

## Assessment of Ghrelin and Testosterone Hormones in Males Patients with Ischemic Heart Disease

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**Abstract:** The present study aims to assess the newly discovered ghrelin hormone level in ischemic heart diseases (IHD) patients and to find out a possible relationship between ghrelin level with different parameters including lipid profile, testosterone level, blood pressure, Body mass index (BMI) IHD patients and compared with healthy controls as a tool for monitoring and even possibly prediction or diagnosis of these diseases. Seventy six patients are diagnosed with ischemic heart disease and twenty control healthy subjects having no history of diabetic mellitus and hypertension and other disease participated in this study. The patients are men only aged between (40\_69) years old. Patients of ischemic heart disease are collected from AL-sader teaching city in AL\_Najaf AL\_Ashraf Governorate during the period from 2/8/2012 to 2/12/2012. The measured parameters in serum included: cholesterol (Ch) and its different fractions, triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) were measured spectrophotometrically, Ghrelin and testosterone measurements were carried out using ELISA technique. Results of the present study in general revealed that there is low ghrelin and testosterone level in ischemic heart diseases (IHD) patients comparing with control group and in hypertensive IHD patients comparing to normotensive IHD patient and high level cholesterol, TG, LDL with low level of HDL. Also the result shows negative correlation between ghrelin and cholesterol, TG, LDL, blood pressure and body mass index (BMI) and positive correlation between ghrelin and testosterone, HDL. From the present study, it can be concluded that the decrease in ghrelin level is associated mostly with the risky parameters including risks of atherosclerosis, heart disease, obesity, hypertensive and low testosterone level.

**Keywords:** Octave Wave, PIE.

### I. INTRODUCTION

Ghrelin is a novel GH (growth hormone)-releasing peptide, isolated from the stomach, which has been identified as an endogenous ligand for the GHS-R (GH secretagogue receptor) (Kojima et al. 1999). Ischemic heart disease (IHD) is the most common cause of death in most Western countries as well as worldwide (Barton et al. 2000). Generally IHD is a result of atherosclerosis. Atherosclerosis is characterized by endothelial dysfunction, lipoprotein oxidation, leukocyte infiltration, release of various chemotactic, growth factors, the building up of cholesterol, lipids and calcium. In IHD, several hormones and other regulatory mediators are recruited that either worsen or ameliorate cardiovascular alterations following restricted supply of blood to the heart. It has been indicated that myocardial ischemia could cause acute alterations in circulating ghrelin levels (Baxter et al. 2004). That is, cellular metabolism was manipulated by regulatory mediators and several hormones, including recently discovered acylated peptide hormone ghrelin (Kojima et al. 1999). Studies in humans (Nagaya et al. 2001) and animal models (Zhang et al. 2006) have exhibited beneficial effects of ghrelin in the cardiovascular system such as a gain in left ventricular mass, an increase in left ventricular ejection fraction and the administration of an acylated peptide hormone ghrelin causes a significant

decrease in mean arterial blood pressure (MABP) and has a strong inotropic action, which suggests its important regulatory role in cardiovascular homeostasis (Matsumura et al. 2004). Some studies have examined the relationship between a low plasma ghrelin concentration and risk factors of atherosclerosis such as high blood pressure, obesity and insulin resistance in cross-sectional population-based studies and also increased oxidative stress in obese subjects (Pöykkö et al. 2003, Suematsu et al. 2005, Zwirska-korcza et al. 2007).

The lifetime risk of ischemic heart disease is much larger in men compared to women, suggesting that testosterone or the lack of estrogens play an important role. On the other hand, testosterone levels decrease with age, coincident with the age-related increase in atherosclerotic disease. The results obtained from cross-sectional studies suggest that men with IHD might have lower testosterone levels. While intervention studies with testosterone in older men with IHD suggest an improvement of ECG. In addition, testosterone exerts significant effects on several risk factors for IHD. The aim of this study is to elucidate the role of ghrelin and testosterone as risk factors of ischemic heart disease and the association with another criteria such as lipid profile, BMI, hypertension and diabetic.

## II. MATERIALS AND METHODS

### A. Patients and Healthy Groups

76 patients are diagnosed with coronary heart disease and 20 control healthy subjects having no history of diabetic mellitus and hypertension and other disease participated in this study. The patients are men only aged between (40\_69) years old. Patients of ischemic heart disease are collected from Al Sader Teaching city in AL\_Najaf AL\_Ashraf Governorate during the period from 2/8/2012 to 2/12/2012 . Patients are divided into subgroups according to hypertension ,normotension, diabetic, nondiabetic , body mass index and according to ages.

### B. Blood Sample

8 milliliters were taken from ante cubital vein puncture using G23 needle were drawn from IHD and control subjects. The blood was allowed to clot in plain test tube at room temperature. The serum was aspirated after centrifugation at 3000 rpm for 10 minutes, divided into aliquots in epindroff tubes and stored at -20°C until the measurement of the study parameters.

## III. BIOCHEMICAL MEASUREMENT

**Measurements Of Serum Ghrelin:** Ghrelin ELISA kit for quantitative determination of Ghrelin in human serum was supplied by USBiological, USA.

**Measurements Of Serum Testosterone:** 2 Testosterone ELISA kit for quantitative determination of Testosterone in human serum was supplied by Accu-Bind, USA.

**Measurements of total cholesterol (TC):** Total cholesterol kit for quantitative determination of total cholesterol in human serum was supplied by Biolabo SA, France.

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (standard)}} \times \text{Standard concentration} \quad (1)$$

Standard concentration= 200 mg /dl.

**Measurements of HDL- Cholesterol (HDL-C):** (Tietz, 1999) Serum HDL-Cholesterol level was measured by HDL-Cholesterol phosphotungstic acid (PTA) precipitant kit (Biolabo SA, France), which was based on the following principle: This reagent was only for treatment of specimens before determination of HDL Cholesterol with a reagent for total cholesterol. Low density lipoproteins (LDL-C), very low density (VLDL-C) and chylomicrons from specimens were precipitated by phosphotungstic acid and magnesium chloride. HDL-Cholesterol obtained in supernatant after centrifugation is then measured with total cholesterol reagent. Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (standard)}} \times \text{Standard concentration} \times 1.1 \quad (2)$$

Standard concentration= 100 mg /dl

**Measurements of Triglycerides:** (Fossati and Prencipe, 1982) Triglycerides kit for quantitative determination of

triglycerides in human serum was supplied by Biolabo SA, France.

Calculate the result as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (standard)}} \times \text{Standard concentration} \quad (3)$$

Standard concentration= 200 mg /d

**Calculation Of Very Low Density Lipoprotein Cholesterol:** Very low density lipoprotein was estimated using the following formula:

$$\text{VLDL-C} = \text{TG (mmol/l)} / 2.2 \quad (4)$$

**Calculation Of Low Density Lipoprotein:** Low density lipoprotein was estimated using the following formula:

$$\text{LDL-C} = \text{TC (mmol/l)} - \text{VLDL-C (mmol/l)} - \text{HDL-C (mmol/l)} \quad (5)$$

**Body Mass Index:** Body mass index values were calculated from the following equation (Eknoyan 2008): BMI=Weight(Kg)/Height (m)<sup>2</sup>. It was measured by electronic balance and height apparatus. The BMI of normal weight ranges 18.5-24.9 Kg/m<sup>2</sup> and for overweight ranges 25-29.9 Kg/m<sup>2</sup>. When BMI is larger than 30 Kg/m<sup>2</sup>, the person is definitely obese (Findaza, et al 1972).

**Measurements Of Blood Pressure:** Blood pressure was measured by sphygmomanometer (SK-MINIATUR300B Germany). Both systolic and diastolic blood pressure was recorded in mm Hg, the value of the last reading of the blood pressure was taken for each subject.

**Statistical Analysis:** Analysis of data was performed by using SPSS (Version 17) in the home computer. The results were expressed as (mean ± • standard deviation) .

## IV. RESULTS

### A. Comparison between Patients and Control Groups Lipid Profile

The serum concentration of lipid profile parameters in different ischemic heart diseases patients and control groups is presented in the table (1). The results of lipid profile in table (1) indicated significant (P<0.05) increase in cholesterol and LDL, significant (P<0.05) decrease in triglyceride and non significant (p>0.05) decrease in HDL.

**TABLE I: Lipid Profile in IHD Patients and Control Groups**

Parameters	Control	IHD
Cholesterol (mmol/l)	3.801±0.1607	4.595±0.154*
TG (mmol/l)	1.513±0.581	2.591±1.137*
HDL-C (mmol/l)	1.01±0.06	0.95±0.20
LDL-C (mmol/l)	2.172±0.44	2.620±0.84*

(\*): significantly different in comparing with control group.

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**Ghrelin and Testosterone:** The serum concentration of ghrelin and testosterone in different ischemic heart diseases and control groups are presented in fig.1, 2. The results of IHD patient in fig.1 indicated significant ( $P < 0.05$ ) decrease in ghrelin comparing with control group. Also The results of IHD patient in fig.2 indicated significant ( $P < 0.05$ ) decrease in testosterone when compare with control group.

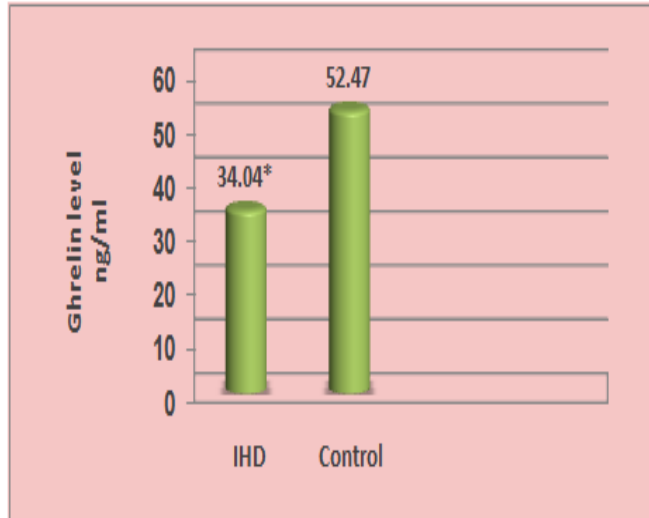


Fig.1. Ghrelin level between IHD patients and control group.

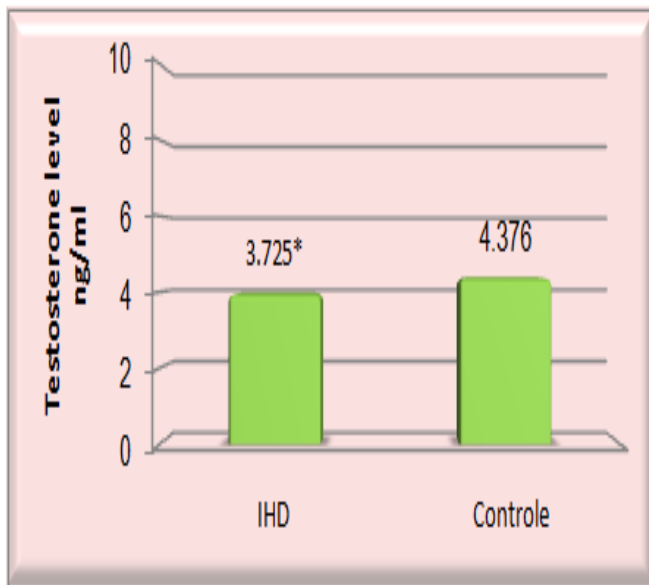


Fig.2. Testosterone level between IHD patients and controls group.

### B. Comparing Ghrelin And Testosterone Level Between Hypertensive And Normotensive IHD Patients

The serum concentration of ghrelin and testosterone in different hypertensive and normotensive ischemic heart diseases groups is presented in fig.3, 4. The result of hypertensive patient in fig.3 indicates significant ( $P < 0.05$ ) decrease in ghrelin level comparing with normotensive

group. Also result in fig.4 indicates significant ( $p < 0.05$ ) decrease in testosterone level of hypertensive IHD patient comparing with normotensive group.

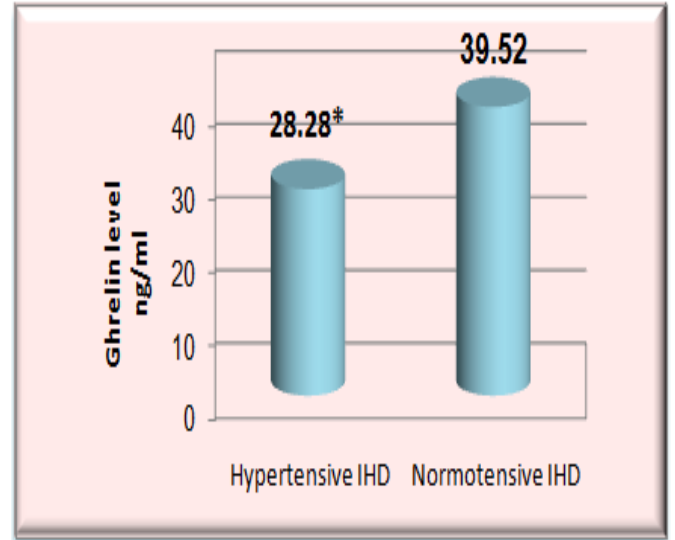


Fig.3. Ghrelin level in hypertensive and Normotensive IHD patients.

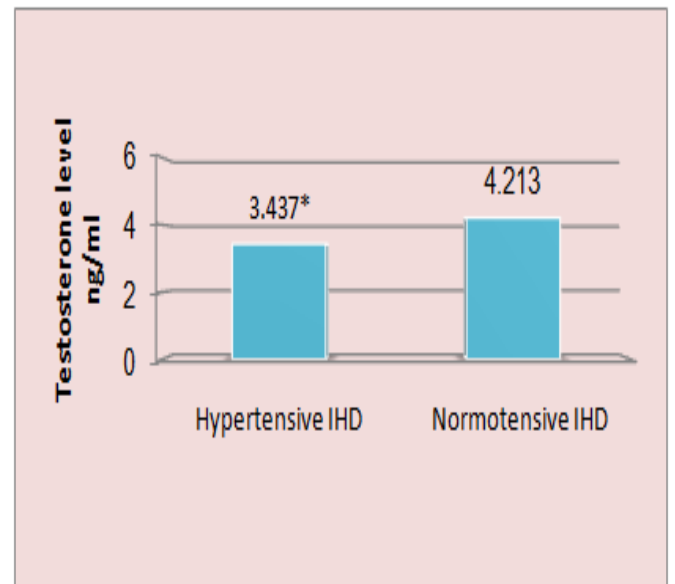


Fig.4. Testosterone level in hypertensive and Normotensive IHD patient.

### C. Comparison Between Ghrelin And Testosterone Level In IHD Patients According BMI

The serum ghrelin and testosterone level of ischemic heart diseases in different group according to BMI are presented in figs.4-9. The result in figs.5 and 6 reveals significant ( $p < 0.05$ ) decrease in ghrelin and testosterone in both over weight and obese IHD patients comparing to normal weight group, no significant differences ( $p > 0.05$ ) show between over weight and obese group, the obese group indicate asignificant decrease ( $p < 0.05$ ) than normal weight group.

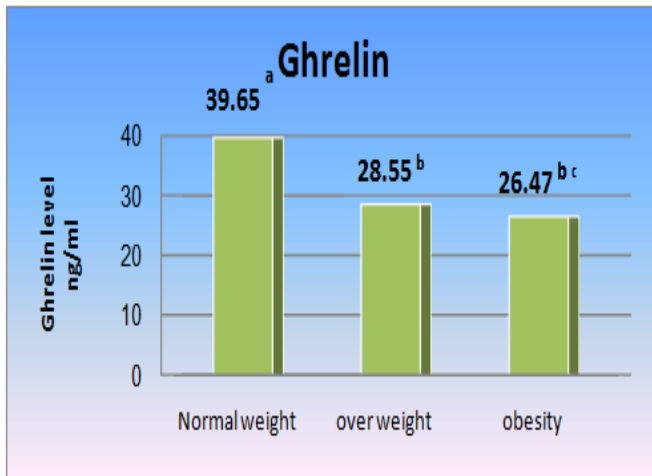


Fig.5. Ghrelin level in IHD patients according BMI.

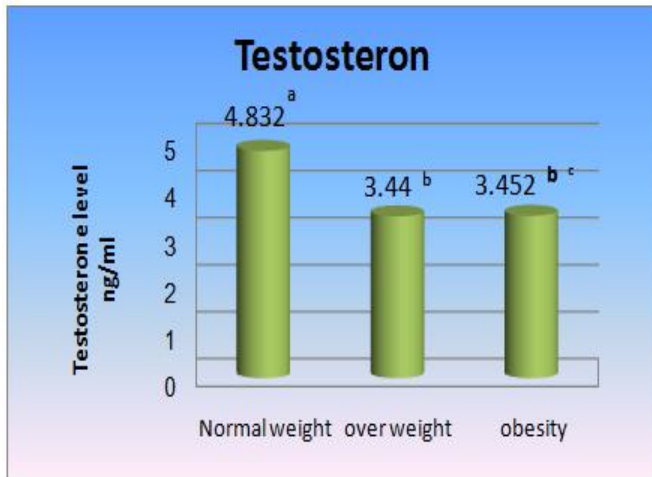


Fig.6. Testosterone level in IHD patients according BMI [ the same letters refer to no significant ,the different latter refer to significant difference at level(p< 0.05)].

**D. Relation Between Study Parameters In IHD Patients**

The results of correlation between study parameters in IHD patient.

**Ghrelin & Testosterone:** The presence of a significant positive correlation ( $r = 0.283$ ,  $p=0.049$ ) between Ghrelin and Testosterone is shown in fig.7.

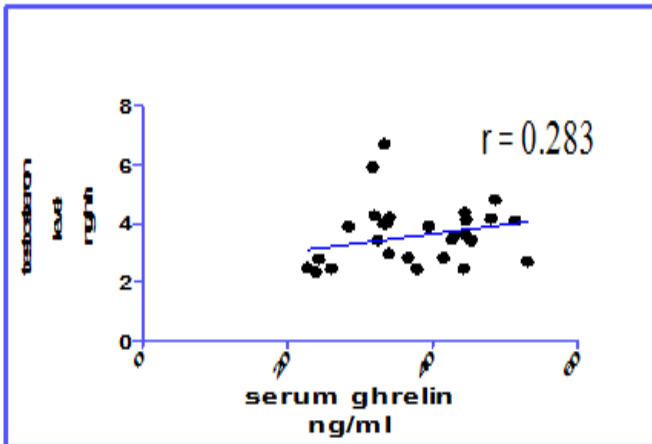


Fig.7. The correlation between ghrelin level and serum testosterone level in IHD patients.

**Ghrelin & Triglyceride:** The presence of a significant negative correlation ( $r = -0.249$ ,  $p=0.049$ ) between Ghrelin and triglycerides (TG) is shown in fig.8.

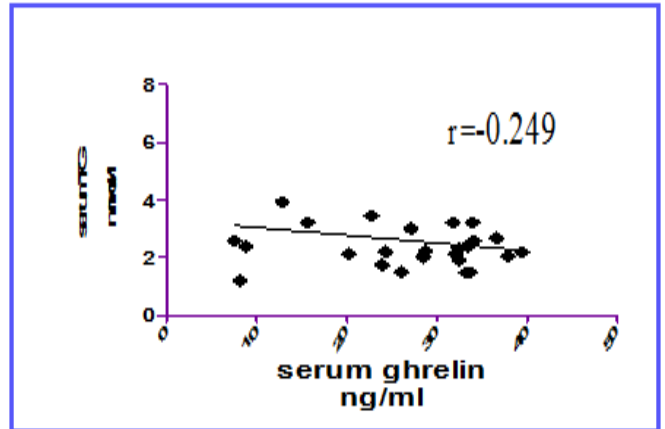


Fig.8. the correlation between ghrelin level and serum triglyceride level in IHD patients.

**Ghrelin & Colesterol:** The presence of a significant negative correlation ( $r = -0.323$ ,  $p=0.044$ ) between ghrelin concentration and cholesterol (Ch) is shown in fig.9.

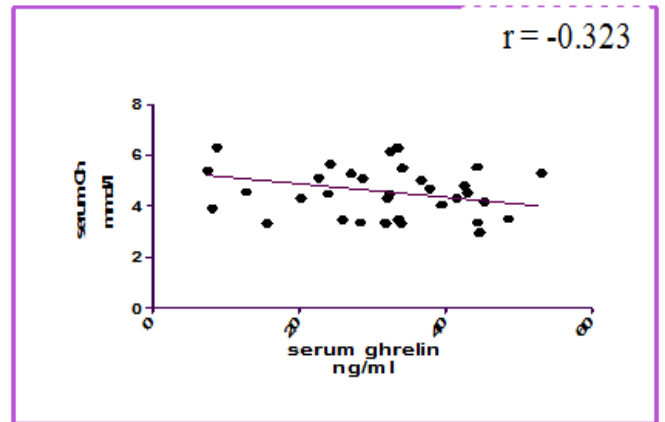


Fig.9. the correlation between ghrelin level and serum cholesterol level in IHD patients.

**Ghrelin & HDL:** The presence of a significant positive correlation ( $r = +0.337$ ,  $p=0.022$ ) between Ghrelin and HDL is shown in fig.10.

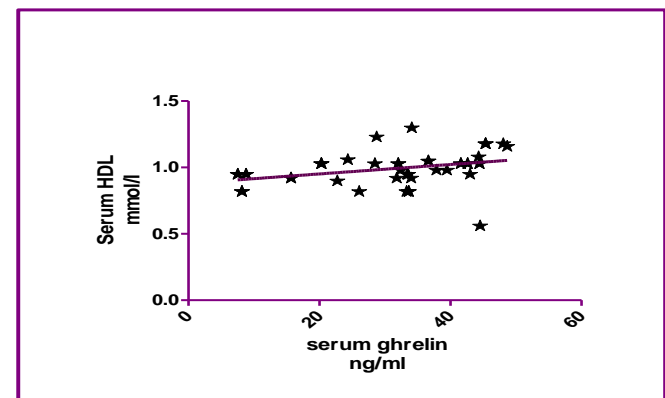


Fig.10. the correlation between ghrelin level and serum HDL level in IHD patients.

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**Ghrelin & LDL:** The presence of a significant negative correlation ( $r=-0.315, p=0.047$ ) between ghrelin concentration and LDL is shown in fig.11.

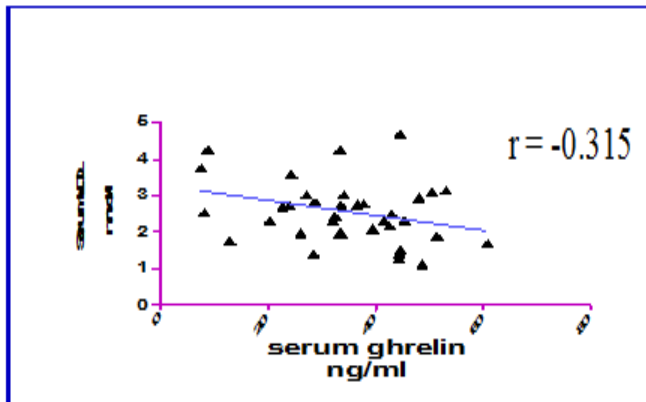


Fig.11. the correlation between ghrelin level and serum LDL level in IHD patients.

**Ghrelin and BMI:** The presence of a significant negative correlation ( $r = -0.318, p=0.042$ ) between ghrelin concentration and BMI is shown in fig.12.

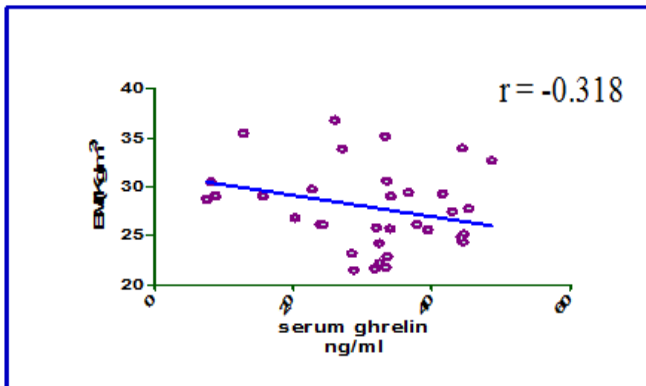


Fig.12. the correlation between ghrelin level and BMI level in IHD patient.

**Ghrelin And Systolic Pressur:** The presence of a significant negative correlation ( $r = -0.336, p=0.039$ ) between ghrelin concentration and systolic pressure is shown in fig.13.

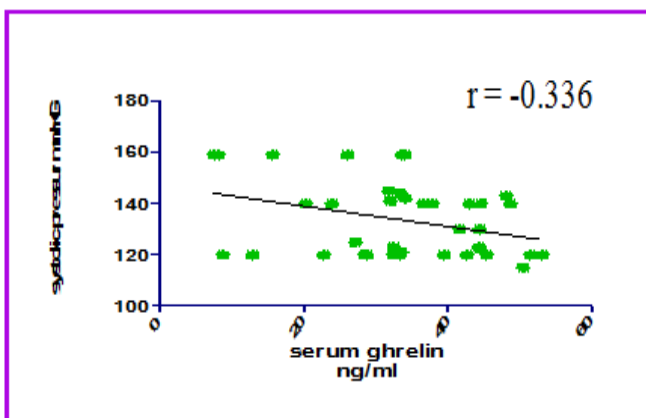


Fig.13. the correlation between ghrelin level and systolic pressure in IHD patients.

### Ghrelin & Diastolic:

The presence of a significant negative correlation ( $r = -0.302, p=0.038$ ) between ghrelin concentration and diastolic pressure is shown in fig.14.

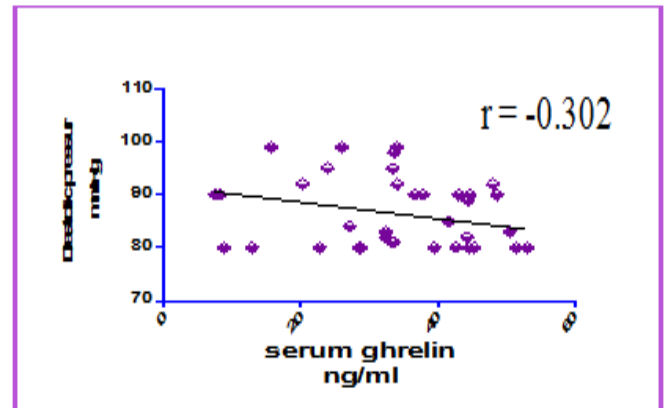


Fig.14. the correlation between ghrelin level and diastolic pressure.

## V. DISCUSSION AND CONCLUSION

Results in table (1) shows significant increase in cholesterol, triglyceride and LDL, no significant decrease in HDL in IHD patient comparing with control group (Esterbauer et al., 1992). These results indicated a higher atherogenic risk in IHD patients than healthy subjects extracted from the high values of cholesterol, TG, and LDL. Cholesterol has been shown to interrupt and alter vascular structure and function as it builds within the lining of the vascular wall, and can interfere with endothelial function leading to lesions, plaques, occlusion, and emboli; along with a reduction in healing, recovery, and appropriate management of ischemia/reperfusion injury (Hayakawa et al. 1999, Huijgen et al. 2008). An elevated level of plasma LDL is a major risk factor for developing atherosclerosis (Jialal et al., 1992). The result in fig.1. shows significant decrease of ghrelin in IHD patient comparing with control group. Ghrelin plays a number of roles in the cardiovascular system the have protective effects by inhibiting cardiomyocyte and endothelial cell apoptosis (Baldanzi et al., 2002), and to improve left ventricular (LV) function during ischemia reperfusion (I/R) injury (Frascarelli et al., 2003). In the vasculature, ghrelin exerts vasodilatory effects (Nagaya et al., 2001a). Apart from vasodilatory effects, ghrelin may also have other vasoactive and anti-inflammatory properties. It has been shown that ghrelin has inhibitory effects on cytokine release and it activates nuclear factor kb and mononuclear cell binding in cultured human umbilical vein endothelial cells (HUVEC), which could potentially be important, (Li et al., 2004).

The result in fig.3 show significant decrease in ghrelin level of hypertensive IHD comparing to normotensive IHD patients. In a study by Kangawa and collaborators, a single injection of ghrelin caused a significant decrease in blood pressure (Nagaya et al., 2001a). One of the first studies on this subject demonstrated an antagonistic action of ghrelin on the

vasoconstrictor effect of endothelin-1 in isolated mammary arteries (Wiley & Davenport, 2002). The vasoconstrictive effect of ghrelin was proposed to be expressed through inhibition of a tonic coronary  $\beta$ 2-adrenergic receptor-mediated, vasodilatory effect related to the release of nitric oxide (Grossini et al., 2007). The result in fig.5 show significant decrease in ghrelin level in IHD patient when the BMI increase. The obese has increased hunger and take longer to reach satiety (Delgado et al.,2004), ghrelin levels correlate negatively with body mass index (BMI) (Shiyya , et al.2002, Tschop et al.2001) and increase following weight loss (Hansen et al.,2002). ghrelin might participate in the long-term regulation of body mass. In humans, studies suggest that weightloss increases circulating ghrelin levels (Hansen et al.,2002, Cummings et al.,2002). The result in fig.2 show significant decrease in testosterone level of IHD patient comparing to control group.

Testosterone is recognized to have important effects on metabolism and vascular behavior beyond the accepted effects on secondary sexual characteristics. Male sex is a risk factor for vascular disease, ( Njolstad et al.,1996). The role of testosterone in development of atherosclerosis has been evaluated in several studies. Males in animal studies tend to develop atherosclerosis earlier and more rapidly independent of lipid levels and evidence of intimal injury. (Hak et al.2002). The result in fig.4 show significant decrease in testosterone level of hypertensive IHD patient comparing to normotensive patient. The mechanisms responsible for the increase in blood pressure in the males are unknown,. Men with low testosterone levels have been reported to have higher blood pressure (Dobryeki et al, 2003, Svartberg et al, 2004) and low testosterone levels correlated with the higher blood pressure (Fogari et al,2002). Low testosterone levels have also been associated with increased risk of cardiovascular diseases and stroke (Robert and Griffth, 2003). The result in fig.6 shows significant decrease in testosterone when the BMI is increase. Obese males have low free testosterone levels . An inverse relationship has been identified between total testosterone levels and BMI. Free testosterone levels decrease as BMI increases. Serum total and free testosterone levels have an inverse relationship with visceral fat mass (Dandona et al.2010).Also, the decrease in testosterone levels is correlated with greater abdominal fat (reviewed in Vermeulen, 2000).

The result in fig.7 shows significant positive correlation between ghrelin and testosterone in IHD patient. there is a positive correlation between serum testosterone level and ghrelin level (Pagotto et al., 2003). Inhibitory effect of elevated plasma ghrelin concentration on testosterone production, which was observed in rats (Tena-Sempere et al., 2002), gonadal tissues have been proposed as being a target for ghrelin based on a high number of binding sites in testis(Papott et al.,2000). The result in the fig.8, 9 and 11 shows significant negative correlation between ghrelin and TG, cholesterol and LDL and the result in figure (10) shows significant positive correlation between ghrelin and HDL. Ghrelin has been shown to have a significant role in lipid

metabolism in the liver, skeletal muscle and adipose tissue (Soares & Leite-Moreira 2008). Total ghrelin favours triglyceride deposition in the liver over skeletal muscle (Barazzoni et al. 2005) and acts directly on adipocytes to stimulate lipogenesis (Patel et al. 2006).

The result in the fig.13, 14 shows significant negative correlation between ghrelin and systolic , diastolic pressure Ghrelin administration was shown to decrease blood pressure (BP) in animal models (Shinde et al., 2005) and ghrelin plasma levels are correlated with BP in humans, ( Makino et al., 2002) vascular actions of ghrelin could provide a link between obesity and hypertension(Rahmouni et al., 2005) Ghrelin causes vasorelaxation in rats and it improves endothelial function by increasing endothelial nitric oxide (NO) bioavailability (Shimizu et al.,2003). The result in the fig.12 show significant negative correlation between ghrelin and BMI. Studies have found that plasma ghrelin levels are lower in obese subjects than in control subjects (Tschop et al.,2001, Rosicka et al.,2002, Shiyya et al.,2003). Plasma ghrelin concentration is negatively associated with percent body fat (Tschop et al.,2001, Bunt et al., 2003) serum ghrelin level is negatively correlated with body mass index (BMI); it is decreased in obesity (Nijhuis et al.,2004, Paik et al.,2004 ).

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