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Original Article

Relationship between glucagon like peptide-1 and non-alcoholic fatty liver disease in diabetic and non-diabetic patients

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1. Introduction

Nonalcoholic Fatty Liver Disease (NAFLD) was found to be an epidemic in the last 30 years**.** It is defined as an increase in the fat content of the liver by more than 5% without significant alcohol intake or the use of steatogenic medications [1]. Non-alcoholic steatohepatitis (NASH) is considered to be a subtype of NAFLD defined by the existence of both hepatic steatosis and inflammation [2]**.**

Risk factors for NAFLD include features of Metabolic Syndrome; obesity, insulin resistance (IR) and Type 2 Diabetes (T2DM) [3]. Up to 70% of obese Type 2 diabetic patients have NAFLD. Insulin resistance being a major risk factor promotes the flux of FFA into the liver by inducing lipolysis in adipose tissues [4]**.**

Incretin hormones are peptides released from enteroendocrine cells of[1] the gut. They are two main hormones; Glucose-dependent insulinotropic peptide (GIP, also named Gastric inhibitory polypeptide) and Glucagon-like peptide-1 (GLP-1) [5]**.** Incretin hormones control postprandial release of insulin form B-cells of pancreas this is called "incretin effect" [6]**.** It can improve insulin sensitivity and lower plasma glucose level by increasing insulin secretion, decreasing glucagon release and delaying gastric emptying [7].

Other actions of GLP-1 include increase in satiety, decrease in energy intake [8] and weight loss [9]**.** There is a role of incretin hormones in NAFLD that is not totally understood. Experimental data suggest a relationship between GLP-1 and hepatic steatosis. It can be prevented or reversed by different GLP-1 agonists in rodents [10]. In humans, data is limited, liver biopsies of NAFLD patients has shown decrease in GLP-1 receptors and increase of DPP-4 receptors [11]. Also, improvement of hepatic lipid deposition has been reported in patients with T2DM taking GLP-1 agonists when assessed by liver proton magnetic resonance spectroscopy [12]**.**

Aim: We aimed to assess the relation between GLP-1 and Non-alcoholic fatty liver disease in subjects with and without Type 2 diabetes.

2. Patients and methods

A cross sectional study conducted on 75 subjects collected from the internal medicine clinics of El-menshawy general hospital from September 2016 till February 2017. Before inclusion, a consent was obtained from each patient after full explanation of the study protocol. The study was approved by the ethical committee of Faculty of medicine Ain Shams University. Subjects were divided into three groups: Group I included 25 subjects with NAFLD without type 2 diabetes (14 males and 11 females), Group II included 25 subjects with NAFLD and type 2 diabetes (12 males and 13 females) and group III included 25 healthy control subjects (11 males and 14 females). Exclusion criteria included patients with alcoholic liver disease or liver cirrhosis, history of intake of steatogenic drugs, patients with type 1diabetes and subjects aged less than 20 or more than 60 years.

Full medical history was taken from all subjects. Thorough clinical examination including anthropometric measurements; weight, height and body mass index (BMI) calculation (kg/m2)

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[1] **ALT**:alanine transaminase,**AST**:aspartate aminotransferase,**DPP-4:**dipeptidyl peptidase-4, **ELISA**: Enzyme-linked immunosorbent one-step process assay **FFA**: free fatty acids, **FIB-4**:fibrosis-4,**GIP**: Gastric inhibitory polypeptide,**GLP-1**:: Glucagon like peptide-1,**HgbA1c**:hemoglobin A1c,**2HPG**:2 h prandial glucose, **NAFLD**: Non-alcoholic fatty liver disease, **NASH**: Non-alcoholic steatohepatitis,**NGT**: normal glucose tolerance,**PPG**: post prandial glucose,**T2DM**:type 2 diabetes.

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were done. Fasting plasma glucose (FPG), 2 h prandial glucose (2hPG),Hemoglobin A1c (HbA1c),ALT, AST, platelet count, and 30 min postprandial (75 g of glucose) GLP-1by using ELISA technique (EIA-4141) kit [13] were assessed in all subjects. Abdominal Ultrasonography (US) to diagnose hepatic steatosis by measuring the degree of brightness of liver parenchyma (semi-quantitative) and Fibrosis 4 score (FIB-4) calculation were done to subjects with NAFLD.

$$\text{FIB} - 4 = \frac{\text{age in years x AST level (U|L)}}{\text{Platelet Count (109/L) x}\sqrt{\text{ ALT (U/L)}}}$$

FIB-4 score <1.30 = F0–F1, FIB-4 score >2.67 = F3–F4 [14].

F0: No fibrosis, F1: Mild fibrosis, F2: Moderate fibrosis, F3: Severe fibrosis, F4: Cirrhosis.

3. Statistical analysis

Data analysis was performed using the SPSS (Statistical Package for the Social Sciences) program (version 18, 2012, IBM Corporation, USA). Data were expressed as follows; mean ± standard deviation (SD) were used for quantitative data, whereas number and percent (%) were used for qualitative data. Independent-samples *t*-test was used when comparing between two groups. One-way analysis of variance (ANOVA) was used when comparing between more than two groups. Chi-square test was used to compare qualitative variables between groups. Pearson's correlation coefficient (r) test was used for correlating data. Stepwise multiple regression analysis was performed to define the independent predictors that affect serum GLP-1 levels. ROC (receiver operator characteristic curve is used to find out the best cut off value of certain variable.) Probability (p-value) less than 0.05 was considered significant and less than 0.01 was considered as highly significant.

4. Results

A statistical significant difference was found among the studied groups regarding GLP-1 values.($P < 0.001$) being highest in group III (control) compared to groups I and II (NAFLD) (936.520 pg/ml ± 149.380) vs. (577.680 pg/ml ± 161.681) and (485.520 pg/ml ± 145.430) respectively. On comparing Groups I and II GLP-1 was non significantly higher in group I than in group II (P value > 0.005). Table 1.

There was also a statistical significant difference between the three groups as regards BMI ($P < 0.001$) it was higher in group I compared to groups II and III (34.078% ± 2.976) vs. (33.370% ± 3.802) and(24.024% ± 1.028) respectively. On comparing groups II &III BMI was significantly higher in group II compared to group III ($p < 0.001$). Table 1.

Age, FBS, 2HPG and HgbA1c were significantly higher in group II compared to groups I and III (51.440 ± 5.738) vs.(45.880 ± 6.044) and (31.320 ± 6.980), (181.800 mg/dl ± 27.353) vs. (88.920 mg/dl ± 10.920) and (82.800 mg/dl ± 5.485), (307.080 mg/dl ± 37.586) vs.(126.920 mg/dl ± 8.679) and (114.000 mg/dl ± 8.221) and(9.569% ± 1.681) vs. (5.032% ± 0.397) and (4.808% ± 0.283) respectively ($p < 0.001$). Table 1.

There was NO statistical significant difference between three groups regarding gender, AST,ALT, platelet count and no statistical significant difference between groups I& II regarding FIB-4 score (P value > 0.005). Table 1.

On doing a correlation study between GLP-1 and all other variables in the study there was a significant positive correlation with Age ($P < 0.001$) and a significant negative correlation with BMI, 2HPG, HbA1C, AST and FIB-4 score ($p < 0.001$, $p = 0.002$,$p < 0.001$,p 0.016 and $p = 0.004$ respectively) On doing multivariate regression analysis, BMI was the only independent determinant of GLP-1 levels. Table 2.

5. Discussion

Our study showed a statistical significant decrease in GLP-1 levels in type 2 diabetic groups when compared with control group. Many studies agreed with our results Faerch et al., 2015 [15],Novaes et al., 2015 [16] and Lastya et al., 2014 [17] who aimed to assess glucose stimulated GLP-1 levels in type 2 Diabetic subjects and healthy controls. They found that GLP-1 concentrations were significantly lower in subjects withT2DM than those with normal glucose tolerance (NGT).

On the contrary, these results disagreed with Yeow et al., 2017 [18] and Aulinger et al., 2016 [19] who studied incretin hormones in young subjects with T2DM and control group. They reported no differences between the two groups as regard plasma GLP-1 levels during oral glucose tolerance test. This variability may be due to the young age, short duration of diabetes, relative good health and well controlled HbA1c levels of the subjects involved in these studies, which allowed evaluation of the effects of diabetes and obesity on the incretins at a very early stage.

As regards glucose stimulated GLP-1 concentrations in non diabetic NAFLD group, our study showed a statistically significant decrease in peptide concentrations when compared with controls($p < 0.001$).

These results agreed with Bernsmeier et al., 2014 who conducted a study on 52 patients with NAFLD and 50 matched healthy controls. Standardized oral glucose tolerance test was performed and GLP-1plasma levels were measured sequentially for 120 min after glucose administration. They reported significantly lower GLP-1 concentrations in NAFLD subjects than controls [20].

On the other hand, our results about GLP-1 in all subjects disagreed with Junker et al., 2016 who aimed to asses incretin

Table 1
Comparison among 3 groups regarding all data using ANOVA test.

	Group I		Group II		Group III			
	range	Mean ± SD	range	Mean ± SD	range	Mean ± SD	F	P value
Age (years)	36–55	45.880 ± 6.044	41–60	51.440 ± 5.738	20–44	31.320 ± 6.980	68.512	<0.001*
BMI %	29.3–38.9	34.078 ± 2.976	27.3–40.3	33.370 ± 3.802	22.06–25.36	24.024 ± 1.028	96.899	<0.001*
FBS mg/dl	70–105	88.920 ± 10.920	120–217	181.800 ± 27.353	73–94	82.800 ± 5.485	257.177	<0.001*
PPS mg/dl	110–139	126.920 ± 8.679	235–360	307.080 ± 37.586	100–128	114.000 ± 8.221	561.697	<0.001*
HbA1C %	4.4–5.6	5.032 ± 0.397	7.3–12.3	9.569 ± 1.681	4.4–5.4	4.808 ± 0.283	176.619	<0.001*
Postprandial GLP1 pg/ml	250–850	577.680 ± 161.681	200–701	485.520 ± 145.430	712–1200	936.520 ± 149.380	61.178	<0.001*
AST U/L	10–54	29.160 ± 10.597	12–58	32.600 ± 11.874	12–39	28.640 ± 8.592	1.062	0.351
ALT U/L	12–39	27.960 ± 6.931	16–49	28.920 ± 8.082	16–39	27.320 ± 6.053	0.324	0.724

* high statistical significance.

Table 2
Correlation between GLP1 versus other variables among all subjects.

Correlations		
	GLP-1	
	r	P value
AGE(years)	0.478	<0.001*
BMI(kg/m^2)	−0.817	<0.001*
FBS(mg/dl)	−0.463	0.071
PPS(mg/dl)	−0.425	0.002
HgA1c(%)	−0.497	<0.001*
AST(U/L)	−0.340	0.016
ALT(U/L)	−0.071	0.622
FIB -4 groups I&II	−0.400	0.004

r:spearman's rank correlation coefficient.
* high statistical significance.

hormones levels during oral glucose tolerance test in non-diabetic NAFLD subjects, type 2 diabetic NAFLD subjects and control group. They reported no differences in secretion of GLP-1 and GIP in any of the groups. This discrepancy may be due to their selection of control group that included more obese (BMI 28% ± 1) individuals than in our study (BMI 24.024% ± 1.028) [21].

Our study reported that BMI was significantly higher in both NAFLD and T2DM + NAFLD groups compared to controls. This is in agreement with Bernsmeier et al., 2014 and Lastya et al., 2014 studies. Bernsmeier et al. found that Weight and BMI were significantly higher in 52 NAFLD patients compared to 50 controls [20]. Lastya et al. conducted a study on 40 subjects who were native Indonesian citizens and 18–70 years of age; Twenty subjects with T2DM and 20 subjects as the control group. They found that body mass index in T2DM subjects was higher than those with NGT [17].

In this work no significant difference was found between NAFLD group and NAFLD + T2DM group as regard fib-4 score. This is in agreement with Junker et al., 2016 who conducted a study on 20 subjects (matched for age, sex and BMI); 10 subjects with normal glucose tolerance + NAFLD and 10 subjects with T2DM + NAFLD. Fibrosis score was calculated in all subjects of the study and there was no difference among any of the groups [21].

In the present study, we also found that glucose-stimulated GLP-1 was negatively correlated with BMI. These current results are in agreement with Faerch et al., 2015 [15] and Muscelli et al., 2008 [22] who studied the impact of obesity on the incretin effect and they found that glucose-stimulated GLP-1 was depressed with increasing the degree of obesity and after weight reduction, GLP-1 levels increased apparently.

In the present study, we found that glucose-stimulated GLP-1 in NAFLD and T2DM + NAFLD subjects was negatively correlated with the degree of liver fibrosis, that was detected by FIB-4 score calculation. This is in agreement with Miyazaki et al., 2012, who studied hepatic expression of dipeptidyl peptidase-4(DPP-4) in non-alcoholic fatty liver disease and its association with insulin resistance and glucose metabolism. They found that NAFLD patients might have increased DPP-4 activity resulting in lower levels of biologically active GLP-1 and this was found also to be in a negative correlation to the severity of liver fibrosis [11].

On doing a multivariate regression analysis BMI was the only independent negative determinant of glucose induced GLP-1 level (P-value <0.001). This is in agreement with Vollmer et al., 2008 who studied predictors of incretin concentrations in subjects with normal, impaired glucose tolerance and diabetes mellitus and found that body weight was negative predictor of GLP-1 secretion [23]. Also is it similar to that found by Nauck et al. 2011who studied secretion of glucagon-like peptide-1 in type 2 diabetes and found that BMI is a negative predictor of GLP-1 secretion [24].

6. Conclusion

Glucose stimulated GLP-1 levels are lower in NAFLD patients with and without T2DM than controls. The levels were significantly lower with increasing degree of obesity, post prandial plasma glucose, Hemoglobin A1c or FIB-4 score. BMI was the only negative predictor of GLP-1 level in the studied population. This draws the attention that GLP-1 analogues may be beneficial in the treatment of T2DM patients with NAFLD and T2DM patients with obesity.

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Declaration of interest

None.

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