Is serum amylase normal in women with polycystic ovarian syndrome?

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Abstract:

Background : Polycystic ovary syndrome (PCOS) is the most common cause of infertility in reproductive-age women , it is an important harbinger of metabolic disorders. It has been reported that hyperamylasemia can be used as marker of ovarian cancer patients . The current study was conducted to evaluate amylase activity and to estimate the correlation of this enzyme with insulin and insulin resistance in PCOS patients.

Methods: This study was conducted on forty five patients with PCOS in comparison to twenty five women as control. Fasting blood sample was taken from each subject and analyzed for amylase activity , FSH,LH, Insulin , proteins, and blood sugar , meanwhile insulin resistance was determined by HOMA-IR index.

Results: The results of the study showed a significant increase ($p<0.001$) in amylase activity , amylase specific activity , BMI, LH, Insulin, and HOMA-IR for patients group in comparison with control group. There was significant correlation between insulin levels and HOMA-IR with specific activities of amylase in PCOS group while there were no significant correlation between insulin levels and HOMA-IR with specific activities of amylase in control group.

Conclusion: The current study suggested that metabolic disorders in PCOS patients includes hyperamylasemia , so high levels of amylase cannot be used as tumor marker for ovarian tumors.

Key words: Poly cystic ovarian syndrome , Serum amylase, Insulin , Insulin resistance.

Introduction:

Polycystic ovary syndrome (PCOS) is the most common gynecological endocrine disorder that affects approximately 10% of all women [1-3], it is considered as heterogeneous condition with a complex pathophysiology, the principal features are chronic anovulation and hyperandrogenism [4]**,** Hirsutism or acne, or both , insulin resistance, obesity, hypertension and dyslipidemia, defining so called syndrome X, abnormality of insulin secretion and insulin resistance play a critical role in the syndrome's [5,6] . 55–75% of women with PCOS had a high LH to FSH ratio due more to increased levels of LH than low levels of FSH, meanwhile LH/FSH ratio is normally about 1:1 in premenopausal women , but with PCOS a ratio of greater than 2:1 or 3:1 may be considered diagnostic [3].

Amylase (a-1,4-glucan-4-glucanohydrolase, EC 3.2.1.1) is a heterogeneous calcium-dependent metalloenzyme of molecular weight of 54-62 kDa , it is a digestive enzyme helps break down starches into simpler sugar molecules that are ultimately absorbed into the bloodstream, thus influencing blood glucose levels [7,8]. It consists of two families of isoenzymes, pancreatic amylase (P-

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type) and salivary amylase (S-type) [9]. Amylase enzymes made primarily by the pancreas and salivary glands but it is also produced by the small intestine mucosa, ovaries, placenta, liver, and fallopian tubes [10,11] . The clinical relevance of hyperamylasemia has been extensively studied in relation to various conditions such as acute pancreatitis and as a result of tumor-producing amylase especially in pancreatic, lung, stomach , uterine , and ovarian cancers , non-epithelial amylase-producing osteosarcoma and multiple myeloma as well as non-malignant ovarian disease [12-17] . To our knowledge there is no literature deals with the measurement of amylase activity in sera of PCOS patients, therefore, the aim of this study was to estimate serum amylase level and to assess any correlations of serum amylase with Insulin and HOMA-IR in PCOS patients as compared to healthy women .Meanwhile, polyacrylamide gel electrophoresis (PAGE) was used to differentiate protein patterns and the changes in amylase isoenzymes in serum of the studied groups.

Materials and Methods:

1.Subjects:

The present study comprises forty five women suffering from PCOS ranging in age mean± SD (27.53±3.96) years from Al-Elweaa and Karbalaa hospitals / Iraq in comparison with twenty five healthy women ranging in age mean ±SD (28.96±3.24) years as control . Women with PCOS were divided into two groups : patients group I and patients group Π according to the type of infertility (primary and secondary, respectively).Women with hyperprolactinemia and androgen-secreting tumors were excluded from the study, and none of these patients received medicines.The criteria for healthy control women were absence of menstrual irregularities, hirsuitism and major medical illness and there was no history of diabetes mellitus or hypertension.

2. Protocol:

The serum fasting blood sugar (F.B.S), total serum protein (T.S.P) and Albumin levels were measured by spectrophotometric methods supplied by Human Diagnostic, Germany. Globulin concentration and [Albumin]/ [globulin] ratio in sera samples of the studied groups in this study were calculated .Serum amylase levels were performed using the direct substrate kinetic enzymatic method manufactured by Human Diagnostic, Germany, the mean absorbance change per minute (Δ A/min) was calculated in terms of units per liter with a normal range of 120 IU/L. The hormones (FSH, LH and Insulin) were measured using commercially available Enzyme Linked Immunosorbent Assay (FSH and LH : BioCheck, USA; insulin: Diagnostic Automation Company, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using to the formula {Fasting serum insulin (mU/L) × Fasting blood glucose (mmol/L)/22.5} [18] .Body mass index (BMI) was calculated as the ratio of weight (kg)/height $(m)^2$. Conventional polyacrylamide gel electrophoresis (7.5%) was carried out for separation of proteins and amylase isoenzymes using Tris-glycine buffer pH 8.9 as electrode buffer.After electrophoresis, the first part of the gel was stained for protein using Coomassie Brillant Blue G-25. while the second part of the gel was incubated in starch solution for 2 hours at 37 °C for amylase isoenzymes detection, then the gel was brief water-rinsed and stained with KI-iodine solution, until resolution of the amylase banding was evident. Photographs were taken immediately after bands appearance.

3.Statistical analysis:

Statistical analysis was performed using the SPSS version 16.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA).Correlation analysis was used to test the linear relationship between amylase and insulin and HOMA-IR ,respectively.

Results and Discussion:

Seventy subjects comprising of forty five PCOS patients and twenty five control were included in the present study. Table 1 shows mean and standard deviation of BMI, FSH,LH, Insulin , proteins, F.B.S ,HOMA-IR in addition of amylase activity with its specific activity for the control and PCOS patients groups. There was a significant different in mean value of BMI, LH, Insulin, HOMA-IR amylase activity and amylase specific activity for patients group in comparison with control group.

Table 1: Some biochemical parameters levels in the control and patients groups included in this study.

Characteristic		Control group [n=25]	Patients group [n=45]	P Value
Age	(year)	**28.96±3.24**	**27.53±3.96**	>0.05
BMI	(kg/m^2)	**25.43±2.11**	**29.77±3.53**	<0.001
LH	(mIU/mL)	**8.69± 2.21**	**6.16±2.22**	<0.001
FSH	(mIU/mL)	**5.13±0.72**	**4.65±1.17**	>0.05
LH/FSH		**1.37± 0.29**	**1.38±0.53**	>0.05
Insulin	(mIU/L)	**7.04±1.02**	**18.11±6.31**	<0.001
HOMA-IR		**1.45±0.26**	**3.76±1.47**	<0.001
F.B.S	(mg/dL)	**85.84±8.05**	**86.18±11.49**	>0.05
Amylase activity	(IU/L)	**50.33± 20.97**	**144.75±74.84**	<0.001
Amylase specific activity $*10^{-4}$	(IU/mg)	**6.95± 2.96**	**19.81±10.16**	<0.001
T.S.P	(g/dL)	**7.28±0.44**	**7.27±0.52**	>0.05
Albumin	(g/dL)	**4.17± 0.31**	**4.09±0.48**	>0.05
Globulin	(g/dL)	**3.11±0.32**	**3.18±0.66**	>0.05
Albomin/Globulin		**1.35± 0.18**	**1.37±0.46**	>0.05

PCOS is the most common hormonal disorder among women of reproductive age and is a leading cause of infertility, some researchers believe that abnormal levels LH and high levels of androgens prevent the ovaries from functioning normally [19] , in the current study, the LH levels in sera samples of patient group was found markedly decreased ($p<0.001$), when compared with that of the control group , also there were no significant decreases in FSH levels ($p<0.05$) ,this parallel decreasing in both pituitary hormones will lead to non significant differences in LH/FSH ratio between the two studied groups (Table 1) . Obesity (BMI > 27 kg/m^2) has also been recognized as a feature of PCOS [20]. The results in Table (1) reveal the presence of a highly significant increase ($P < 0.001$) in patient's BMI in comparison with that of the control group. Several studies indicated that prevention and treatment of obesity is important for the management of PCOS [20,21], however treatment should focus on restoring menstrual regularity, decreasing androgen excesses, and decreasing insulin resistance [22].

PCOS is not only a fertility problem, but recently it has been disclosed that it is a metabolic disorder, thus many patients with PCOS also have features of the metabolic syndrome, including insulin resistance, obesity, and dyslipidemia, suggesting an increased risk for diabetes mellitus and

cardiovascular disease [23-25] but insulin resistance and hyperinsulinemia play a critical role in the syndrome's pathogenesis [26], in this study a significant increase in fasting serum insulin and HOMA-IR ($p<0.001$) were observed in these PCOS cases in comparison with control ,Table (1) ,while there was non- significant difference in F.B.S that may be because the association of blood sugar with hyperinsulinaemia (Insulin is the principle hormone that lowers blood sugar). For many years, Hyperamylasemia was thought related to malignant tumors such as pancreatic tumors as well as non-malignant ovarian disease [12-17] and although it has been reported that Hyperamylasemia can be used as marker in the diagnosis and follow up cases of ovarian cancer patients [27], the results in this study indicated that amylase activities and specific activities increased significantly ($P < 0.001$) in patients group with PCOS (Table 1) when compared with those of control group, this may be because reduced amylase clearance (the kidney plays the main role in eliminating circulating amylase [28] or may be because the abnormal fallopian tubes secretion of amylase . Meanwhile it is clear from the results in Table 1 that proteins revealed no significant differences ($p> 0.05$) when PCOS patients was compared with healthy individuals group ,this may be mean that such increase in amylase activities is probably biologically insignificant according to the decreasing in other enzymes levels.

The patients group was subdivided into two groups: patients group I ;with primary infertility; [n=25] and patients group Π ;with secondary infertility; [n=20] , the results of determination of the biochemical parameters are given in Table 2. Differences between the two subgroups were statistically non significant in all biochemical parameters levels included in the present study. However, difference between the control group and patients I and Π groups was significant in BMI, LH , Insulin, HOMA-IR, amylase activity, amylase specific activity and was not significant in other parameters except FSH levels in patients Π groups in comparison with control.

Table 2: Some biochemical parameters levels in the three groups included in this study (Mean± SD).

Characteristic		**Control group [n=25]**	**Patients group I [n=25]**	**Patients group Π [n=20]**
Age	(year)	28.96±3.24	27.40±3.80	27.70±4.26
BMI	(kg/m^2)	25.43±2.11	29.29±4.45 [a]	30.38±1.79 [b]
LH	(mIU/mL)	8.69± 2.21	6.12±1.89 [a]	6.22±2.63 [b]
FSH	(mIU/mL)	5.13±0.72	4.81±1.41	4.45±0.77 [c]
LH/FSH		1.37± 0.29	1.34±0.44	1.43±0.63
Insulin	(mIU/L)	7.04±1.02	19.02±7.01 [a]	16.98±5.28 [b]
HOMA-IR		1.45±0.26	3.93±1.62 [a]	3.55±1.27 [b]
F.B.S	(mg/dL)	85.84±8.05	86.08±1.342	86.30±8.84
Amylase activity	(IU/L)	50.33± 20.97	148.48±75.49 [a]	140.08±75.72 [b]
Amylase specific activity $*10^{-4}$	(IU/mg)	6.95± 2.96	20.23±10.34 [a]	19.28±10.16 [b]
T.S.P	(g/dL)	7.28±0.44	7.32±0.56	7.21±0.46
Albumin	(g/dL)	4.17± 0.31	4.11±0.51	4.07±0.45
Globulin	(g/dL)	3.11±0.32	3.21±0.66	3.14±0.67
Albomin/Globulin		1.35± 0.18	1.37±0.51	1.37±0.41

Results were expressed as the mean±SD.
a ***P*** <0.001 compared with control group.
b ***P*** <0.001 compared with control group.
c***P*** <0.01 compared with control group.

In order to clarify the correlation between amylase specific activity and insulin within each of the studied groups, the results were reanalyzed by using linear regression analysis. High correlation was observed between amylase specific activities and insulin levels in patients with PCOS (n=45, r=0.71, p<0.01) but low in the control (n=25, r=0.3, p>0.05) , Figure 1. Moreover, Figure 2 shows medium correlation between amylase specific activities and HOMA-IR in patients with PCOS (n=45, r=0.67, p<0.01) but very low correlation was indicated in the control (n=25, r =0.06, p>0.05).

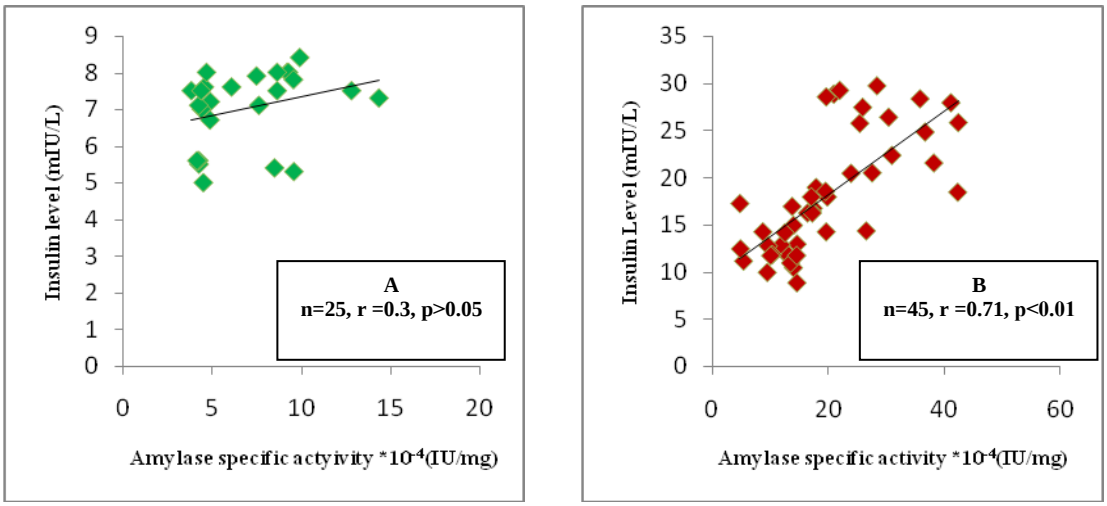

Fig 1: Scatter plot between serum amylase specific activities with Insulin levels for A: control group, and B: PCOS patients group.

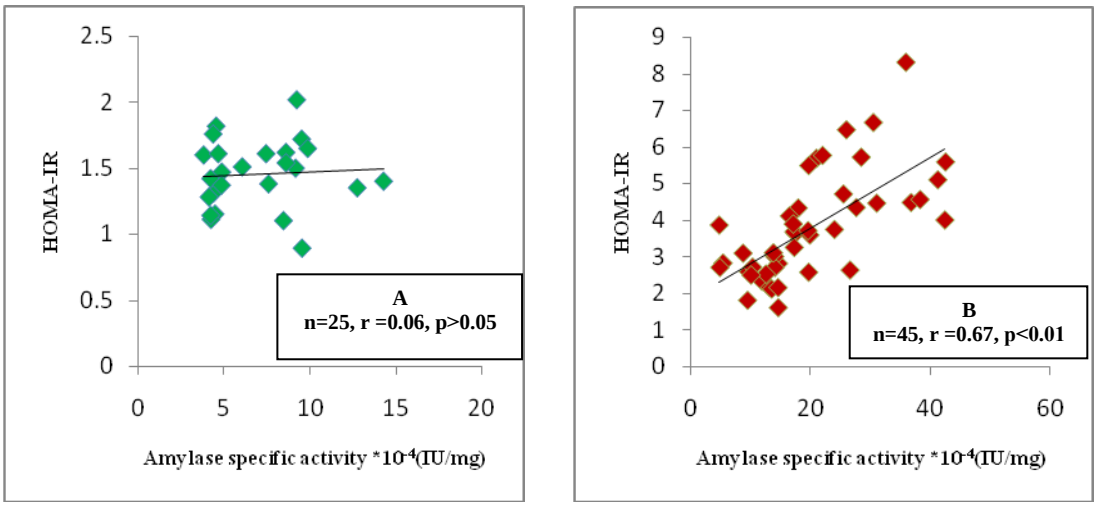

Fig 2:Scatter plot between serum amylase specific activities with HOMA-IR levels for A: control group, and B: PCOS patients group.

Meanwhile, we report a positive correlation between amylase specific activities and Insulin as well as between amylase specific activities and insulin resistance in sera of PCOS patients group, but not for control group (Figures 1&2 ,respectively) .This result is expected because serum amylase levels reflect metabolic abnormalities and abnormal glucose metabolism, both of which are associated with insulin action due to insulin resistance and/or inadequate insulin secretion [29,30] (the mechanisms underlying these associations remain unclear), moreover

Insulin plays a major role in the control of pancreatic amylase biosynthesis, also development of severe insulin resistance was associated with impairment of amylase-gene expression [31]. Additionally a marked positive association between serum amylase and body mass index was indicated in Table 1, this result is disagree with previous epidemiological studies [29,32] .

In order to detect the differences in total protein and amylase activity present in the studied groups, conventional polyacrylamide gel electrophoresis was carried out on crude sera samples of control and PCOS patients groups; Figure (3A and 3B), respectively. It is obvious from the comparison of the proteins profile of the two studied group (Figure 3A) that the sera was separated into distinct bands by which the separation of different proteins is based on the differences of both molecular size and the charge of these proteins [33] .The same figure indicates that there is no clear difference in proteins band intensity, which reflects the non significant variation in proteins concentration among the studied groups, Table 1. Serum amylase present in the sera samples actually is the total amylase i.e. consist of both P-type and S-type, therefore the elevation of sera alpha amylase (total amylase) may be attributed to the two types . Deep look at the electrozymogram indicated that the serum amylase activity located in two different parts of the gel, one with slow mobility (S-type) and the other with fast mobility (P-type), and each of them in turn consist of more than one band. Figure 3B shows increasing of band's intensity in the salivary type regions for the sera of PCOS patients in comparison to the control .i.e. the heavy bands were appeared in the PCOS patient group especially in the slowest fraction (according to their mobility to the anode) that may be explained the high levels of amylase, Table 1, and the abnormal secretion of this enzyme from different sources.

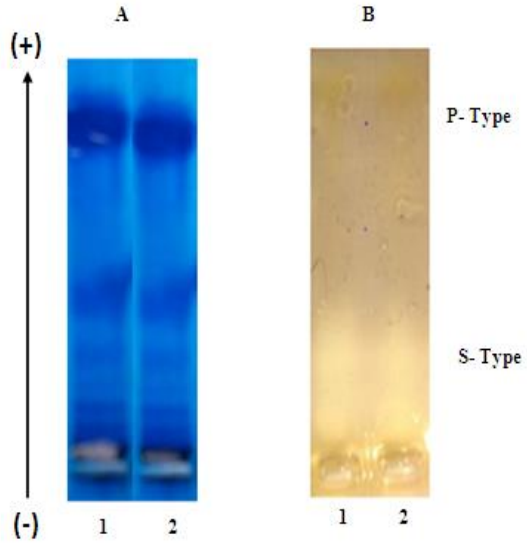

Fig 3 :Electrogram of (A): Proteins profile samples , (B): Amylase activity. The crude samples that applied were:1: pooled crude sera (control) ; 2: pooled crude sera (PCOS patients).

In conclusion, the present study demonstrated that serum amylase levels are increased in PCOS patients, and are associated mainly with insulin and HOMA-IR which are acutely disturbed in PCOS, suggesting a possible relationship with metabolic disorder .Moreover high levels of amylase cannot be used as tumor marker for ovarian tumors. The mechanisms remain to be elucidated, this can be confirmed by determining level of amylase found in the fluids from cysts associated with human fallopian tubes.

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هل مستوى الأميليز طبيعي في مصل النساء المصابات بمتلازمة التكيس المبيضي؟

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الخلاصة :

خلفية البحث: تعد متلازمة التكيس المبيضي السبب الأكثر شيوعا للعقم عند النساء في سن الإنجاب، وهو نذير مهم للاضطرابات الأيضية في الجسم، وقد أفيد أنه يمكن استخدام فرط أميليز الدم كدالة لسرطان المبيض . تهدف الدراسة الحالية الى تقييم نشاط الأميليز في المريضات اللاتي يعانين من متلازمة التكيس المبيضي بالاضافة الى تقدير العلاقة الترابطية لهذا الانزيم مع الأنسولين ومقاومة الأنسولين في نفس المريضات.

طريقة العمل: تضمنت الدراسة الحالية قياس فعالية أنزيم الاميليز والبروتين الكلي و مستوى الكلوكوز الصيامي ومستويات هرمونات ال LH, FSH ,Insulin بالاضافة الى حساب مقاومة الانسولين في أمصال خمسة وأربعون مريضة مصابة بمتلازمة التكيس المبيضي ومقارنتها بمجموعة الضبط المكونة من 25 امراة من الأصحاء.

النتائج: أظهرت الدراسة زيادة معنوية في الفعالية والفعالية النوعية لأنزيم الاميليز و كتلة الجسم و حساب مقاومة الانسولين بالاضافة الى مستويات هرموني ال Insulin وال LH ، كما لوحظ وجودعلاقة ترابطية معنوية بين مستويات الانسولين ومقاومة الانسولين مع مستويات الفعالية النوعية لانزيم الأميليز في النساء المصابات بمتلازمة التكيس المبيضي في حين لم يكن هناك علاقة ترابطية معنوية لمجموعة الضبط.

الاستنتاج: تقترح الدراسة الحالية بأن الاضطرابات الأيضية عند المصابات بمتلازمة التكيس المبيضي تشمل أرتفاع مستويات أنزيم الأميليز في الدم وهذا يعني بأنه لا يمكن أعتبار أرتفاع مستويات هدا الانزيم كدالة لأورام المبيض فقط.