Original Article

Ghrelin Levels in Male Patients with Hyperlipoproteinemia I, II versus Type 2 Diabetes Mellitus

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Summary:

Background; Hyperlipoproteinemia (HLP) are divided in primary and secondary subtypes. Primary HLP is usually due to genetic causes. Secondary HLP is resulting from another underlying disorder such as diabetes mellitus that leads to alterations in plasma lipid and lipoprotein metabolism, HLP may be idiopathic .

Objectives; The aims of this paper were to evaluate the differences in the ghrelin hormone (Ghr) levels between healthy control and patients with primary hyperlipoproteinemia [hyperlipoproteinemia I (HPLI) , hyperlipoproteinemia II (HPLII)] and secondary hyperlipoproteinemia [type 2 diabetes mellitus(DM2)], and study the relation of (Ghr) with other parameters.

Patients and Methods; Ninety male individuals (age 30-45)years were enrolled in this study which were divided into three groups as follows:- (G1) consist of 30 healthy male individuals as a control group, (G2) consist of 30 male patients with (HLP) without any other disease (15 of them were HLP1,the other were HLP11), (G3) consist of 30 male patients with DM2 without any other disease .(Ghr), fasting blood glucose (FBG), c-peptide, Insulin, Insulinresistance (IR), Insulin sensitivity (S%), Beta cell function(B%) ,Glucose/ Insulin ratio, triacylglycerol(TG),total cholesterol (TC), low density lipoprotein(LDL),high density lipoprotein(HDL),very low density lipoprotein(VLDL),TC/HDL ratio, LDL/HDL ratio and atherogenic index of plasma(AIP) were evaluated .

Results; The mean level of Ghr was significantly lower (P<0.05) in DM2 compared with control group, significantly higher(P<0.05) in HLP1 compared with HLP11 and DM2 and significantly higher(P<0.05) in HLPII compared with DM2. There were significant correlations between Ghr level and (insulin, c-peptide ,IR,S% ,TG ,VLDL ,AIP) in patients with DM2 and significant correlations between Ghr level and (TG ,LDL ,VLDL ,LDL/HDL ratio ,c-peptide ,S%)in patients with HLPI.

Conclusion; We conclude that low plasma Ghr level is closely related to atherogenicity in DM 2 patients while there is no significant relationship between them in HLPI and HLPII.

Keywords: Ghrelin, Hyperlipoproteinemia I, Hyperlipoproteinemia II, Diabetes mellitus II.

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Introduction:

Hyperlipoproteinemia (HLP) type I,II is a metabolic disorder characterized by abnormally elevated concentrations of specific lipoprotein particles in the plasma. HLP I is elevation chylomicron concentration. HLP II is elevation of low density lipoprotein (LDL) or very low density lipoprotein (VLDL) and LDL(1).HLP are divided in primary and secondary subtypes. Primary HLP is usually due to genetic causes. Secondary HLP is resulting from another underlying disorder such as diabetes mellitus that leads to alterations in plasma lipid and lipoprotein metabolism, HLP may be idiopathic (2).

Type2 diabetes mellitus(DM2) encompasses individuals who have insulin resistance and usually have relative rather than absolute insulin deficiency. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance .The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity (3). Ghrelin(Ghr) is a 28-amino-acid peptide produced primarily in the stomach and so called for its property of stimulating growth hormone (GH) secretion in human , increases food intake, and produces weight gain . Fatty acid modification of Ghr is essential for Ghr-induced GH release from the pituitary and appetite stimulation . Blood concentrations of Ghr are lowest shortly after consumption of a meal, then rise during the fast just prior to the next meal . Ghr has been shown to activate the endothelial isoform of nitric oxide synthase in a pathway that depends on various kinases . There is also strong evidence that Ghr has a peripheral appetite modulatory effect on satiety by affecting the mechanosensitivity of gastric vagal afferents, making them less sensitive to distension resulting in over eating . Obese individuals have lower Ghr levels than those who are thin (4). Obesity, which has become a global public health problem, is one of the major risk factors for development of DM2(5).The aim of this study was to study the correlation between Ghr and insulin resistance (IR), β-cell function (B%)

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, insulin sensitivity (S%) , atherogenic index(AIP) , in patients with DM2 and HLP type I-II.

Patients and Methods:

The range of body mass index (BMI) for the patients and control was (30-35) Kg/m2, and the range of body fat percentage (BFP) for patients and control was (28-36),blood was collected from patients attended in Al-kindy Hospital from October 2012 to June 2013. Patients and control groups were determined the following parameters: Ghrelin hormone(Ghr) was assayed using ELISA Kit supplied by RayBio (6).Total cholesterol(TC) was determined using enzyme-catalyzed colorimetric method (7). Total triglyceride (TG) was determined using enzyme-catalyzed colorimetric method (8). Serum HDL was measured using Burstein separation method using HDL-C kit (9). C-peptide was assayed by sold phase enzyme-linked immunosorbent assay ELISA method by DEMEDITEC c-peptide ELISA Kit (10). Glucose was determined using enzymatic colorimetric method (Glucose oxidase-peroxidase) (11). Mathematical formulas were used to measure:atherogenic index of plasma (AIP)= Log(TG/ HDL)(12) ,IR, B% and S% by use an updated HOMA model(HOMA2)(13) ,LDL by using the Friedwald equation, LDL = TC-[TG/5 + HDL] ,VLDL=TG/5 (14).Insulin; Fasting glucose (mg/dL)×fasting Insulin(µU/mL)/405 = IR, which used in HOMA-IR(15).Statistical Analysis: Data are presented as mean ±SD using SPSS program. The differences between two groups were analyzed by t-test. P-value less than 0.05 considered significant .Pearson's correlation coefficient was used to examine between Ghr and other parameters in patient groups.

Results:

The results in table (1) shows that :

Table (1): (Means±SD)and P-values of Ghr,FBG,C-peptide,Insulin,Insulin resistance,Insulin sensitivity and ß-cell function for patients and control groups

Parameters Groups	Mean±SD							
	Ghr ng/ mL	FBG mg/dL	C-peptide ng/mL	Insulin µU/mL	Insulin resistance	Insulin sensitivity	β-cell function	Glucose/ Insulin
C (n=30)	0.81±0.06	96.2±9.6	0.19±0.04	2.21±1.94	0.51±0.43	173.5±49.4	20.7±4.4	67.9±34.6
HLP (n=30)	0.60±0.09	95±16.0	0.26±0.01	1.88±1.16	0.42±0.24	174.2±53.1	19.8±2.5	66.7±34.3
HLP 1 (n=15)	0.67±0.08	94.2±11.3	0.23±0.01	1.51±0.48	0.37±0.11	178.9±22.8	19.2±1.2	71.5±22.2
HLP 11 (n=15)	0.53±0.13	95.6±8.6	0.3±0.1	2.12±1.40	0.46±0.29	171.2±38.7	20.19±3.05	63.7±40.7
DM 2 (n=30)	0.38±0.06	202.6±70.7	3.6±0.2	5.28±2.73	2.84±1.74	46.62±25.2	38.14±27.9	64.8±51.5
	P-Value							
C-HLP	P<0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
C-HLP 1	P<0.05	P>0.05	P>0.05	P<0.05	P<0.05	P>0.05	P>0.05	P>0.05
C-HLP 11	P<0.05	P>0.05	P<0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
C-DM2	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P>0.05
HLP1-HLP11	P<0.05	P>0.05	P>0.05	P<0.05	P<0.05	P>0.05	P>0.05	P>0.05
HLP1- DM2	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P>0.05
HLP11-DM2	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P>0.05

Significant decrease in mean level of Ghr in DM2 group as compared to control group (P<0.05), significant increase in mean level of Ghr at HLP I group as compared to HLP II group (P<0.05) and DM2 (P<0.05),alsosignificant increase in mean level of Ghr at HLP II group as compared to DM2 (P<0.05).In DM2 patients group, the mean of fasting blood glucose was significantly greater than that in the control group at (P<0.05),and there were no significant differences in means between normal control group and other different groups There was a highly significant increase in C-peptide levels (P<0.05) in DM2 patients group as compared with the control group, also there was significant increase in C-peptide level (P<0.05) in HLP II group as compared with control group .As a comparison between pairs of patients groups, there was significant increase in C-peptide level (P<0.05) in DM2 group as compared with HLP I and HLP II groups.There were a highly significant increase in insulin levels (P<0.05) in DM2 patients group and significant decrease (P<0.05) in HLP I patients group as compared with control group. For a comparison between pairs of patients groups, there was significant increase (P<0.05) in insulin level of HLP II group as compared to HLP I group which has the lowest value among

groups, and significant highly increase (P<0.05) in insulin level in DM2 patients group as compared to HLP I and HLP II groups.There was significant increase in IR (P<0.05) in DM2 patients group and significant decrease (P<0.05) in HLP I group (which has lowest mean value) as compared to control group. There was significant increase in IR of DM2 patients group as compared to HLP I and HLP II groups (P<0.05), HLP II group also had significant increase when compared with HLP I group (P<0.05).With the S%, the lowest mean value estimated was at the patients of DM2 group ,there was significant decrease in mean of DM2 patients group as compared to control group (P<0.05) .As a comparison between study groups, there was no significant difference in mean of S% between HLP I and HLP II groups (P>0.05),while there were significant decrease differences in means of DM2 group as compared to HLP I and HLP II groups (P<0.05).The majority mean of B% values estimated was at the DM2 patients group, which record significant increase difference as compared to control group (P<0.05) . When comparison among study groups, significant increase in mean of B% at DM2 patients group as compared to HLP I and HLP II groups (P<0.05). And the results in table (2) shows that:

Table (2) : (Means±SD)and P-values of TAG,TC,LDL,HDL, VLDL, TC/HDL, LDL/HDL ,AIP for patients and control groups

Parameters Groups	Mean±SD							
	TG mg/dL	TC mg/dL	LDL mg/dL	HDL mg/dL	VLDL mg/dL	TC/HDL	LDL/HDL	AIP
C (n=30)	129.6±32.8	166.0±27.3	101.0±15.9	44.1±9.2	25.2±6.5	4.01±0.92	2.40±0.71	0.34±0.1
HLP (n=30)	207.1±73.21	197.6±35.4	115.5±40.2	42.1±11.4	41.4±14.6	5.02±1.18	2.91±1.10	0.68±0.02
HLP 1 (n=15)	248.4±50.9	168.6±23.9	80.6±23.2	41.3±12.9	49.6±10.1	4.52±0.85	2.49±0.67	0.81±0.15
HLP 11(n=15)	165.8±69.7	226.6±15.0	150.3±14.8	43.0±10.3	33.1±13.8	5.53±1.28	3.67±0.90	0.56±0.12
DM 2 (n=30)	196.1±88.30	216.5±68.0	132.0±55.3	42.4±7.2	39.2±17.6	5.20±1.88	3.13±1.39	0.63±0.21
	P-Value							
C-HLP	P<0.05	P<0.05	P>0.05	P>0.05	P<0.05	P>0.05	P>0.05	P<0.05
C-HLP 1	P<0.05	P>0.05	P<0.05	P>0.05	P<0.05	P>0.05	P>0.05	P<0.05
C-HLP 11	P>0.05	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05
C-DM2	P<0.05	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05
HLP1-HLP11	P<0.05	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05
HLP1- DM2	P<0.05	P<0.05	P<0.05	P>0.05	P<0.05	P<0.05	P<0.05	P<0.05
HLP11-DM2	P>0.05	P>0.05	P<0.05	P>0.05	P<0.05	P>0.05	P>0.05	P<0.05

The majority of mean values of TG estimated were at the HLP I group which has greater significant difference, also DM2 group have increase significant difference as compared to normal control group. Greater significant difference in means of TG between HLP I group and HLP II group at (P<0.05),also significant increase difference between HLP I group and DM2 group at (P<0.05).highly significant increases in means of TC in HLP II group and DM2 group as compared to control subjects group at (P<0.05) .There were significant decrease in means of TC in HLP I group when compared to HLP II group and DM2 group at (P<0.05) .In all patient groups , the means of serum LDL were significantly difference as compared to control subjects group at (P<0.05). There were significant increase differences in HLP II group and DM2 group as compared to control subjects group and significant decrease difference in HLP I group as compared to control subjects group . There were significant decrease difference in HLP I group as compared to HLP II group and DM2 group at (P<0.05).Serum VLDL and AIP levels showed significant increase differences as compared to control subjects group at (P<0.05),significant increase difference in HLP I group as compared to HLP II and DM2 groups at (P<0.05).The results of TC/HDL ratio showed significant increase differences in means for HLP II and DM2 groups at (P<0.05) .The results showed significant increase in means in HLP II group and DM2 group as compared to HLP I group at (P<0.05) .The mean values of LDL/HDL ratio showed, that the lowest value was found in control group. There were significant increase differences (P<0.05) in HLP II and DM2 groups as compared to control subjects group. There were significant increase differences in HLP II and DM2 groups as compared to HLP I group(P<0.05).

The results in table (3) shows that :

Table (3): Correlation coefficients and P values between Ghrelin hormone levels and other parameters for all groups.

Groups Ghrelin	Control		HLP		HLP1		HLP 11		DM2	
	r	p-value	r	p-value	r	p-value	r	p-value	R	p-value
T C (mg/dL)	0.114	P> 0.05	-0.0614	P> 0.05	-0.32	P>0.05	-0.08	P>0.05	0.015	P>0.05
TG (mg/dl)	0.083	P>0.05	0.089	P> 0.05	-0.36	P<0.05	0.026	P>0.05	-0.424	P<0.05
LDL (mg/dL)	0.168	P> 0.05	-0.079	P> 0.05	-0.44	P<0.05	-0.12	P>0.05	-0.0345	P> 0.05
VLDL (mg/dL)	0.084	P> 0.05	0.0900	P> 0.05	-0.36	P< 0.05	0.028	P> 0.05	-0.428	P< 0.05
HDL (mg/dl)	-0.004	P> 0.05	-0.089	P> 0.05	-0.19	P> 0.05	0.008	P> 0.05	-0.327	P> 0.05
TC/HDL	0.028	P> 0.05	-0.08	P> 0.05	-0.14	P> 0.05	-0.11	P> 0.05	0.166	P> 0.05
LDL/HDL	0.04	P> 0.05	-0.109	P> 0.05	-0.37	P< 0.05	-0.12	P> 0.05	0.080	P> 0.05
AIP	0.037	P> 0.05	0.062	P> 0.05	0.28	P>0.05	0.036	P>0.05	-0.433	P<0.05
FBG (mg/dl)	-0.115	P> 0.05	-0.009	P> 0.05	0.06	P>0.05	-0.04	P>0.05	0.0347	P>0.05
C-peptide (ng/ml)	0.0062	P>0.05	0.227	P> 0.05	-0.49	P<0.05	0.05	P>0.05	-0.037	P<0.05
Insulin (µU/ml)	-0.0204	P> 0.05	0.0749	P> 0.05	0.165	P>0.05	0.015	P>0.05	-0.210	P< 0.05
IR	0.0064	P> 0.05	0.049	P> 0.05	0.189	P> 0.05	0.05	P> 0.05	-0.177	P< 0.05
S%	-0.149	P> 0.05	-0.0756	P> 0.05	0.52	P< 0.05	-0.1	P> 0.05	0.345	P< 0.05
B%	0.0064	P> 0.05	0.050	P> 0.05	0.189	P> 0.05	0.05	P> 0.05	0.116	P> 0.05
Glu/Insulin	-0.308	P> 0.05	-0.234	P> 0.05	-0.31	P> 0.05	-0.13	P> 0.05	-0.119	P> 0.05

There was significant positive correlation between Ghr and S% in HPLI and DM2 groups. Significant negative correlation between Ghr and TG,LDL,VLDL,LDL/HDL,C-peptide in HPLI group and significant negative correlation between Ghr and TG,VLDL,AIP,c-peptide,Insulin ,IR,S% in DM2 group.

Discussion:

It has been shown that Ghr concentrations are reduced in different pathophysiological conditions including obesity, DM2, and other conditions with metabolic disturbances. Ghr may play an important role in adipogenesis and storage of energy in adipose tissue. Chronic Ghr administration has been shown to increase body fat content in humans(16). We found, that ghrelin concentrations of subjects with type 2 diabetes were lower than those of subjects with HLP1 and HLP2. Circulating Ghr concentration is inversely associated with the body mass index; thus, obese individuals have lower levels than lean individuals. (17) Therefore, the lower ghrelin concentration in type 2 diabetic subjects could be due to their higher adiposity. The negative correlation between fasting ghrelin and insulin has also been reported earlier. Ghrelin inhibits glucose-induced insulin release via a paracrine mechanism (18). Our findings were in accordance with these results, showing that ghrelin level is negatively associated with insulin concentrations and the prevalence of type 2 diabetes and positively associated with insulin sensitivity(18) . An increase in insulin after the oral or intravenous glucose administration could contribute to the inhibitory effect of glucose on ghrelin concentrations(19). However, it is still unclear whether insulin and glucose play a direct inhibitory role in ghrelin secretion.(20)Our findings are in accordance with these results which showing that ghrelin level is negatively associated with HOMA-IR(18)(21). our study also was in agreement with a study which stated that Ghr may be associated with the beta-cell hypofunction and first-phase insulin secretion defects in DM2.(18)(22) Our findings are in accordance with study which announced that Ghr negatively correlated with C-peptide.(23) in our study, Ghr is negatively correlated with AIP in DM2 group .This result is closely related to the results obtainded from the study by Min Zhang in 2012 (24) . We conclude that low plasma Ghr level is closely related to atherogenicity in DM 2 patients while there is no significant relationship between them in HLPI and HLPII.

Authors' contributions:

Salma Abdul Rudha designed the study, performed experimental work, paper writing and drafting, read and approved the final manuscript, Samir Laybi Shkhaier experimental work, paper drafting, Baydaa Ahmed participated in experimental work.

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