AND METHODS

Study Design: Observational case-series study.

Ethical Consideration: The ethical clearance of this study had been approved by bioethics unit of Ivano-Frankivsk National Medical University.

Procedure of Study: 180 patients with type 1 diabetes (111 males, 69 females), treated at the endocrinology department of Ivano-Frankivsk Regional Clinical Hospital, were randomly selected to undergo complete physical examination after signing the informed consent form to participate in a clinical trial. Group I included 74 (40 males, 34 females; the average age was 40.5±1.4 years) patients with symptoms DG and Group II included 106 (71 males, 35 females; the average age was 40.5±1.0 years) subjects with type 1 diabetes. Clinical data were collected for all subjects, including body mass index (BMI), Kerdo autonomic index (KAI), duration of diabetes, and presence of diabetic complications.

To diagnose of type 1 diabetes as well as to evaluate the degree of carbohydrate metabolism compensation, national standards were used (the Order of the MOH of Ukraine of 29.12 2014 No 1,021).
Exclusion criteria for participation in the study included taking medications of symptomatic treatment affecting gastric motility, liver dysfunction, organic diseases of the gastrointestinal tract, cholelithiasis, and viral hepatitis, demyelinating diseases of the central nervous system, thyroid disorders, and renal impairment.

The prandial and postprandial glucose levels were measured by glucose oxidase method using an automatic glucose analyzer AGKM-01K (Kvertimed, Ukraine). Glycated hemoglobin (HbA1C) levels were measured using ion exchange liquid chromatography method. Baseline sample blood, biochemistry panel, thyroid function (TSH) were all assessed. The identification of PMP22, ghrelin, CKK level were made using the enzyme-linked immunosorbent assay (ELISA, USA).

Laboratory Assay: Patient's blood sample was collected from a median cubital vein for 3 mL and stored in ethylene diamine tetraacetic acid (EDTA) coated tube for PMP22, ghrelin and CCK assay. Blood samples were centrifuged 10.000 rpm for 10 minutes. Supernatan was collected. Solid phase sandwich ELISA (Human PMP 22, ghrelin, CCK ELISA kits) were used to analysis concentration of SDH. Add samples and standards and incubate the plate at 37^0C for 90 minutes, do not wash. Add biotinylated antibodies and incubate the plate at 37^0C for 60 minutes, wash plate 3 times with PBS 0,01 M. Add ABC working solution and incubate the plate at 37^0C for 30 minutes. Wash plate 5 times with PBS 0,01 M. Add TMB colour developing agent and incubate the plate at 37^0C in dark for 20 minutes. Add TMB stop solution and read the OD absorbance at 450nm in a microplate reader. The standard curve was plotted as the OD 450 of each standard solution vs the concentration of standard solution. The human PMP22, ghrelin and CCK level of the samples were interpolated from the standard curve.

The severity of DG was assessed by Gastroparesis Cardinal Symptom Index (GCSI) questionnaire and Gastric emptying rate (GER) with ^{13}C-octanoic breath test (^{13}C-OBT).

Symptoms of DPSN were assessed using the Neuropathy Disability Score (NDS) – the Neurological Symptoms Score (NSS). Mild DPSN or its subclinical form were observed in patients with the NSS score of 5 points; moderate manifestations of DPSN were found in patients with the NSS score of 5-13 points; severe DPN was seen in patients with the NSS score of more than 14 points.

Participants completed a diabetes history questionnaire, including an item to assess frequency of severe hypoglycemia. Severe hypoglycemia was defined as a hypoglycemic episode during which glucose in the blood was so low that self-treatment was not possible because of mental confusion or stupor and external assistance was required [10,15]. The incidence and severity of low blood sugar were assessed using the Hypoglycemia Fear Scale (HFS) - a questionnaire developed to measure the fear of hypoglycemia. The Behavior subscale (HFS-B) measures behaviors used to avoid hypoglycemia and its negative consequences (the HFS-B score ranges from 0 to 60). The Worry subscale (HSF-W) measures different emotional aspects of hypoglycemia (the HFS-W score ranges from 0 to 72). The total assessment of hypoglycemic episode severity was made considering the total score of both scales (HFS-T); in the presence of positive symptoms, the minimum score – 33 points and the maximum one – 132 points.

The degree of DG severity was determined applying a three-minute patients' survey – the GCSI which allows the patients to evaluate the severity of symptoms by themselves. The symptoms were rated on a 5-point scale (0-absent, 1-very mild, 2-mild, 3-moderate, 4-severe, 5-extremely severe). The total sum within 1 to 11 points indicates mild degree of severity, while the total sum within 12 to 22 points indicates moderate degree of severity; total sum within 23-33 points indicates severe degree, while the total sum of more than 34 points indicates extremely severe degree.

The GER was determined using the ^{13}C- OBT; normal value for the time by which half of the gastric content is evacuated (T½) to the duodenum is 40-75 minutes; motility acceleration - T½<40 minutes; mild degree of motility acceleration – 79-95 minutes; moderate degree of motility deceleration – 96-115 minutes; severe degree of motility deceleration – more than 115 minutes [18].

The diagnosis of gastroparesis was made based on gastroparesis severity classification (Abell T.L. et al., 2006) [6,14]. Grade 1 – mild gastroparesis – is characterized by easily controlled symptoms of dyspepsia. The patients can maintain an adequate body weight and nutritional status following a regular diet or a diet with some minor modifications. If there is an optimal control of carbohydrate metabolism, hyperglycemia has no negative effect on the stomach function. Grade 2 – compensated gastroparesis – is characterized by moderately severe symptoms which may be partially controlled with pharmacological agents (the combination of prokinetic and antiemetic agents). The patients can maintain adequate nutrition after their diet and life style correction. Patients with grade 3 (gastric failure) gastroparesis are medication-unresponsive. They cannot maintain adequate nutrition or hydration. Intensive care is needed including hospitalization for intravenous hydration and insulin infusion, intravenous administration of prokinetic and antiemetic agents and/or surgery.

Statistical Analysis: The data was analyzed using SPSS 23. The relationship among PMP22, ghrelin, CCK level and the severity gastroparesis in patients with type 1 diabetes were analyzed by spearmen correlation, with 95% confidence interval. The data was shown descriptively in narration, table, and percentage.

RESULTS

According to the questionnaire results, physical examination data as well as additional laboratory and instrumental investigations, mild DG was found in 16.7% patients with type 1 diabetes, moderate DG was observed in 15.5% patients and the signs of severe gastroparesis were present in 8.9% patients only.

The main clinical and laboratory as well as instrumental indicators which were used to perform the initial examination are presented in Table 1.

Table 1 shows the incidance DG was higher in male than female, but the prevalence this complication was upper in the female. The early diagnostic of slow GER in male was 54.1% and the incidance DG in female only 45.9%.

Diabetes duration and HbA_1C were comparable between patients with and without gastroparesis (11.6± 0.9 vs. 9.8± 0.8 years, 9. 2± 0.1 vs. 9.7±0.2 % respectively) although HbA_1C was higher than the target level for patients with compensation form of diabetes (Table 1).

Increase level of PMP 22, CCK and decrease ghrelin in patients I group are significantly more as compared to subjects II group.

Table: Characteristics of studied patients

Indicators	Group I (n=74)	Group II (n=106)
Gender		
Male, n [%]	40 [54.1%]	71 [66.9%]
Female, n [%]	34 [45.9%]	35 [33.1%]
Age, years	40.5±1.4	40.5±1.0
DM duration, years	11.6±0.9*	9.8±0.8
Body mass index, kg/m^2	29.5±0.3*	25.8±0.4
KAI, c.u.	-0.8±0.1	-0.5±0.1
Neuropathy Disability Score (NDS), points	17.3±0.8*	10.7±0.8
Results of questionnaire survey		
GCSI total score, points	22.9±0.8*	0
Carbohydrate metabolism		
Glycated hemoglobin, HbA$_1$C, %	9.2±0.1	9.7±0.2
Fasting glucose in the blood, mmol/L	7.9±0.1	8.9±0.2
Postprandial glycemia, mmol/L	9.9±0.2*	11.1±0.2
Symptoms hypoglycemia, HFS-Total score, points	22.9±0.8*	17.4±1.5
Criterion for nerve fiber demyelination		
PMP 22, ng/mL	14.5±2.2*	2.0±0.4
Gastrointestinal peptides		
Ghrelin, ρg/mL	18.9±7.9*	83.8±3.4
CCK, ng/mL	2.6±0.5*	0.2±0.01
Instrumental evaluation of the GER		
^{13}C-OBT, T½ , min	115.7±2.7*	58.7±2.9
Chronic complications of type 1 diabetes		
Peripheral polyneuropathy, n [%]	74 [100.0]	102 [96.2]
Retinopathy, n [%]	70 [94.6]	89 [83.9]
Nephropathy, n [%]	49 [64.5]	40 [37.7]

*Mean ± SD, * Kruskal-Wallis test p < 0,05*

Table 2: The relationships between peptides level and ^{13}C-OBT

Characteristics	The severity of DG			Total	r*	p
	Mild (n=30)	Moderate (n=28)	Severe (n=16)			
PMP 22, ng/mL	8.9±1.4 (6.2-12.6)	8.9±1.4 (10.9-12.1)	11.7±3.2 (19.9-23.1)	14.5±2.2 (7.1-19.9)	0.93	0.000
Ghrelin, ρg/mL	31.8±1.3 (6.9-70.7)	4.7±0.8 (3.1-5.3)	1.7±0.3 (1.4-1.7)	18.9±7.9 (3.1-26.7)	- 0.92	0.000
CCK, ng/mL	1.8±0.8 (1.6-1.9)	2.4±0.2 (2.2-2.3)	5.1±0.8 (2.4-5.2)	2.6±0.5 (1.8-2.4)	0.91	0.000

** Spearman rho test (significancy p < 0.05); if r = 0: no correlation; r > 0-0.25: weak correlation; r > 0.25-0.5: moderate correlation, r > 0.5-0.75: strong correlation, > 0.75-0.99: very strong correlation.*

To explore the neurophysiological characteristics of patients with DG were positive moderate or severe stage of DPSN in result of severity NDS, (in the patient I group: NDS is 17.3± 0.8 vs. 10.7± 0.8 resently in subjects II group).

Above figure 1 shows, strong correlation results of questionnaire (GCSI) and interpretation of GER using the ^{13}C-OBT.

The symptoms of hypoglycemia was more present rare in I Group patients than II Group subjects. We also found that symptoms of hypoglycemia (HFS-Total) correlated significantly with result of gastric motor function parameters of ^{13}C- OBT (r= 0.49 p < 0.05) (figure 2).

The PMP 22, ghrelin, CCK level had very strong correlation with the result of ^{13}C- OBT (table 2). In summary, symptoms of DG is associated with increased level of PMP 22, CCK and decrease concentration of ghrelin.

DISCUSSION

Several studies have documented the ghrelin was reported to inhibit gastric accommodation in healthy volunteers. However, this neuropeptide has been shown not to affect meal related symptoms in healthy obese or lean patients [17,19].

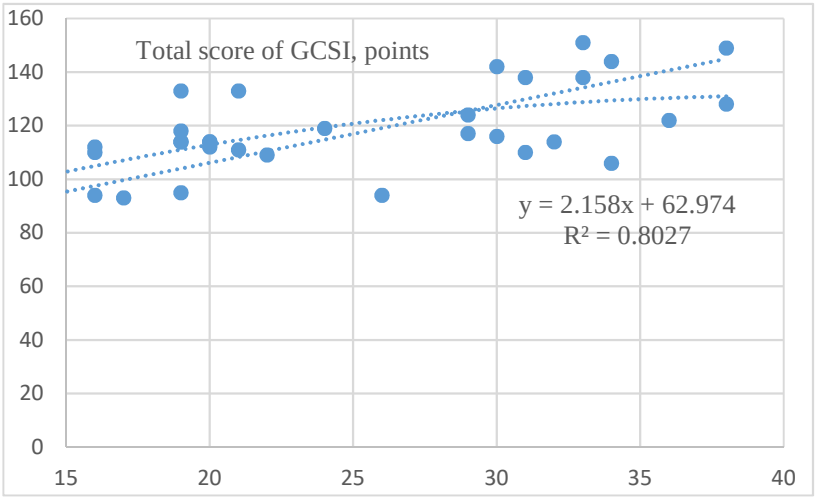

Figure 1. Correlations between results of total score of GCSI (points) and ^{13}C-OBT(T ½, min) in patients with DG, Spearman's correlation coefficient.

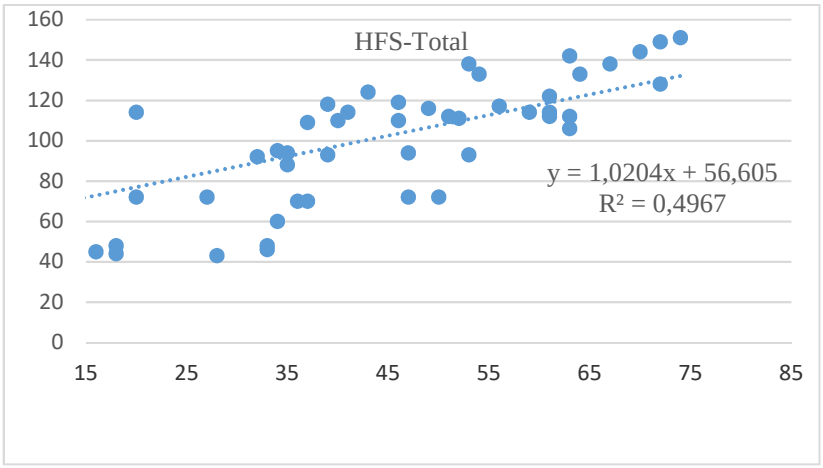

Figure 2: Correlations between results of total score of HFS (points) and ^{13}C-OBT(T ½, min) in I group patients with symptoms of gastroparesis, Spearman's correlation coefficient.

Choung R.S. et al. reported higher prevalence of DG in female patients than male and due this gastric dysfunction to the independent factors of decompensation of type 1 diabetes. In other scientific works, the impact of age, gender, disease duration, a positive family history of DM, cigarette smoking, concomitant cardiovascular and gastrointestinal diseases, disorders of the nervous system, the severity of polyneuropathy, the state of carbohydrate and lipid metabolism compensation, the incidence and fear of future hypoglycemic episodes, the degree of obesity are the predictors of the slowing down of gastric function in patients with type 1 diabetes [4]. In our study subjects with very high risk hypoglycemia was present in the diabetics who belong to the slow GER. Patients with DG have significantly better level of postprandial glycemia as compared to patients without gastrointestinal symptoms, but this effect is caused by high risk of hypoglycemia in this group subjects with a decrease stomach motility. We could not find any other study that has compared the results like this [3,5,8].

In the current work, CCK's action on the pancreas is mediated by the vagal nerve is based upon solid data. We found no clear evidence, however, that increased secretion of this hormone is a cause of the reduced appetite and food intake that accompanies GER in patients with DG [12,20].

Increase concentration of PMP 22 have direct implications for nerve dysfunction and neuropathy in subjects with diabetes. The correlation between hight level of PMP 22 and result of ^{13}C-OBT observed in our study could be attributed to the simultaneous small and large

nerve fibre damage due to common underlying biochemical mechanism.

The most common duration of diabetes for longer 10 years associated with increased risk factor of DG. The slow GER according to decrease level of grelin and increase level of PMP 22, CCK are based on the narrowing of the stomach dysfunction, the early progression of difficult symptoms of gastroparesis in patients with type 1 diabetes.

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Results of this study showed that hypercholecystokininemia, hypoghrelinemia, increase level of PMP 22 were a diagnostic markers of early grade of DG.

CONCLUSSION

The level of PMP 22, ghrelin, CKK, when combined with the GCSI and/or ^{13}C-OBT, may further improve the predictive accuracy with early diagnostic of DG. These findings need to be confirmed in larger studies.

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Conflict of Interest
There is no conflict of interest in this study.

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