GHRELIN IN POLYCYSTIC OVARIAN SYNDROME WITH AND WITHOUT DIABETES MELLITUS IN EGYPTIAN POPULATION

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ABSTRACT

Background and aim: Ghrelin is a 28 amino acid peptide, which is primarily produced by the stomach. Ghrelin is a strong secretagogue of growth hormone (GH).The polycystic ovary syndrome (PCOS) is traditionally characterized by chronic anovulation, functional hyperandrogenism. Obesity of the central type and Insulin Resistance (IR) are highly associated with the syndrome, predisposing women with PCOS to the development of glucose intolerance and, ultimately, type 2 diabetes mellitus. In the current study, we investigated fasting plasma ghrelin levels in a group of patients with PCOS, with and without type 2 DM, and compared with an age matched control group.

Results: Fasting plasma ghrelin was significantly lower in PCOS patients than in control groups.

Conclusion: Fasting plasma ghrelin levels were significantly lower in PCOS groups as compared to the control group. Decreased plasma ghrelin levels correlated significantly with Insulin resistance in the patients groups.

INTRODUCTION

Ghrelin is a 28 amino acid peptide, which is primarily produced by the stomach [1]. Ghrelin is a strong secretagogue of growth hormone (GH), exerting its action on the hypothalamic-pituitary system via the GHSR-1A (growth hormone secretagogue receptor-1A)[2]. Besides its GH-secretagogue properties, ghrelin has also been found to induce the hypothalamic secretion of prolactin and adrenocorticotrophic hormone[3], exert a negative effect on the hypothalamic-pituitary-ovarian axis [4], stimulate food intake and positive energy balance [5] and interact with insulin, most probably in a negative feedback circuit, affecting glucose homeostasis [6]. Intriguingly, decreased ghrelin levels have been associated with obesity and states of insulin resistance (IR) [7].

The polycystic ovary syndrome (PCOS), probably the most common endocrine disorder in women of reproductive age, is traditionally characterized by chronic anovulation, functional hyperandrogenism with or without elevated total androgen levels and polycystic ovaries on ultrasound examination [8].

Obesity of the central type and IR are highly associated with the syndrome, predisposing women with PCOS to the development of glucose intolerance and, ultimately, type 2 diabetes mellitus [9].

Assuming an involvement of ghrelin in the modulation of steroidogenesis as well as glucose and energy balance, we investigated fasting plasma ghrelin levels in a group of patients with PCOS, with

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and without type 2 DM, and compared with an age matched control group.

MATERIALS & METHODS

The study included sixty females from Elshatby University Hospital distributed as follows: Twenty five with PCOS without DM (Group I), Twenty five with PCOS with type II DM (Group II) and ten healthy controls (Group III).

All females were subjected to complete history taking, thorough clinical examination including anthropometric measurements and radiological investigations including Ultasound to establish the diagnosis of PCOS.

Laboratory investigations performed included blood glucose measurements fasting and post prandial, glycosylated hemoglobin, renal function tests and hormonal profile to measure the FSH/LH ratio at day 3 of the menstrual cycle. Fasting serum Insulin was determined and Homeostasis Model Assesment (HOMA index) was calculated using the following formula

$$\text{HOMA} = \frac{\text{Fasting seruminsulin (}\mu\text{u / ml) x Fasting plasma glucose (mmole / L)}}{22.5}$$

Fasting plasma ghrelin was measured using an ELISA kit (Bioscience Europe SA).Data were processed and analyzed by the software statistical package for social sciences (SPSS). Data were expressed as mean ±SD. Comparison of means between the three groups was done using one way analysis of variance and within groups using paired *f-test*. Correlation was tested using pearson correlation coefficient. Linear regression analysis was used to compare the different variables. Significance was set at $p < 0.05$.

RESULTS

Clinical Data

In group I, the age ranged from 17-32 years with a mean value of 24.8± 3.85 years. In group II, the age ranged from 26-37 years with a mean value of 31.92 ± 2.6 years. In group III, the age ranged from 18-29 years with a mean value of 22.7± 3.53 years.

Four (16%) subjects in group I had primary amenorrhea, 13 (52%) subjects had irregular menses and 8 (32%) subjects had regular menses. In group II, 13 (52%) subjects had irregular menses and 12 (48%) subjects had regular menses. In group III all subjects had regular menses.

There was growth of terminal hair in women in the same pattern and sequence as seen in normal men. The hair was present in the hirsute subjects in face, axilla, limbs and trunk. Facial hair was present in moustache and beard with change in quality, size, length and degree of pigmentation. It was present in 15 (60%) subjects in group I and 14 (56%) subjects in group II. There was a statistical significant difference between the three groups (p=0.004).

Visceral obesity was present in 16 (64%) subjects in group I, 17 (68%) subjects in group II and none of the subjects in group III. There was a statistical significant difference between the three groups (p=0.0006).

In group I, Waist-hip ratio ranged from 0.72-1.05 with a mean value of 0.83 ± 0.08. In group II, it ranged from 0.76-0.94 with a mean of 0.87± 0.06. In group III, it ranged from 0.72-0.82 with a mean of 0.78±0.03. There was a statistical significant difference between the 3 groups (f=5.965, p=0.004).

Laboratory investigations

In group I, FBG ranged from 78-114 mg/dl with a mean value of 90.68± 9.82. In group II, it ranged from 168-309 mg/dl with a mean value of 210.6± 37.7. In group III, it ranged from 74-92 mg/dl with a mean value of 83.4± 6.28. There was a statistical significant difference between the 3 groups (f=168.451, p<0.0001).

FSH/LH ratio in group I ranged from 0.23-1.33 with a mean value of 0.57± 0.29. In group II, it

ranged from 0.26-1.68 with a mean value of 0.69± 0.36. In group III, it ranged from 2-2.72 with a mean value of 2.28 ± 0.27. There was a statistical significant difference between the 3 groups (f=114.272, p<0.0001) (Table I , Figure1).

In group I Fasting plasma insulin ranged from 6.8-43 mIu/mL with a mean value of 21.012 ± 10.62 In group II, it ranged from 7.4-56 with a mean of 28.32 ± 13.35. In group III, it ranged from 6-11mIu/mL with a mean value of 8.35 ± 1.51. There was a statistical significant difference between the 3 groups (f=11.743, p<0.0001) (Table II , Figure2).

In group I, HOMA index ranged from 1.44-9.12 with a mean value of 4.6313 ± 2.29. In group II, it ranged from 3.06-34.05 with a mean value of 14.82 ± 7.73. In group III, it ranged from 1.13-2.38 with a mean value of 1.703 ± 0.33. There was a statistical significant difference between the 3 groups (f=33.482, p<0.0001) (Table III , Figure 3).

In group I. serum fasting ghrelin ranged from 0.3-26.5 F.moL/mL with a mean value of 7.27 ± 8.5. In group II. it ranged from 1-31 F.moL/mL with a mean value of 9.28 ± 9.3. In group III, it ranged from 16.6-38.2 F.moL/mL with a mean value of 25.03 ± 7.8. There was a statistically significant difference between the 3 groups (f= 15.587, p<0.0001) (Table IV, Figure 4) .

There was a significant increase in level of HOMA index in correlation with a decreased level of FSH/LH ratio i.e. PCOS in all three groups (Figure V).

There was a significant decrease in FSH/LH ratio i.e. PCOS in correlation with decreased level of fasting plasma ghrelin (Figure VI).

DISCUSSION

The syndrome of polycystic ovaries (PCOS) is associated with adiposity and metabolic changes predisposing to insulin resistance and D.M. Women with (PCOS) are at increased risk of type 2 Diabetes mellitus. The overall increase in the incidence of diabetes in women with PCOS is as much as seven times higher than that in control females subjects [10]. The recently discovered GH-secretagogue ghrelin is intimately involved in the control of appetite and weight regulation [11]. Plasma ghrelin levels have been reported to be lower in PCOS women as compared to control subjects [12].

The present study was designed in order to assess the correlation of plasma ghrelin with different parameters of the PCOS including metabolic and hormonal features. PCOS patients were divided into two groups: group I including patients without DM and group II including PCOS with type 2 DM. A control group without PCOS was included. Patients with PCOS in group I and II had characteristic features of primary amenorrhea, irregular menstrual cycle and also hirsutism.

Insulin resistance was noted among PCOS groups, as was noted by a high HOMA index, especially in patients with type 2 DM. Fasting insulin levels were significantly higher in those patients. PCOS is a major risk factor for type 2 diabetes mellitus in women regardless of age. O'Meara, et al 1993[13], found that fasting hyperinsulinemia is present in obese women with PCOS. This is in part secondary to increased basal secretion rate. The most compelling evidence suggests that beta-cell dysfunction in addition to insulin resistance are features of PCOS.

In our study, fasting plasma ghrelin levels were significantly lower in PCOS groups as compared to the control group. Decreased plasma ghrelin levels correlated significantly with Insulin resistance in the patients groups.

Ghrelin has attracted attention for its involvement in the control of food intake and energy balance, when administrated centrally or peripherally to humans, ghrelin enhances appetite, reduces fat utilization and causes adiposity[14]. Plasma ghrelin concentration had been shown to be lower in obese patients when compared with normal subjects.

Therefore conditions characterized by a positive energy balance are associated with low circulating ghrelin concentration[12]. Schofl et al, 2002[15] studied the circulating ghrelin levels in patients with PCOS. They confirmed that in PCOS women serum ghrelin levels were significantly lower than in healthy lean or obese controls. In insulin resistant PCOS women ghrelin levels were significantly lower. There was close correlation of ghrelin to insulin sensitivity. In summary, ghrelin levels are decreased in PCOS women and are highly correlated to the degree of insulin resistance. It was confirmed also by Schofl et al 2002[15] that in PCOS women, serum ghrelin levels were significantly lower than in healthy learn or obese controls. Seppo et al [16], confirmed that low ghrelin was associated with high waist circumference, high Body mass Index (BMI), high systolic and diastolic B.P, high fasting blood glucose and plasma insulin. Low ghrelin level is also associated with prevalence of type 2- diabetes and insulin resistance. Low ghrelin level is, independently associated with type 2 diabetes , insulin concentration , insulin resistance and elevated B.P. Therefore it might have some role in type 2 diabetes and regulation of blood pressure.

In conclusion, fasting Ghrelin level decreases in PCOS patients with insulin resistance, with and without DM. It is likely hat mechanisms that result in different clinical and biochemical manifestations of the syndrome might also affect ghrelin concentrations.

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Table I : FSH / LH ratio .

	Group I	Group II	Group III
Range	0.23 - 1.33	0.26 - 1.68	2-2.72
Mean	0.57	0.69	2.28
SD	± 0.29	± 0.36	± 0.27

Table II : Fasting insulin levels .

	Group I	Group II	Group III
Range	6.8 - 43	7.4 - 56	6-11
Mean	21.012	28.32	8.35
SD	± 10.62	± 13.35	± 1.51

Table III : HOMA index .

	Group I	Group II	Group III
Range	1.44 - 9.12	3.06 - 34.05	1.13 - 2.38
Mean	4.631	14.820	1.703
SD	± 2.29	± 7.73	± 0.33

Table IV : Fasting plasma Ghrelin .

	Group I	Group II	Group III
Range	0.3 - 26.5	1 - 31	16.6 - 38.2
Mean	7.27	9.28	25.03
SD	± 8.5	± 9.3	± 7.8

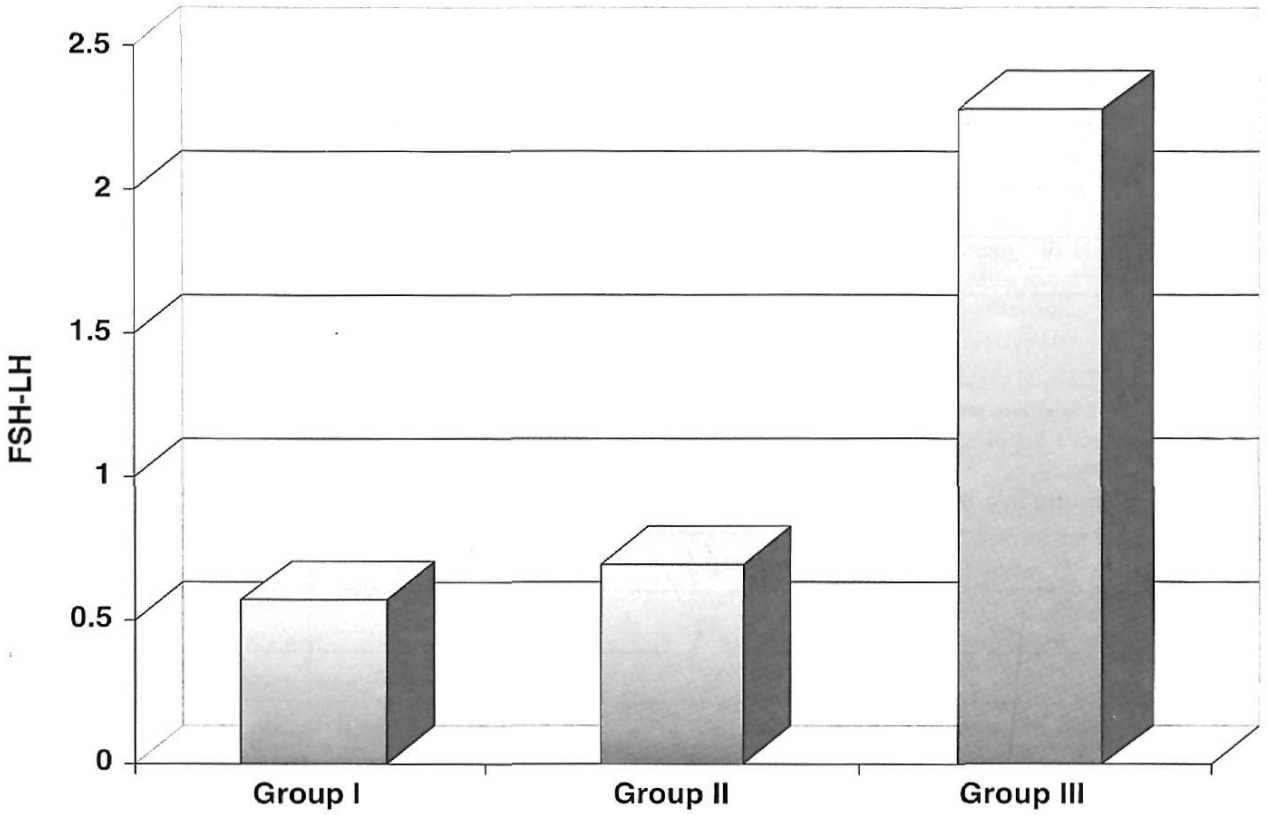

Figure 1: FSH-LH in the 3 studied groups

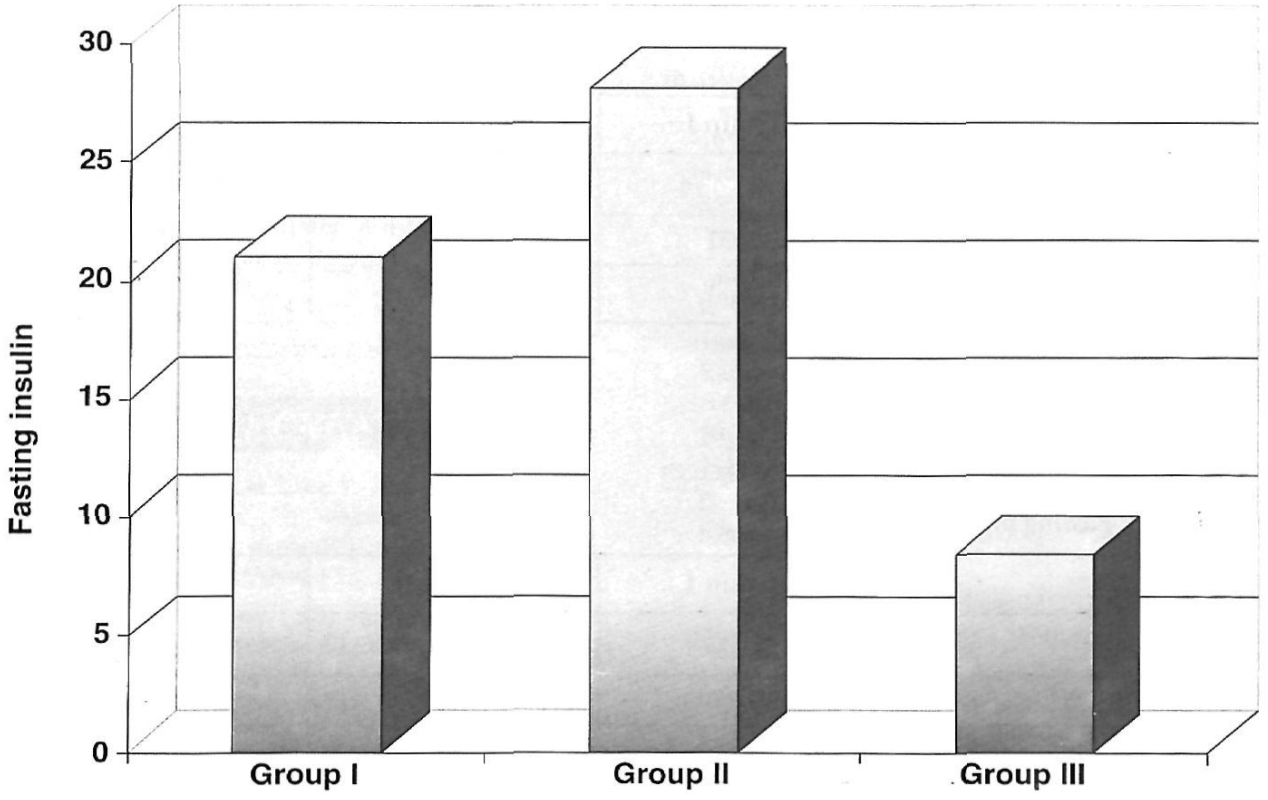

Figure 2 : Fasting plasma insulin in the 3 studied groups

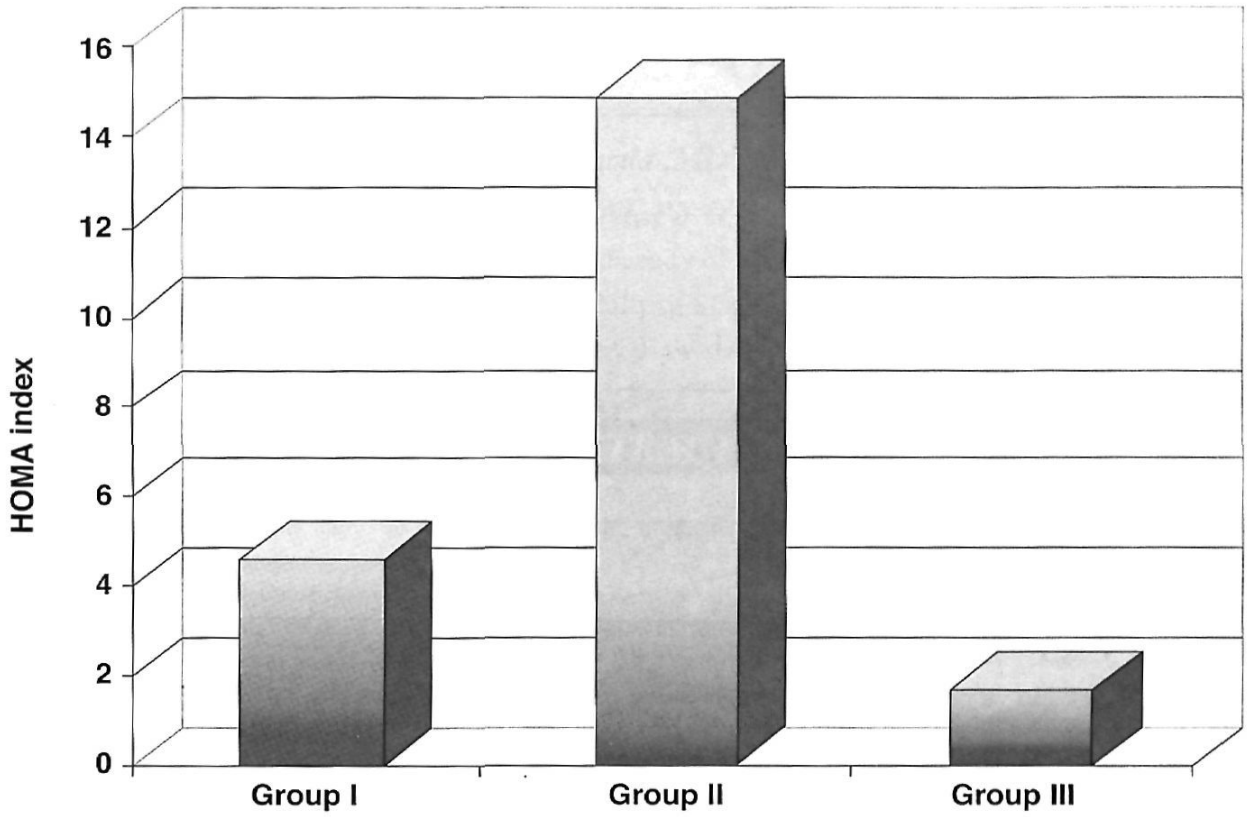

Figure 3: HOMA index in the 3 studied groups

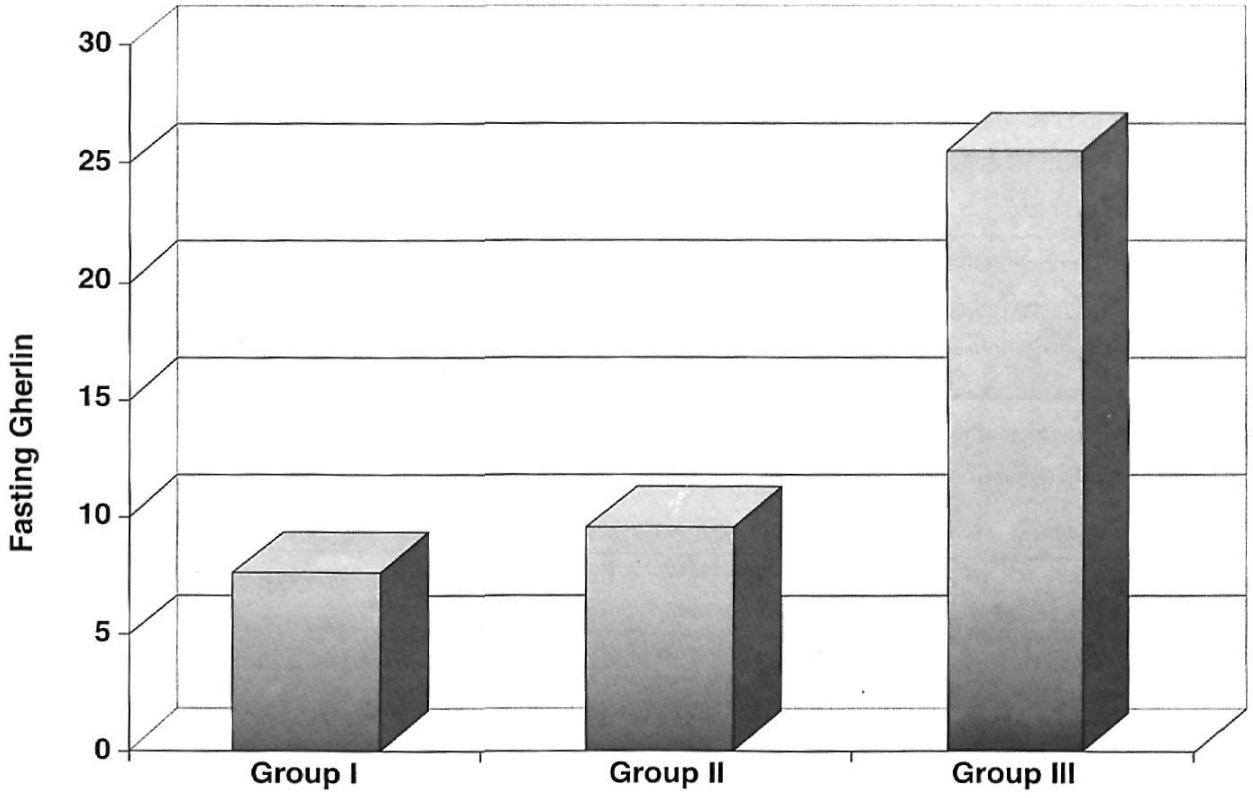

Figure 4: Fasting plasma Ghrelin in the 3 studied groups

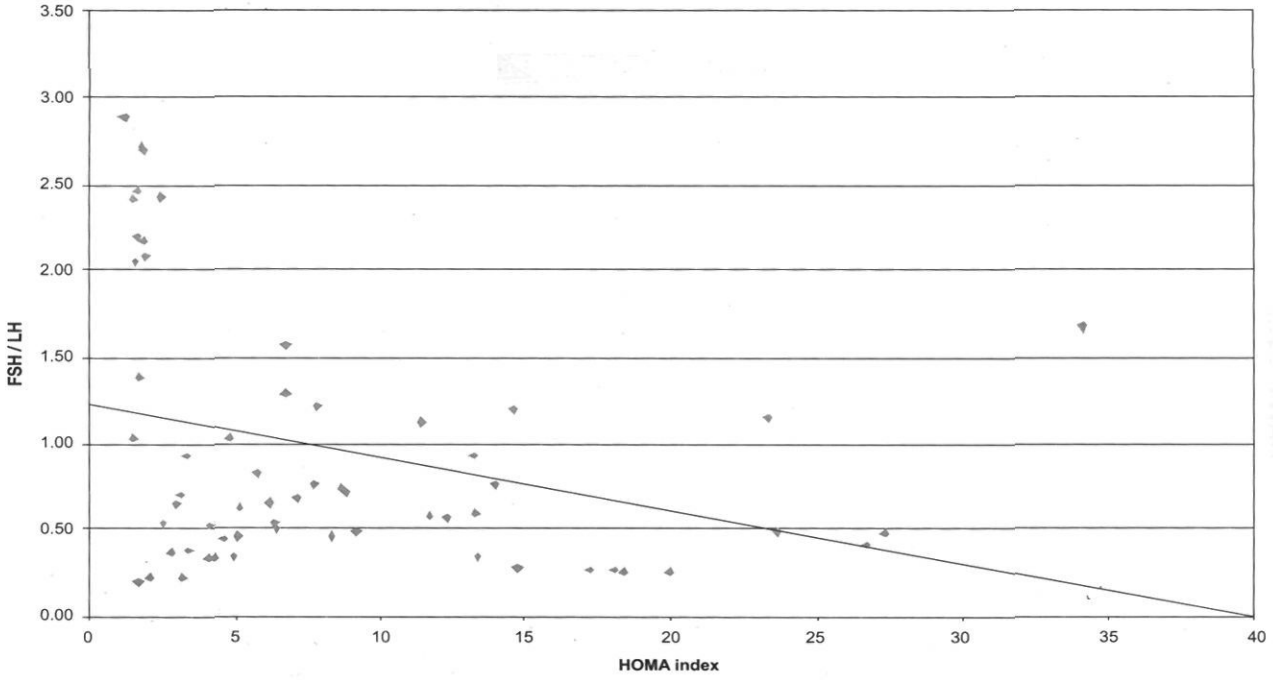

Figure 5 : Correlation between HOMA index level and FSH/LH in all groups.

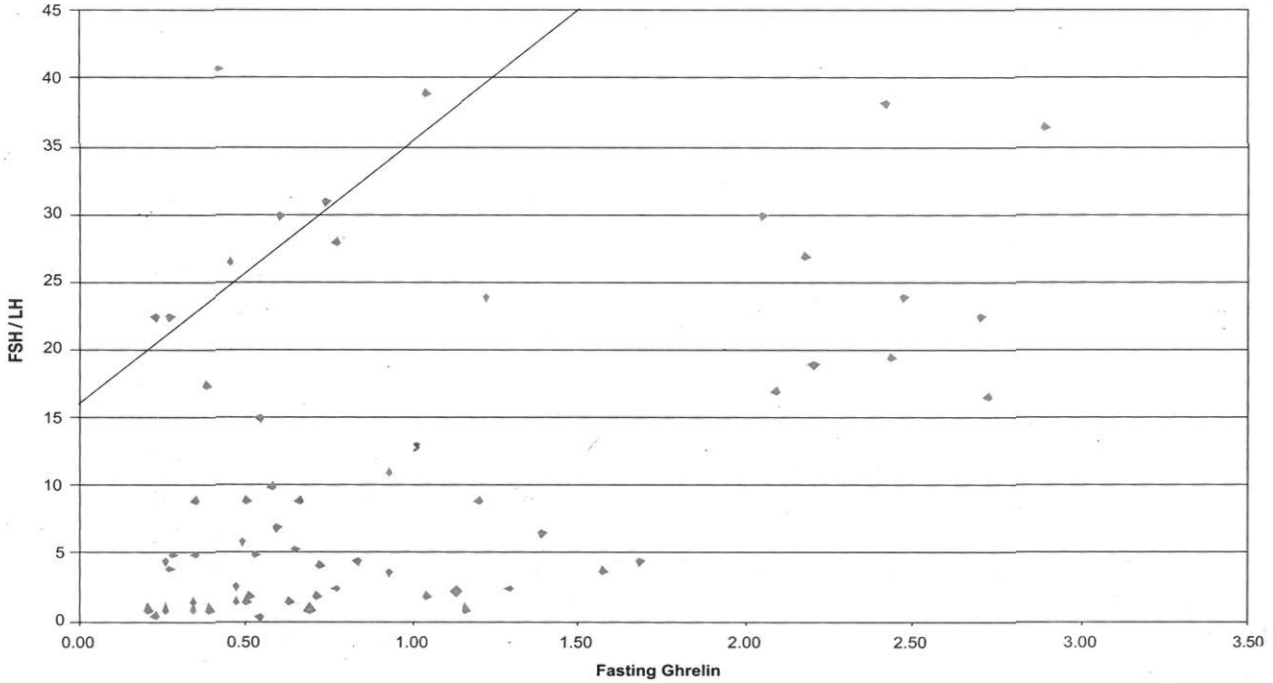

Figure 6 : Correlation between FSH/LH and Fasting ghrelin level in all groups.