

Association of waist circumference, serum ghrelin level and insulin resistance in polycystic ovary syndrome: a cross-sectional study

Madhur M. Gupta^a, Chaitanya V. Bhojane^b, Sonali B. Rode^c, Harsh V. Salankar^d

^aDepartment of Biochemistry, NKPSIMSRC & LMH, Nagpur, ^bMedical Student, NKPSIMSRC & LMH, Nagpur, ^cDepartment of Pharmacology, SBIMS, Raipur (C.G.), ^dDepartment of Pharmacology, NKPSIMSRC & LMH, Nagpur

Correspondence to Harsh Salankar, Department of Pharmacology, NKPSIMSRC & LMH, Nagpur (M.S.), India. Tel: +91-8889500320; Fax: +91-7104-665011; e-mail: harshsalankar@gmail.com

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Context

Ghrelin is mostly secreted by enteroendocrine cells of stomach. It is responsible for appetite, carbohydrate metabolism, and improving muscle mass and bone density. Ghrelin also plays a perilous part in reproductive and energy-related activities. In the recent past, Polycystic ovary syndrome (PCOS) is a key root of infertility due to abnormal hormonal activities. Insulin resistance (IR) is more common in PCOS.

Aims

To find the connection between waist circumference, serum ghrelin concentrations, and insulin resistance in PCOS patients.

Settings and design

A cross-sectional study was carried out at a tertiary care teaching hospital.

Materials and methods

Patients within the range of 20–35 years of age group and suffering from PCOS were recruited from Gynaecology OPD. The waist circumference was measured in centimeters. 5 ml fasting blood sample was collected and analyzed for – Plasma Ghrelin, Plasma glucose, Plasma Insulin, and Insulin resistance by HOMA-IR score.

Statistical analysis used

SPSS version 26 was utilized. Data of the measured variables were entered in the Excel sheet and expressed as mean \pm standard deviation. Co-efficient of correlation denoted the relation between the variables.

Results

We analysed the waist circumference, serum ghrelin, plasma insulin level and HOMA-IR score of the subjects. Waist circumference was positively correlated with HOMA-IR and serum ghrelin level. Serum ghrelin and insulin resistance also showed a strong association.

Conclusions

Results obtained from our study state that in PCOS patients waist circumference is directly proportional to serum ghrelin level and insulin resistance. Also, serum ghrelin has a correlation with insulin resistance.

Keywords:

Keywords, Homeostatic model assessment, insulin resistance, polycystic ovary syndrome, serum ghrelin, waist circumference

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Key Message: Serum ghrelin could serve as a diagnostic marker for early detection and effective management of PCOS.

Introduction

Ghrelin, a peptide hormone with twenty-eight amino acids is responsible for appetite, carbohydrate metabolism etc [1]. It is mainly formed by enteroendocrine cells of the stomach and is abbreviated as Hunger hormone. Its serum level varies according to diet. It stimulates gastric motility. The nonacylated ghrelin constitutes a major proportion of circulating ghrelin affecting cardiovascular function by its role in carbohydrate

and fat metabolism. Acylated ghrelin is concerned with ghrelin release by its glucose metabolism. Polycystic ovary syndrome (PCOS) is due to abnormal hormonal activities resulting in enlarged ovaries with a small cyst on the outer boundary. It results in prolonged menstrual phases. There is a fluid collection forming cyst which may be the cause of the infrequent release of eggs resulting in infertility. It is known that an increase in insulin may be the cause of

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altered ovulatory function [2]. In India, PCOS is a problem for 3.7% to 22.7% of females of reproductive age [3]. Chronic PCOS increases the chances of cardiovascular ailments, type II diabetes mellitus, and endometrial cancer. Irrespective of with or without a history of obesity, insulin resistance is more common in PCOS. Studies have verified a sturdy and direct connection between waist circumference and insulin resistance. This insulin resistance is usually expressed as Homeostatic model assessment (HOMA-IR) score [4]. Insulin resistance is a problem where muscles, fat, and hepatocytes do not respond to insulin and altered glycolysis metabolism occurs which affects energy homeostasis [5]. To override this, beta cells of islets of Langerhans produce more insulin in the acute phase than in chronicity, as beta cells start losing due to overactivity [6]. Blood glucose level starts to increase with accessory problems like obesity, hypertension, high serum cholesterol level, and type II diabetes mellitus.

Ghrelin plays a somber role in energy and reproductive activities. It is said that decreased ghrelin levels affect type II diabetes mellitus and insulin resistance [7]. Apart from glucose homeostasis, ghrelin also plays important role in other aspects, some of which are:

Energy homeostasis

Since energy metabolism totally depends upon intake and its expense, dysregulation of which can produce fat deposition leading to obesity. Ghrelin because of its orexigenic nature (i.e to increase the appetite) increases energy intake,[7–11] and administration of the same increases lipogenesis and decreases lipolysis [12,13].

Action on the heart and its functions

In late phases of myocardial infarction ghrelin prevents excessive stimulation of the sympathetic system by lowering adrenaline and noradrenaline which improves cardiac function [14].

Muscle atrophy

According to many studies on ghrelin it is found that it elevates satiety levels thus it indirectly helps in improving muscle mass by increasing the urge to intake proteins (food). Thus, ghrelin is beneficial in any muscular atrophic conditions [15,16].

Bone

Ghrelin increases phosphorylation and proliferation of osteoblasts and thereby increases bone density.

Subjects and methods

The current work was done to evaluate the relationships of various parameters of metabolic interest which are of vital importance in PCOS patients.

- (1) Relationship between waist circumference and insulin resistance as measured by HOMA-IR score.
- (2) Relationship between waist circumference and serum ghrelin.
- (3) Any association between serum ghrelin and insulin resistance.

Subjects were recruited from Gynaecology OPD of a rural tertiary care teaching hospital in the central part of India. Patients were within the range of 20–35 years of age group and suffering from PCOS. PCOS was defined as when atleast two of the following features were positive, i.e, oligo or anovulation (less than six cycles of menstruation in the preceding year), Ferriman-Gallwey score of than eight and/or biochemical signs of hyperandrogenism (abnormal LH/FSH ration and or elevated testosterone levels) and polycystic ovaries on ultrasound. All females had normal levels of thyroid, prolactin, renal and hepatic functions. Exclusion criteria included pregnancy, pre-diagnosed cases of diabetes mellitus, usage of oral contraceptives, hormones, oral hypoglycemic, statins, steroids and any disorder apart from PCOS which will alter the measured biochemical parameters. After approval from the institutional ethics authority vide letter number 14/2022 the study was planned, written informed consent was obtained during July to August 2022 from 30 PCOS patients before enrolment in the study.

The waist circumference was measured in centimeters. 5 ml fasting blood sample was collected and analysed for – Plasma Ghrelin (ELISA), Plasma glucose (by enzymatic glucose oxidase peroxidase), Plasma Insulin (chemiluminescent enzyme assay), and Plasma Insulin resistance by HOMA-IR score. HOMA-IR was measured by the values obtained of plasma glucose and serum insulin i.e : fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5.

Estimation of blood glucose (mg%)

Substrate β -D glucose is acted upon by glucose oxidase to liberate hydrogen peroxide. The action of peroxidase enzyme hydrogen peroxide yields nascent oxygen [O]. The coupling of Nascent oxygen with 4-amino antipyrine in a phenolic medium gives a red quinoneimine dye. The colour obtained was in direct

proportion to the concentration of glucose present which is measured colorimetrically at 530 nm and compared with that of a standard treated similarly.

Estimation of serum insulin ($\mu\text{IU/ml}$) and serum Ghrelin (ng/ml)

Both the parameters were measured on the principle based on ELISA using the double sandwich technique. All the steps were followed as per guidelines and measured in the ELISA reader. The intensity of colour was in direct relation to the concentration of analyte in the blood. A standard curve was prepared by measuring the colour strength to the amount or concentration of analyte before the investigation.

SPSS version 26 was utilized for statistical analysis. Data of the measured variables were entered in the excel sheet and expressed as mean \pm standard deviation.

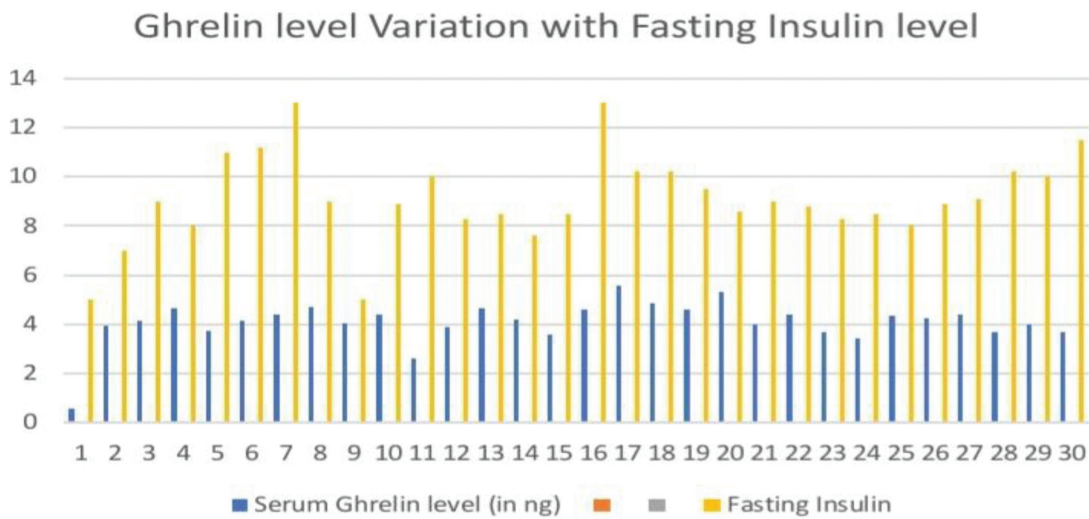
Co-efficient of correlation denoted the relation between the variables.

Results

We analyzed waist circumference, serum ghrelin level, fasting plasma glucose and insulin levels. HOMA-IR score of the patients was calculated and the obtained information was postulated in the form of graphs. The mean \pm SD for age, waist circumference, HOMA-IR, and serum ghrelin was 27.28 ± 3.26 , 94.53 ± 10.76 , 1.99 ± 0.27 and 4.09 ± 0.87 respectively. Waist circumference was positively correlated with HOMA-IR and serum ghrelin level. Serum ghrelin and insulin resistance also showed a strong association in our patients with PCOS.

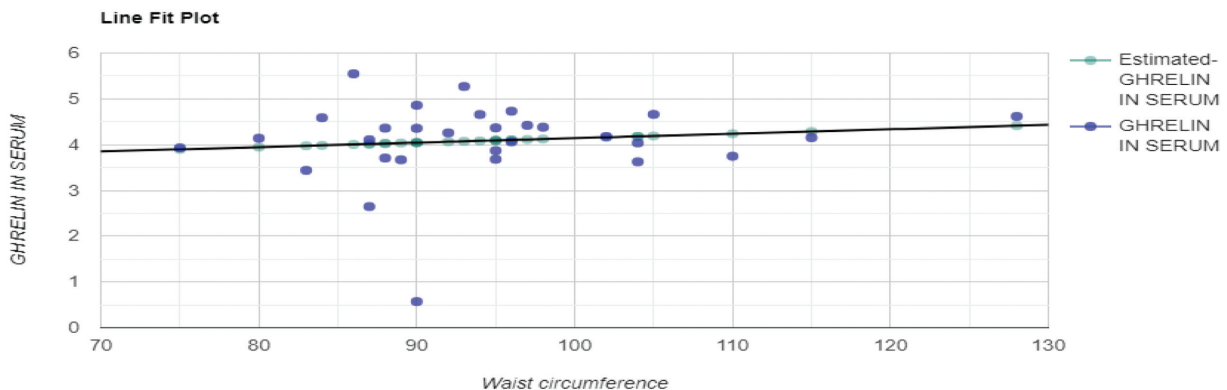
Results obtained from our study state that in PCOS patients ghrelin level is directly proportional to the

Figure 1



Correlation of serum Ghrelin level with fasting Insulin level in PCOS patients.

Figure 2



Correlation of waist circumference with serum Ghrelin level in PCOS patients.

fasting Insulin level but up to a certain extent then the relation is arbitrary i.e do not follow any specific trend (Fig. 1).

As depicted in Fig. 2, the correlation between waist circumference and serum ghrelin levels is weak (Correlation (R)=0.1204, P value=0.5263).

As shown in Fig. 3, waist circumference and HOMA-IR score are related but the correlation is weak. (Correlation (R)=0.2527, P value=0.1779).

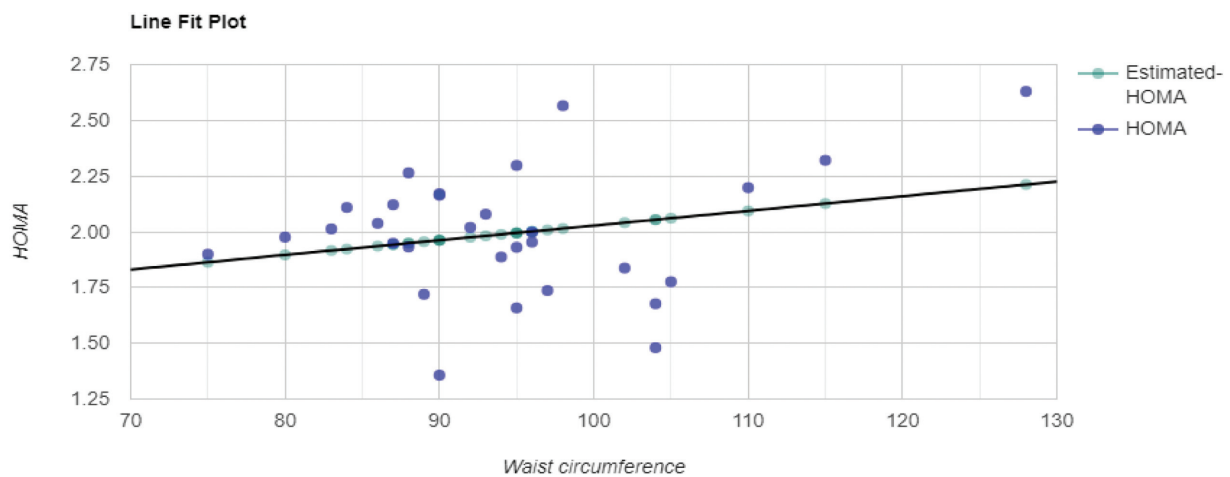
As depicted in Fig. 4, the correlation between serum ghrelin levels and HOMA-IR score is strongly positive (Correlation (R)=0.3874, P value=0.03442 which is significant).

Discussion

Insulin resistance and hyperinsulinemia are said to have a decisive role in the causation of PCOS and are linked with an augmented risk of diabetes, emergent truncal obesity and metabolic syndrome when compared with the normal subset population [17].

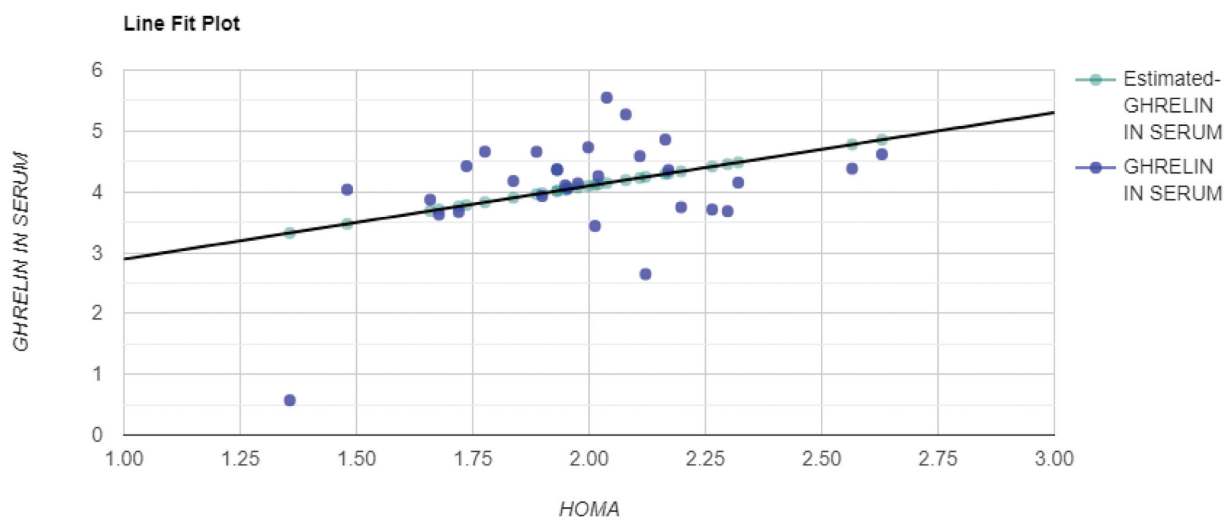
Our study states that waist circumference is in linear proportion to insulin resistance and serum ghrelin levels. Hans Wahrenberg et.al. stressed the importance of waist circumference which is associated to insulin resistance. This is presently viewed as a principal element of metabolic syndrome [18]. Quantification of nanomolar concentration levels of ghrelin hormone is important and is said to evoke as biochemical parameter in development of insulin

Figure 3



Correlation of waist circumference with HOMA-IR score in PCOS patients.

Figure 4



Correlation of serum Ghrelin level with HOMA score in PCOS patients.

resistance. Studies on the connection between serum ghrelin levels and insulin resistance in PCOS subjects present conflicting results. Schofl C *et al.*[19] have demonstrated that plasma ghrelin levels are decreased in PCOS while Pourghassem *et al.* did not show any association [20].

As per our study, ghrelin level in PCOS patients is higher in the case of insulin-resistant PCOS patients. So, this establishes a positive bond between ghrelin level and insulin resistance in PCOS which requires further diagnostic investigations to obtain more information on this aspect.

Also, results obtained in our study state that in PCOS patients ghrelin level is directly proportional to the fasting insulin level, but up to a certain extent then the relation is arbitrary which is contrary to the study conducted by Jonathan D Purwell *et al.* which states that serum ghrelin level is indirectly proportional to fasting insulin level in non-PCOS patients and decreased ghrelin level in PCOS patients proved facilitatory to emergence of type 2 diabetes mellitus and resistance to insulin [21].

We calculated (HOMA-IR) score which gives us an idea about resistance to insulin (normal range: – 2.23–3.1). We found the constant fashion of high HOMA-IR scores in our subjects with varying age groups, and a positive correlation with serum ghrelin levels in PCOS which suggests that there is developing insulin resistance.

Ghrelin has shown to inverse the down-regulating outcome of insulin on the enzyme phosphoenolpyruvate carboxykinase which regulates gluconeogenesis [22]. A central and peripheral effect of ghrelin on the hormone which decreases glucose and blood glucose homeostasis [23]. As there is a known relationship between insulin, glucose and PCOS, it is conjectured through numerous studies that ghrelin may predispose to the development of PCOS. Insulin level is a vital parameter in regulating ghrelin level. So, this could build an affiliation between insulin in terms of sensitivity and ghrelin level.

The key constraint of our study is the study duration and hence the relatively minor number of subjects involved; however, the subjects were of homogenous nature.

Considering the findings, which though suggest an association of serum ghrelin with insulin resistance, it seems to be imperative to discover the underlying connection arising, and hence future research needs to be carried out.

Conclusion

Androgen level estimation, ultrasound and pelvic examination constitute the common practice to diagnose PCOS. Since there is no such reliable diagnostic biomarker/indicator available in the market, it leads to an increase in the chance of a delayed diagnosis of PCOS patients. According to our study, serum ghrelin level varies significantly in the case of PCOS which makes it a diagnostic biomarker of this disease. This indicator will help in the early diagnosis of the patients because of which the patient would get a chance to get an effective treatment.

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Conflicts of interest

There are no conflicts of interest.

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