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Research Article

ASSOCIATION BETWEEN INSULIN, GHRELIN, HOMEOSTASIS MODEL ASSESSMENT-INSULIN RESISTANCE, HOMEOSTASIS MODEL ASSESSMENT-β, WAIST-TO-HIP RATIO AND BODY MASS INDEX TO PLASMA GLUCOSE AND GLYCOSYLATED HEMOGLOBIN AND ITS CLINICAL USEFULNESS IN TYPE 2 DIABETES MELLITUS PATIENTS WITH OBESITY

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ABSTRACT

Objectives: To evaluate new diabetic control monitoring parameters insulin, ghrelin, body mass index, waist-to-hip ratio, homeostasis model assessment-insulin resistance (HOMA-IR), and HOMA-β for Type 2 diabetes mellitus (T2DM) with obesity and to compare them with the existing diabetic control markers plasma glucose and glycosylated hemoglobin (HbA1c) and to recommended these additional tests to assess complications associated with kidney, liver, cardiac, and pancreas.

Methods: A total of 100 T2DM patients with obesity who attended the sugar clinic attached to Apollo Speciality Hospitals, Vanagaram and who were on standard treatment and 50 age and sex matched controls attending the routine master health check in the same hospital were enrolled for the study. Fully automated analyzers and reagents and controls were used for all assays to ensure validity of the results obtained. For insulin and ghrelin assays, established commercial kits were used and all other parameters were calculated using formulae established previously. Graphpad online calculator was used to calculate t and p values.

Results: The results obtained for both controls and patients for the set of additional parameters were compared with fasting plasma glucose (FPG), post prandial plasma glucose (PPPG), and HbA1c between controls, patients and controls with patients by calculating r and p values. Highly significant correlations were obtained in all comparisons.

Conclusions: Very good associations ($p<0.0001$) were found between FPG, PPPG, and HbA1c to each of the new parameters for controls, patients and between controls and patients. These additional parameters may be done at fixed intervals of time to evaluate kidney, liver, cardiac and pancreatic complications/dysfunction in T2DM patients with obesity.

Keywords: Diabetes mellitus, Glycosylated hemoglobin, Plasma glucose, Waist-to-hip ratio, Body mass index, Homeostasis model assessment-insulin resistance, Homeostasis model assessment-β.

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INTRODUCTION

Among the various types of diabetes mellitus (DM), Type 2 DM (T2DM) predominates worldwide due to life style and eating disorders. The first test done in clinical laboratories to diagnose DM is qualitative urine micro, sugar and albumin using a random urine sample and if sugar is present fasting plasma glucose (FPG) and post prandial plasma glucose (PPPG) will then be measured to correlate. Based on that, glucose tolerance test (GTT) will be done to confirm DM. After treatment, monitoring the control of DM will mostly be based on the level of glycosylated hemoglobin (HbA1c) and occasionally doing FPG and PPPG. However, all these routine tests will not be able to detect kidney, liver, cardiac and pancreatic complications. Therefore, there is an urgent need to establish additional organ complications markers such as insulin, ghrelin, body mass index (BMI), waist-to-hip ratio (WHR), homeostasis model assessment-insulin resistance (HOMA-IR), and HOMA-β so as to use them occasionally to monitor the functioning of kidney, liver, cardiac and pancreas.

Literature review

The prevalence of diabetes is rising all over the world due to population growth, aging, urbanization, sedentary life style an increase of obesity and physical inactivity as the main factors leading to DM and is disproportionately high among Asians in young to middle-aged adults. This could have long-lasting adverse effects on a nation's health and economy, especially for developing countries. The International Diabetes Federation (IDF) estimates that the total number of people in India with diabetes to be around 50.8 million in 2010, and may raise to 87.0 million by 2030 [1].

The primary objective is in the management of DM for the attainment of near-normal glycemia. In India, more than half of the patients have poor glycemic control and have vascular complications due to obesity-induced DM. Therefore, there is an urgent need to develop novel therapeutic agents for DM to arrest the development and progression of complications without compromising on safety. DM shows positive and independent associations with age, BMI, WHR, IR, and oxidative stress, a family history of diabetes, socioeconomic status and sedentary physical activity. The routine markers used as of now are FPG, PPPG, and GTT to confirm DM and HbA1c to monitor diabetic control [2].

Oral hypoglycemic agents have a very important clinical impact on treating T2DM. Many complications develop if the disease is not treated early enough and with the proper pharmacological agents. These complications include diabetic retinopathy, nephropathy, and neuropathy. Other cardiovascular risk factors must also be addressed in

patients with T2DM. The clinical benefits of all pharmacological agents become more complete when accompanied with nonpharmacological treatments [3].

Ghrelin and DM

The recently discovered gastric endocrine agent ghrelin, the only potent hunger inducing factor circulating may serve as an additional diagnostic tool to monitor DM and its control based on its properties of targeting the neuroendocrine regulation of energy balance. It is quite possible that a ghrelin antagonist will either fail to cure obesity due to the existence of compensatory mechanisms or undesired effects might reveal the true biological function of ghrelin [4]. Suppression of serum ghrelin was found to be strongest after meal challenge suggesting further investigation of the significance of ghrelin suppression in patients with diabetes [5] and ghrelin level may be associated with poor diabetes control, and bad prognosis parameters including dyslipidemia (high cholesterol, low-density lipoprotein-cholesterol, triglycerides, and relatively low high-density lipoproteins-cholesterol), IR parameters (high HOMA-IR and low HOMA-β) in obese diabetic patients. Therefore, it can be concluded that low ghrelin levels are the indicator of bad consequences in obese diabetic patients [6].

HbA1c and DM

The HbA1c test checks the long-term control of blood glucose (2-3 months) levels in people with established DM who are on treatment. Most doctors use HbA1c level as the best way to check how well a person is controlling his or her diabetes [7]. The primary action of insulin is to stimulate glucose disappearance. Insulin helps control post prandial glucose in three ways. Initially, insulin signals the cells of insulin-sensitive peripheral tissues, primarily skeletal muscle, to increase their uptake of glucose. Second, insulin acts on the liver to promote glycogenesis. Finally, insulin simultaneously inhibits glucagon secretion from pancreaticβ-cells, thus signaling the liver to stop producing glucose via glycogenolysis and gluconeogenesis. All of these actions reduce blood glucose [8,9]. There is a significant correlation between PPPG and HbA1c values. Validation of results in the large cohort of patients in multicenter study will make them generalizable. Since PPPG is performed routinely, its interpretation in terms of long-term glycemic control will help clinicians to tailor their therapeutic strategies [10].

HOMA-β and DM

By studying the correlations between serum ghrelin levels to both BMI, and insulin, a statistically significant reverse relation between them was found, within each group, suggesting that, ghrelin concentrations were affected in T2DM patients with obesity. Hence, it may play a significant role in DM. Many studies have linked ghrelin to obesity [11,12]. IR is a key factor in metabolic disorders such as hyperglycemia and hyperinsulinemia, which are promoted by obesity and may later lead to T2DM. Systematic assessment of barriers of insulin therapy, individualized diabetes treatment plans and information of patients may help to overcome such negative attitudes, leading to quicker initiation of therapy, improved adherence to treatment, and a better quality of life [13,14].

BMI, WHR, and DM

HOMA-β predicts the basal state of insulin and glucose in terms of resistance and β-cell function. HOMA solutions might indicate 100% β-cell function and 100% insulin sensitivity, or, in the case of a thin, fit individual with high sensitivity, 50% β-cell function and 200% insulin sensitivity. β-cell data are reported in isolation, one might conclude erroneously that the subject had failing β-cells, as opposed to appropriately low secretion because the sensitivity of the body is high. The HOMA-β model has proved be a robust clinical and epidemiological tool in descriptions of the pathophysiology of diabetes. Already quoted in 500 publications, it has become one of the standard tools in the armamentarium of the clinical physiologist. HOMA-β analysis allows assessment of inherent β-cell function and insulin sensitivity and can characterize the pathophysiology in those with abnormal glucose tolerance [15]. By providing information on the trend of diabetes in certain community, important clues about the magnitude and structure of the primary and secondary intervention programs may help to effectively manage this disease. Weight loss, regular exercise, modification of diet, and quitting smoking could prevent the majority of cases of T2DM. Weight control would appear to offer the greatest benefit [16].

Studies have demonstrated that WHR is the strongest anthropometric index that associates with T2DM in both sexes, and this parameter should be used in routine practice for the follow-up of such patients [17], the association of WHR to DM is real and independent of age, sex, family history of diabetes [18]. Measurements of the simple calculate parameter using height and weight will be very useful to monitor and control of T2DM which is directly linked to obesity. A significant correlation was found between ghrelin concentration with BMI and abdominal circumference [12]. Elevated BMI was also associated with progressively higher risk for all DM complications. The relationship between excess weight and being diagnosed with a DM complication was stronger for women than for men. These results suggest a stronger association between BMI and onset of DM than was previously documented in similar studies [19].

METHODS

This study is an open, parallel, prospective, nonrandomized, concurrent control group, (experimental group before and after) study in comparison with sex and age matched controls.

Conforming to ethics

Approval from Institutional Ethics Committee (Apollo Hospitals Chennai) was obtained for this study before the commencement of patient's recruitment. All data were identified by a unique identification number and patient initials. Patient's information and the results obtained on blood were kept confidential.

Inclusion criteria

- All patients in the age group of 26-55 years.
- Both males and females without family history of DM.
- Who are under insulin or any other oral antidiabetic drug also included for this study.
- Who are diagnosed to have T2DM.
- Who do not have liver, kidney and cardiac related diseases.
- Patients who have FPG>126 mg/dL, PPPG >220 mg/dL and HbA1c >6.5%.
- Patient who have BMI >30 confirming obesity.

Exclusion criteria

- Patients in the above age group <26 to >55 who have family history of DM.
- Who have liver, kidney and cardiac related diseases.
- Who are not having T2DM.
- Who have normal FPG, PPPG, and HbA1c.
- Who have BMI <30.

Subject recruitment and data collection

For the evaluation of biochemical parameters involved in this study, the research diabetic group was compared with normal group and/ individuals. Individuals for the research group were recruited from Apollo Speciality Hospitals referral from consultant sugar clinic. The normal group comprised willing individuals who were not diabetic as well as obese. Both groups, in addition to completing underwent blood tests, which were compared.

Experimental group

Newly diagnosed diabetic patients With obesity age group of 18-55 years.

- Males-50
- Females-50.

Controls group

Collecting data from normal persons undergoing Master Health Checkup-50.

For the measurement of plasma glucose Dirui CS 1300B analyzer and kit supplied by Iris health-care organization was used. For the measurement of insulin, Roche e411 analyzer and the kits supplied by the company were used. Ghrelin was measured using ELISA technique using Raybiotech Company. For measuring HbA1c, Biorad D-10 (HPLC method) instrument and the kit supplied by them was used.

BMI, WHR, HOMA-IR, and HOMA-β were calculated using the following formula

$$\text{BMI} = \frac{\left(\text{Weight in kilograms}\right)}{\left(\text{Height in meters}^2\right)}$$

$$\text{WHR} = \frac{\text{Waist cirumferance}}{\text{Hip circumference}}$$

$$\text{Homa} - \text{IR} = \frac{\text{Insulin} * \text{FBS}}{22.5}$$

$$\text{Homa} - ^2 = \frac{20 * \text{Insulin}}{\text{Glucose} - 3.5}$$

Glucose in mmol/L.

Insulin in μIU/mL [20-22].

Biorad accuracy controls at two levels were used every time when assays were carried out to validate the accuracy of the results obtained in this study.

Statistical calculations

A software www.graphpad.com downloaded from the website was used to calculate t and p values.

RESULTS

Table 1 shows the mean and SD obtained for all normal (males 25 and females 25) and study group (males 50 and females 50). All the 9 analytes studied shows elevated levels compared to normal population.

Table 2 presents the mean and SD for the normal males and study group males along with t and p values. All the analytes compared between normal and study group patients shows highly significant ($p<0.0001$) indicating that all the analytes studied for normal and study group patients shows significant differences confirming the clinical usefulness of the additional 6 analytes included in this study.

Table 3 shows similar data presented in Table 2 for normal males versus study group males. All analytes compared between normal and study group females shows highly significant correlation indicating that the additional 6 analytes studied are clinically useful irrespective of sex.

Table 4 presents the statistical parameters t and p for the study group patients before and after treatment. Based on the p values, it is confirmed that the analytes studied are indeed useful to monitor the improvement of the patients analytes values (reduced for all analytes).

Table 5 presents similar data as shown in the Table 4 for all patients. The observations and interpretations are similar to the one observed for all patients shown in Table 4.

Table 6 presents similar data as shown in the Table 4 for all patients. The observations and interpretations are similar to the one observed for all patients shown in Table 5.

DISCUSSIONS

Many studies have predicted the clinical usefulness of insulin and the usefulness of the calculated parameters HOMA-IR, HOMA-β, WHR, and BMI in evaluating the control of T2DM with obesity. These additional parameters will certainly help in evaluating kidney, liver, cardiac, and pancreatic dysfunctions if done at regular intervals of time. Previous

Table 1: All normal versus study group patients

S. No	Analyte	Normal mean and SD (n=50)	Study group mean and SD (n=100)	t	p	Interpretation
1	FPG	95±6	183±56	11.8	<0.0001	Study group has high FPG
2	PPPG	119±12	269±82	12.93	<0.0001	Study group has high PPPG
3	HbA1c	5.5±0.4	8.7±1.5	15.26	<0.0001	Study group has high HbA1c
4	Insulin	8.6±5	37.7±13.3	16.2	<0.0001	Study group have high insulin
5	Ghrelin	644±107	1516±440	13.9	<0.0001	Study group has high ghrelin
6	BMI	25.2±2.5	31.5±3.0	15.6	<0.0001	Study has high BMI
7	WHR	1.0±0.1	1.2±0.04	17.4	<0.0001	Study group has high WHR
8	HOMA-IR	1.9±1.0	12.7±6.8	11.1	<0.0001	Study group high IR
9	HOMA-β	28.5±15.2	77±32	11.5	<0.0001	Study group high β function

SD: Standard deviation, FPG: Fasting plasma glucose, PPPG: Post prandial plasma glucose, HbA1c: Glycosylated hemoglobin, BMI: Body mass index, WHR: Waist-to-hip ratio, HOMA-IR: Homeostasis model assessment-insulin resistance

Table 2: Normal males versus study group males

S. No	Analyte	Normal mean and SD (n=25)	Study group mean and SD (n=50)	t	p
1	FPG	95±6	189±54	8.65	<0.0001
2	PPPG	120±13	265±83	8.65	<0.0001
3	HbA1c	5.5±0.3	8.7±1.5	10.53	<0.0001
4	Insulin	10.1±6.4	37.8±9.1	11.87	<0.0001
5	Ghrelin	654±99	1380±407	8.76	<0.0001
6	BMI	25.3±2.3	30.6±2.6	8.63	<0.0001
7	WHR	1.0±0.1	1.2±0.04	12.36	<0.0001
8	HOMA-IR	2.4±1.5	17.4±6.2	12.49	<0.0001
9	HOMA-β	33±19	74±28	6.59	<0.0001

SD: Standard deviation, FPG: Fasting plasma glucose, PPPG: Post prandial plasma glucose, HbA1c: Glycosylated hemoglobin, BMI: Body mass index, WHR: Waist-to-hip ratio, HOMA-IR: Homeostasis model assessment-insulin resistance

Table 3: Normal females versus study group females

S. No	Analyte	Normal mean and SD (n=25)	Study group mean and SD (n=50)	t	p
1	FPG	94±7	177±58	7.11	<0.0001
2	PPPG	118±12	273±83	9.26	<0.0001
3	HbA1c	5.4±0.4	8.6±1.5	10.45	<0.0001
4	INSLUIN	7±2	37.7±16.5	9.24	<0.0001
5	GHRELIN	635±117	1651±434	11.46	<0.0001
6	BMI	24.5±1.5	32.5±3	12.54	<0.0001
7	WHR	1.0±0.1	1.2±0.04	12.36	<0.001
8	HOMA-IR	1.6±0.4	8.1±3.2	10.1	<0.0001
9	HOMA-β	24±9	79±36	7.5	<0.0001

SD: Standard deviation, FPG: Fasting plasma glucose, PPPG: Post prandial plasma glucose, HbA1c: Glycosylated hemoglobin, BMI: Body mass index, WHR: Waist-to-hip ratio, HOMA-IR: Homeostasis model assessment-insulin resistance

Table 4: All patients at diagnosis versus after treatment (study period 6-month)

S. No	Analyte	At diagnosis mean and SD (n=100)	After treatment mean and SD (n=100)	t	p	Interpretation after treatment
1	FPG	183±56	135±40	6.98	<0.0001	Reduced
2	PPPG	269±82	199±54	7.13	<0.0001	Reduced
3	HBA1C	8.7±1.5	7.7±1.3	5.04	<0.001	Reduced
4	Insulin	37.7±13.3	24.5±11.9	7.4	<0.0001	Reduced
5	Ghrelin	1516±440	1100±256	8.17	<0.0001	Reduced
6	BMI	31.5±3.0	28±2.9	8.39	<0.0001	Reduced
7	WHR	1.2±0.04	1.1±0.05	15.61	<0.0001	Slightly reduced
8	HOMA-IR	12.7±6.8	7.2±4.3	6.84	<0.0001	Improved
9	HOMA-β	77±32	67±39	1.98	0.0468	Improved

SD: Standard deviation, FPG: Fasting plasma glucose, PPPG: Post prandial plasma glucose, HbA1c: Glycosylated hemoglobin, BMI: Body mass index, WHR: Waist-to-hip ratio, HOMA-IR: Homeostasis model assessment-insulin resistance

Table 5: Male patients at diagnosis versus after treatment (study period 6-month)

S. No	Analyte	At diagnosis mean and SD (n=50)	After treatment mean and SD (n=50)	t	p
1	FPG	189±54	141±43	4.92	<0.0001
2	PPPG	265±83	204±57	4.29	<0.0001
3	HBA1C	8.7±1.5	7.9±1.5	2.67	0.009
4	INSULIN	37.8±9.1	24.3±12.1	6.31	<0.0001
5	GHRELIN	1380±407	1108±268	3.95	<0.0001
6	BMI	30.6±2.6	27.5±2.6	5.96	<0.0001
7	WHR	1.2±0.04	1.1±0.05	11.04	<0.0001
8	HOMA-IR	17.4±6.2	8.5±5.5	7.59	<0.0001
9	HOMA-β	74±28	64±37	1.52	0.1307

SD: Standard deviation, FPG: Fasting plasma glucose, PPPG: Post prandial plasma glucose, HbA1c: Glycosylated hemoglobin, BMI: Body mass index, WHR: Waist-to-hip ratio, HOMA-IR: Homeostasis model assessment-insulin resistance

Table 6: Female patients at diagnosis versus after treatment (study period 6-month)

S. No	Analyte	At diagnosis mean and SD (n=50)	After treatment mean and SD (n=50)	t	p
1	FPG	177±58	128±35	5.1147	<0.0001
2	PPPG	273±83	194±52	5.7034	<0.0001
3	HBA1C	8.6±1.5	7.6±1.2	3.68	0.0004
4	Insulin	37.7±16.5	24.7±11.8	4.5316	<0.0001
5	Ghrelin	1651±434	1091±245	7.9454	<0.0001
6	BMI	32.5±3	28.3±3.1	6.8843	<0.0001
7	WHR	1.2±0.04	1.1±0.04	12.5	<0.0001
8	HOMA-IR	8.1±3.2	5.9±2.0	4.1224	<0.0001
9	HOMA-β	79±36	71±41	1.0368	0.3024

SD: Standard deviation, FPG: Fasting plasma glucose, PPPG: Post prandial plasma glucose, HbA1c: Glycosylated hemoglobin, BMI: Body mass index, WHR: Waist-to-hip ratio, HOMA-IR: Homeostasis model assessment-insulin resistance

studies have shown correlations between ghrelin to both BMI and insulin, However, they did not recommend its routine use of such parameters [11,12]. We have observed higher IR and insulin in T2DM with obesity similar to previously documented [13,14].

Analysis of HOMA-β has reverted the % of β-cell functioning which is very useful to monitor pancreatic dysfunctions and such observations have been previously documented [15].

WHR and BMI, both of which could be calculated easily will certainly help to monitor obesity level as the treatment progresses and such observations have previously been pointed out [12,18,19]. Hence, the outcome of this study has established the clinical usefulness of the tests done.

CONCLUSIONS

The outcome of this study has strongly established the following conclusions.

Along with routinely used parameters such as FPG, PPPG, and HbA1c, this study has undertaken an additional 6 parameters out of which 4 are based on calculations, all of which were diagnostically useful to monitor various organs such as liver, kidney, cardiac and pancreatic complications.

Significant elevations for patients compared to controls were observed for the measured parameters glucose, HbA1c, insulin, ghrelin and the calculated parameters BMI, WHR, HOMA-IR, HOMA-β.

Additional tests evaluated in this study will certainly help to monitor organ complication/dysfunction if they are investigated at fixed intervals of time along with HbA1c.

The additional diagnostically useful tests measured and established in this study such as ghrelin. Insulin and other calculated parameters may be easily done in any clinical laboratory.

The final conclusions are insulin and ghrelin (measured) and BMI, WHR, HOMA-IR, HOMA-β (calculated) may be grouped as organ complication evaluation tests for T2DM patients with obesity and they may be done at fixed intervals of time to evaluate kidney, liver, cardiac and pancreatic complications.

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