Relation of α-Amylase Activity with Glucose and Anti-Gliadin IgA and IgG in Sera of Patients with Celiac Disease

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Abstract

Celiac disease (CD) is an inflammatory small intestinal disorder that can lead to severe villous atrophy, and malabsorption . Since the measurement of α-amylase activity is the most widely used biochemical test for the diagnosis of pancreatic and non pancreatic disease , therefore serum α-amylase were studied in the present study in an attempt to evaluate the usefulness of this enzyme in the diagnosis of celiac disease and its relationship with anti gliadin IgA and IgG and serum glucose . Thirty one patients with celiac disease were studied and compared with twenty four healthy individuals . Significant elevation of α-amylase activity , glucose and anti gliadin IgA and IgG were observed in the sera of patients with celiac disease compared with the control group . Also a significant positive correlation between α-amylase activity and anti gliadin IgG were found in the present study in the sera of patients with celiac disease while a non significant correlation were found between α-amylase activity and anti gliadin IgA and glucose in the sera of the same patients of celiac disease.

Keywords:- α-amylase , anti – gliadin IgA , IgG , glucose , celiac disease .

الخلاصة

داء الاحتشاء هو التهاب يحدث نتيجة خلل معوي صغير، من الممكن ان يؤدي إلى حدوث ضمور زغابي شديد وسوء امتصاص . ولكون قياس فعالية الفا – اميليز يعد من أكثر الفحوص الكيميائية الحياتية استخداما لتشخيص الأمراض البنكرياسية وغير البنكرياسية , لذا فقد تم قياس هذا الأنزيم في الدراسة الحالية في محاولة لتقدير أمكانية استخدام الفا-اميليز في تشخيص مرض الاحتشاء وعلاقته بكلكوز مصل الدم ومضادات الكلايدن IgA , IgG ، حيث تضمنت الدراسة الحالية جمع ٣١ عينة من مرضى المصابين بداء الاحتشاء ومقارنتها بــ ٢٤ عينة من الأشخاص الأصحاء . أظهرت النتائج وجود زيادة معنوية في فعالية الفا ـ اميليز ومستوى كل من الكلكوز ومضادات الكلايدن IgA , IgG في مصول دم المصابين بداء الاحتشاء مقارنة مع مستوياتها في مصول دم الأصحاء وقد تبين أيضا وجود علاقة ايجابية معنوية بين فعالية الفا-اميليز ومضاد IgG في مصول دم المصابين بداء الاحتشاء في حين تبين عدم وجود علاقة معنوية بين فعالية الفا-اميليز وكل من الكلكوز ومضاد IgA في مصول دم المصابين بداء الاحتشاء .

Introduction

Celiac disease (CD) , also known as celiac sprue or gluten - sensitive entropathy[1],[2]. Is the most common life – long food sensitive entropathy in humans , is characterized by malabsorption , chronic inflammation of small intestine mucosa , villous atrophy and crypt hyperplasia[3],[4],[5] . Currently , the onset of celiac disease is considered to result from interaction between environmental factors , gluten as important genetic predisposition and immune system hyperactivity[6] . The disease can clinically manifest at any age , most commonly in the first few years of life , a few months of introducing gluten in diet[7]. About 20% of cases occur in patients who are older than 60 years of age[8]. Celiac disease may be associated with a wide range of disease[1],[9], including thyroid dermatological , lympho proliferative disorders , and immune disorders[1],[10],[11]. Furthermore , there is a greater than expected prevalence of immune disorders in CD patients as well as of CD in patients with autoimmune diseases[12],[13] . Celiac disease is diagnosed by abnormal blood tests and an abnormal appearing intestine on biopsy and symptoms that resolve with a gluten free diet[14,15] . Testing sera for IgA or IgG immunoreactivity to gliadin is usually one of the first steps in the complex process of diagnosing gluten intolerance[16]. Among laboratory tests , measurement of α-amylase (EC:3.2.1.1) is the test most widely used in the diagnosis of pancreatic and non pancreatic diseases[17]. The serum amylase concentration reflects the balance between the rates of amylase entry into and removal from the blood[18]. α-Amylase is an enzyme that hydrolyses alpha- bonds of large alpha – linked poly saccharides such as starch and glycogen , yielding glucose and maltose[19].

It is the major form of amylase found in humans and other mammals[20]. Amylase is most prominent in pancreatic juice and saliva , each of which having its own isoform of human α-amylase[19].Conflicting data have been reported in previous studies for α-amylase activity in patients with Celiac disease. Some of these data reported elevated levels of serum amylase[21],[22]. While others pointed out that patients with CD have low amylase values[23].The aim of this study was to evaluate the serum concentrations of α-amylase and its correlation with glucose and anti gliadin IgA and IgG antibodies in celiac disease.

Subjects and Methods
Subjects
Thirty one patients (15 male their age 2-50 within mean 14 year and 16 female their age 2-42 within mean 10 year) were involved in this study . The patients were referred to Al-Uarmook Teaching Hospital and Al-Kadhimiya Teaching Hospital. Baghdad , Iraq., diagnosed by Dr. Saad Alsadoon and Dr. Fathel Alabodi. All patients with CD were diagnosed depend on symptoms of diarrhea, weight loss ,anemia, and serologic examination by (anti-gliadin IgA and IgG) . No other diseases associated with CD in those patients. They were all underwent free diet treatment without any drugs. As a control , 24 age and gender matches healthy individual were included in the present study .

Serum Sampling
Five milliliters of samples of venous blood were taken in fasting state and left for 10 minutes at room temperature. After blood coagulation , the sera were separated by centrifugation at 3000 rpm for 10 minutes and then sera stored at -20°C until being used . Hemolyzed samples were discarded .

Methods
Determination of anti gliadin IgA and IgG
Serum concentration of anti- gliadin IgA and IgG were measured by double antibody technique using enzyme – linked immunosorbent assay (ELISA), Biohit Oyi, Finland[24],[25].

Determination of α-amylase activity
The activity of serum α-amylase was determined by colorimetric method[26].

Determination of protein concentration
The total serum protein concentration was determined by Biuret method using total protein kit , Biolab, France[27].

Determination of Glucose
The serum concentration of glucose was measured by enzymatic colorimetric using glucose kit[28].

Statistical Analysis
Data were analyzed using spss (version 10.0). The results are expressed as mean ± standard deviation (SD) . Statistical and correlation analysis were undertaken using t-test, and pearson's correlation coefficients. Values of   P < 0.05 were considered significant.

Results
The concentration of anti- gliadin IgA and IgG were measured in the present study in the sera of control and patients with celiac disease by double antibody technique. The mean values presented in Table (1) reflect a highly significant increase in the serum levels of anti- gliadin IgG (p< 0.001) and IgA (p< 0.001) in patients with CD compared with those of the control group . The activity of serum α-amylase was determined using Caraway method . In order to calculate the specific activity of the enzyme ,total protein concentration was measured in the sera of the studied groups, using Biuret method. A non significant increase (p >0.05) in protein levels was observed in the present study in sera of patients with CD in comparison with that of the control group , Table (2) . When the mean values of both activity and specific activity of α-amylase in sera of patients with CD was compared with that of the control group, the results (Table 3) reflected a highly significant increase in activity (P<0.001) and specific activity (P< 0.01) .The serum concentration of glucose was measured throughout this study in the sera of control and patient groups using enzymatic method. The result presented in Table(4) shows a significant increase (P<0.01) in glucose levels in sera of patients with CD in comparison with that of the control group .Upon analysis of the overall results of the present study, it was found a significant positive correlation (r = 0.709 , P< 0.05) between α-amylase activity and anti - gliadin IgG in the sera of patients with celiac disease (54%) who have increase in there α-amylase activity Figure (1). While a non significant correlation (P>0.05) between α-amylase and anti – gliadin IgA and glucose were found in the sera of the same patients with CD.

Table 1 : Serum anti- gliadin IgG and IgA levels in control and patients with celiac disease .

	Groups	Sample number(n)	Range (Au)	Mean (Au) ± Standard Deviation	P value
IgG	Control patients with CD	24 31	7.6-30.1 15.0-204.1	17.31 ± 8.63 77.90 ± 48.02	P<0.001
IgA	Control patients with CD	24 31	7.1-32.5 10-192.1	15.68 ± 10.154 62.83 ± 57.6	P<0.001

Table 2 : Total protein concentration in the sera of control and patients with celiac disease .

Group	Samples number (n)	Range mg/dl	Mean mg/dl ± Standard Deviation	P value
Control	24	6.7-8.9	7.65±0.78	P>0.05
Patients with CD	31	5.1-12	7.99±1.77	

Table 3 : Mean values of α-amylase activity and specific activity in the sera of control and patients with celiac disease.

Group	Activity U/L		Specific activity U/mg		
	Range	Mean ± Standard deviation	Range	Mean	Standard deviation
Control 24	74.0-175.60	126.10±45.01	0.94-2.62	1.699	0.74
Patients with CD 31	35.3-268.78	174.18* ±60.06	0.24-9.60	2.578**	1.64

*significant increase in amylase activity with P<0.001.
** significant increase in amylase specific activity with P<0.01.

Table 4 : Mean values of glucose levels in the sera of control and patients with celiac disease

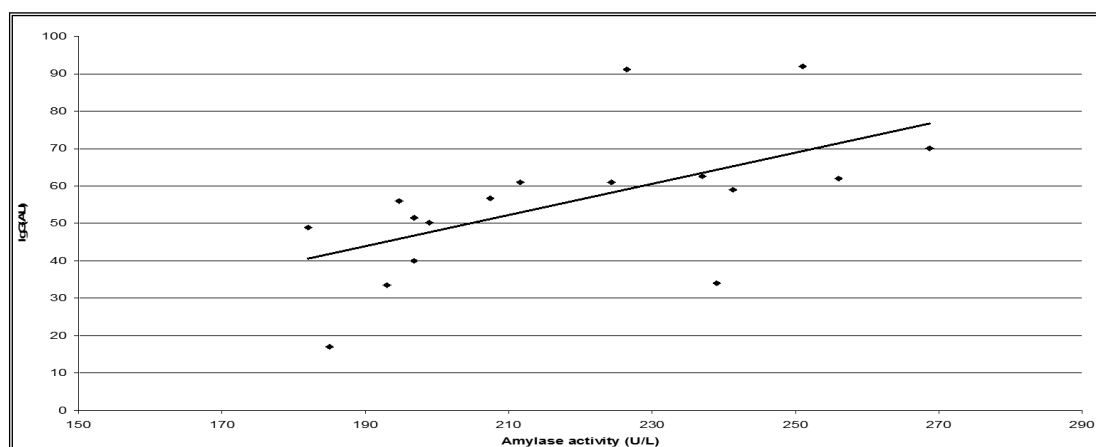| Group	Samples number (n)	Range mg/dl	Mean mg/dl ± Standard deviation	P value
Control	24	60-79.2	70.1 ± 8.11	P<0.001
Patients with CD	31	22.2-234	102.26 ±50.41	

Figure 1 : The correlation between α-amylase activity and anti- gliadin IgG in sera of patients with celiac disease (r=0.709,P<0.05).

Discussion

It is well known that gliadin is directly or indirectly through immune mediated reactions , toxic to small bowel mucosa of relatively small population of genetically predisposed individuals who under this toxic action develop celiac disease[12],[29]. Testing sera for IgA or IgG immunoreactivity to gliadin is usually one of the first steps in the complex process of diagnosing gluten intolerance , because it is well known that antibodies to native gliadin sequences are present in patients with celiac disease[30],[31],[32]. In the present study anti – gliadin IgA and IgG measurements (Table 1) shows a presence of a significant increase of anti – gliadin IgA and IgG in sera of patients with CD. Celiac disease is an autoimmune disorder that can coexist with other diseases , such as diabetes mellitus type 1 , thyroid gland diseases , and Sjogren's Syndrome[7]. Throught this study fasting serum glucose was measured and highly significant increase of its was observed in the patients with CD in comparison with that of the control group. This was in agreement with the result obtained by Galicka latala D. et. al. who reported that hyperglycemia and problems with glucose balance in the serum of patients with celiac disease were observed[33]. While this result was disagreement with the result obtained by Tourniaire J. et. al. who reported that intolerance to gluten can disrupt glucose regulation leading to greater risk of hypoglycaemia[34].Throughout this study highly significant increase (P<0.001) in both activity and specific activity of α-amylase in sera of patients with CD . This is in agreement with the result obtained by Oita T. et. al. who reported that patients with celiac disease showed persistently elevated levels of serum amylase and lipase[21]. Carroccio A. et. al. demonstrated a frequency of about 25% of elevated pancreatic enzymes(amylase, lipase, trypsin, and elastase) values in CD patients[22].while the present study result was disagreement with the result that obtained by Disantagnese P. who reported that patients with celiac disease tend as a group to have low amylase values[23]. The elevated levels of amylase can result either from an increased rate of entry of amylase into circulation and /or a decreased metabolic clearence of this enzyme due to renal failure or macroamylase (a condition in which an abnormally high – molecular – weight amylase is present in the serum)[18]. Torrent Vernetta A. et. al. reported that macroamylaseaemia most often formed due the binding of the amylase to an immunoglobulin and it has been described as a causal finding associated to abdominal pain and to celiac disease[35]. Oita T. et. al. demonstrated that Immunoprecipation assay showed that amylase was bound to poly clonal IgA and IgG[21]. No differences were found , when either the enzyme activity values or the enzyme specific activity values were used at comparison between control and patients with CD since this is due to the non significant difference in the measured protein concentration between control and patients (Table 2) . A significant positive correlation (P < 0.05 , r = 0.709) was found in the present study between α-amylase activity and anti-gliadin IgG in the sera of patients with CD who have increased levels in α-amylase activity while a non significant correlation (P<0.05) were found between α-amylase and fasting serum glucose and anti gliadin IgA in the same patients with CD .

Conclusions

From the results that had been obtained it conclude a significant positive correlation between α-amylase activity and anti- gliadin IgG in the sera of of patients with CD who have elevated in enzyme activity .This finding would appear to suggest a possibility to use the amylase activity as a tool with anti- gliadin IgG for diagnosis of celiac disease.

Acknowledgment

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