

DOI: <http://dx.doi.org/10.21123/bsj.2017.14.3.0557>

## Measurement and purification of Alanine aminotransferase (ALT) enzyme activity in patients with celiac disease

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Received 6/11/2016

Accepted 30/4/2017



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### Abstract:

Celiac disease (CD) is the most common genetically - based disease in correlation with food intolerance. The aim of this study is to measure the activity of ALT enzyme and purify enzyme from sera women with celiac disease. Alanine aminotransferase (ALT) activity has been assayed in (30) women serum samples with celiac disease, age range between (20-40) year and (30) serum of healthy women as control group, age range between (22-38) year. In the present study, the mean value of ALT activity was significantly higher in patients with celiac disease than healthy group ( $p < 0.01$ ). The ALT enzyme was partial purified from sera women with celiac disease by dialysis, gel filtration using Sephadex G- 50 and ion exchange chromatography using DEAE- cellulose A-50 . The results showed a single peak by using gel filtration and the activity reached 31-15 U/L .Two isoenzymes were obtained by using ion exchange chromatography and the purity degree of isoenzymse (I, II) were (5.7) and (5.53) fold respectively.

**Key words:** ALT, Celiac disease, Transaminases, Isoenzymes.

### Introduction:

Celiac disease (CD) is a chronic disease causing inflammation of the proximal small intestine that occurs in genetically predisposed individuals when they eat gluten which is the storage protein in wheat, barley, and rye [1]. A strong association has been observed between celiac disease, generally its silent clinical form, and

autoimmune disorders [2]. Celiac disease is a gluten-sensitive autoimmune disease of the small intestine affecting genetically susceptible individuals worldwide with classical symptoms such as diarrhea, steatorrhea and weight loss due to mal-absorption [3]. Liver dysfunction is related with extra - intestinal manifestation and celiac

disease [4]. Alanine aminotransferase (ALT) [EC.2.6.1.2] is formerly known as glutamic pyruvic transaminase (ALT) that is normally found in the liver, but also found in erythrocytes, heart, muscle cell, pancreas and kidneys. However, when the body cells or an organ such as the liver or heart is damaged, ALT secret from tissues to the blood stream and causing an increase in enzyme level [5]. The high spread of CD in the normal people is an accidental correlation between CD and the liver abnormality cannot be eliminated [6]. The major protein components of gluten-gliadin and glutenin are storage proteins in wheat and they are widely used as an ingredient in food processing [7]. The presence of high levels of transaminases, usually the alanine transaminase (ALT) and Aspartate transaminase (AST) are sensitive to liver damage and may also be increased in other conditions such as thyroid disease, celiac disease and muscle disease. Some studies showed that up to 9% of patients with high enzyme levels are infected with celiac disease [8]. Celiac disease may be connected with abnormal liver function tests in the deficiency of other probable causes of disturbance of liver function and it is required to investigate celiac disease in patients with liver disease and in unknown causes [9]. The main reasons for elevated ALT activity levels are liver injury such as (acute viral fever, alcohol pancreatic disorder and hepatitis), muscles injury, drugs and many toxins [10].

## Materials and Methods:

### Specimens

The samples were collected from subjects attended AL- Yarmuk Teaching Hospital in Baghdad City. Sera were collected from 60 adult females and were divided into two groups; each group includes 30 subjects as follows: Group (1), patients with Celiac disease,

age rang (20-40) year, Group (2) control group (Healthy women) age rang (22-38) year. Venous blood(5ml) was obtained from patients and control subjects. The samples were collected in plain plastic tubes and left at room temperature for 15 min. and then centrifuged at (3000rpm) for 10 mins. Serum was removed and assayed immediately. The sample was stored at -4°C for further analysis.

### Assay of Alanine aminotransferase (ALT) activity .

Serum ALT activity was measured using colorimetric method according to Reitman and Frankel (1957) [11].

### Determination of total protein concentration

Total protein concentration was determined in the serum using a kit provided from a company (SYRBIO) depending on Biuret method [12].

### Purification of ALT enzyme

Purification of enzyme was done by using the following steps:

#### - Precipitation by using Ammonium sulfate (40%).

The method used for precipitation of protein by adding ammonium sulfate. 0.8 gm of ammonium sulphate was added gradually to 5ml of fresh serum in a beaker with constant stirring at (4°C) for one hour until the solution became turbid, and then it was centrifuged at speed (3500rpm) for 10 min. The precipitate was dissolved in a less amount of phosphate buffer solution pH (7.2), then the activity of the enzyme and total protein concentration were measured.

#### -Dialysis

It is an important step for the removal of ammonium sulphate. Two ml of the protein solution, that was obtained from the previous step, was put into a tightly wrapped dialysis bag, then the phosphate buffer solution pH was (7.2). This process was done with constant stirring at 4°C for 15min. The volume of

serum, total protein and enzyme activity were measured.

#### -Gel Filtration

Two ml of the dialyzed solution was added slowly on the surface of sephadex (G-50), column (20x 2 cm ) and left for 5min to be absorbed .Twenty fractions were collected by passing phosphate buffer solution pH (7.2) through the column using the process that was carried out inside a refrigerator and the flow rate was (2 ml /min), then the activity of the enzyme and total protein concentration were measured .

#### -Ion Exchange Chromatography

Ion exchange chromatography technique is used for the separation and purification of proteins .Two ml of fresh filtered serum was passed through a column of diethyl amino ethyl cellulose A-50 (DEAE), column (20x 2 cm). A syringe pump is commonly used to pump various buffers via the column. Forty- five fractions were collected by passing different concentrations of sodium chloride solution (0.1- 0.4)M. The flow rate was (2ml/4 min).

#### Statistical Analysis

The results were analyzed to determine the mean value and standard error of different parameters. The statistical analysis (2012) method was used to study the correlation of different groups with activity of ALT enzyme. Student's t-test was used to compare between two groups and significance between the mean values was considered when  $p < 0.05$  [13].

#### Results and Discussion

The findings in Table (1) show significant differences ( $p < 0.01$ ) in the ALT activity between women with celiac disease ( $28.32 \pm 2.91$ ) U/L and healthy women ( $14.48 \pm 0.83$ ) U/L. Elevated ALT enzyme activity in serum with celiac disease may be related to acute forms of liver illness [14]. Subjects with CD and hypertransaminasemia show a

significant elevation in intestinal permeability compared with normal subjects [15]. Hypertransaminasemia could be the only sign of celiac disease, in the absence of gastrointestinal symptom [16] .The pathogenic mechanism underlying liver injury in celiac disease patients is still unknown, a widely accepted hypothesis depends on increased permeability of the intestine with celiac disease, this may enable the entry of toxins, antigens and inflammatory materials to the portal vein circulation [17]. The higher level of serum ALT is also shown in conditions other than liver injury, such as muscle illness, celiac disease (CD) and healthy subjects [18].

**Table (1): The activity of ALT enzyme in sera of celiac disease patients and healthy women.**

Group	Subjects	ALT activity(U/L) Mean $\pm$ SE	P- value
Celiac disease	30	28.32 $\pm$ 2.91	< 0.01
Healthy women	30	14.48 $\pm$ 0.83	

#### -Purification of ALT enzyme

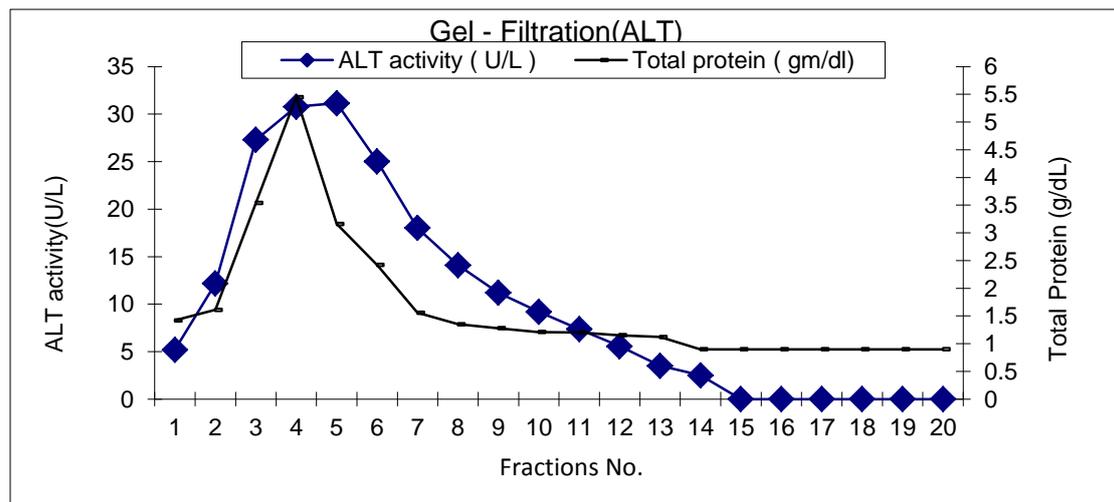
Table (2) presents the isolation and partial purification of ALT enzyme and isoenzymes from sera women with celiac disease. The ALT activity reached (24.86)U/L by using ammonium sulphate saturation (40%). The enzyme was partially purified by using dialysis method with phosphate buffer solution (pH 7.2), the purity fold of ALT was 3.22 folded with a yield (87.69%) , while the purity degree was increased to 4.37fold with a yield (70.95%) using Sephadex G-50column chromatography. This enzyme shows a single peak in (Figure1) and the ALT activity reached 31.15 U/L . The purification by ion exchange chromatography technique offered several distinct advantages over other conventional methods of separation of serum isoenzymes. This enzyme was purified by using DEAE -

cellulose A-50 .Two isoenzymes were obtained as shown in (Figure 2). The purity degree of isoenzyme (I) was 5.7

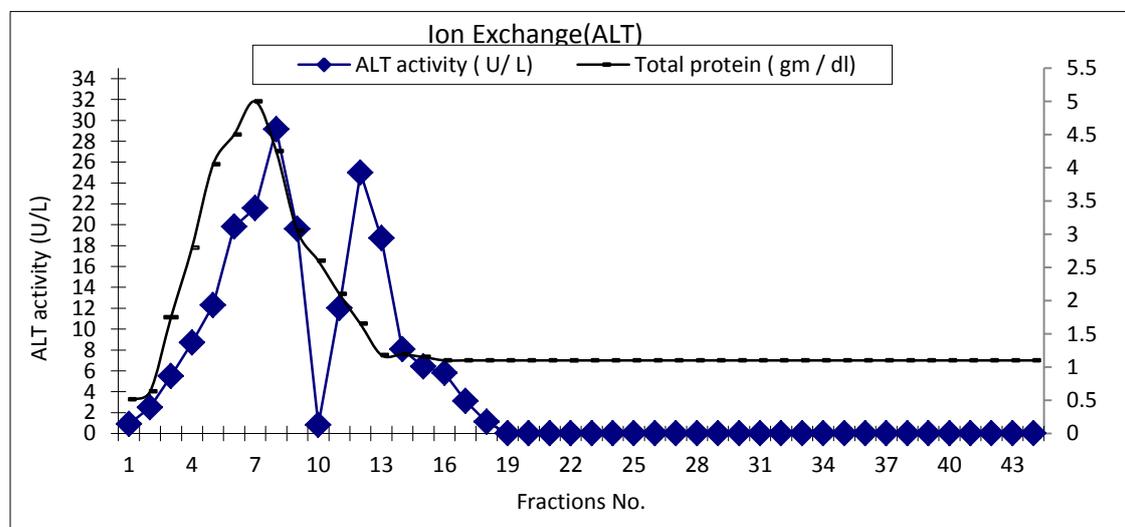
folded with yield (66.40%) and isoenzyme (II) with 5.53 fold with yield (56.94%).

**Table (2): Steps of ALT enzyme partial purification from celiac disease patients**

Step	Volume (ml)	Activity of enzyme U/L	Total activity(U)	Protein Con. (mg/L)	Total Protein(mg)	Specific activity (U/mg)	Fold Purification	Yield %
Crude serum	5	17.56	0.0878	2100	10.5	0.00836	-	-
Ammonium sulphate	2	24.86	0.0497	1548	3.096	0.0160	1.91	55.80
Dialysis	2	38.5	0.077	1420	2.84	0.027	3.22	87.69
Sephadex G-50	2	31.15	0.0623	850	1.7	0.0366	4.377	70.93
Ion exchange Iso. I	2	29.16	0.0583	610	1.22	0.047	5.7	66.40
Ion exchange Iso. II	2	25	0.05	540	1.08	0.046	5.53	56.94



**Fig. (1): Isolation of ALT enzyme from patient with celiac disease by gel filtration**



**Fig.(2):Isolation of ALT isoenzymes from sera celiac disease women by using ion exchange DEAE–cellulose A-50.**

The rises of serum ALT enzyme activity indicates of liver injury depending on the hypothesis that ALT protein is specially and widely reflected in the liver. Yang *et al* [19] found that two isoenzymes (ALT<sub>1</sub> and ALT<sub>2</sub>) are expressed by ALT genes differently in tissues and possibly in the cellular partition, both isoenzymes participate to the total ALT enzyme activity. Less common inherited disease such as Wilson disease and alpha-1-antitrypsin loss; furthermore, slight increase of ALT activity is also shown in the setting of celiac disease (CD) [20]. Enzymes are biochemical macromolecules that catalyst accelerate metabolic processes of organisms [21]. In this study, the elevated of fold purification of ALT enzyme in serum was in agreement with the other study that reported to have raised to 15 fold [22]. The rises of this enzyme in the serum is known to be produced from damage tissues [23]. Celiac disease patients commonly show slightly increased of liver enzymes and this abnormality generally normalizes after gluten-free diet [24]. Mohammed *et al* [9] showed that the inspected of patients with celiac disease had other probable explanations for their abnormal liver tests such as hypertransaminasemia, autoimmune hepatitis and cryptogenic. They did not appear to have an important role in the pathological process and may be associated with CD itself.

### Conclusion:

A significant increase in ALT activity in patients with celiac disease compared with the normal group. Also, we isolated and partial predicated two isoenzymes (I, II) of ALT enzyme from sera women with celiac disease using ion exchange chromatography. The recommendation of the present study is detect the characteristics of isoenzymes.

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## قياس وتنقية فعالية انزيم الانين امينوترانسفيريز(ALT) في المرضى المصابين بحساسية الحنطة

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### الخلاصة :

مرض حساسية الحنطة من الامراض الاكثر شيوعا والذي يرتبط مع عدم القدرة على تحمل الطعام . هدفت الدراسة الحالية الى قياس فعالية انزيم ALT والتنقية الجزئية للانزيم في امصال المرضى المصابين بحساسية الحنطة. في هذه الدراسة تم قياس فعالية انزيم الانين امينوترانسفيريز (ALT) في امصال 30 امرأة مصابة بحساسية الحنطة ، تتراوح اعمارهن بين (20-40) سنة بالمقارنة مع 30 امرأة من الاشخاص الاصحاء (مجموعة السيطرة) تتراوح معدل اعمارهن بين (22-38) سنة. حيث اظهرت النتائج الاحصائية وجود زيادة معنوية ( $P<0.01$ ) في فعالية انزيم ALT لامصال الاناث المصابات بحساسية الحنطة باستخدام اقياس الفرز الغشائي، كروموتغرافيا الترشيح بالهلام (Sephadex G-50) وكروموتوغرافيا التبادل الايوني-DEAE- CelluloseA. كما بينت النتائج وجود قمة واحدة عند استخدام كروموتوغرافيا الترشيح بالهلام، حيث بلغت قيمة فعالية الانزيم 31.15 U/L، كما تم فصل متناظرين انزيمين عند استخدام كروموتوغرافيا التبادل الايوني، وقد بلغت عدد مرات التنقية للمتناظر (1) والمتناظر (2) هي (5.7)، (5.53) على التوالي .

الكلمات المفتاحية : الانين امينوترانسفيريز، مرض حساسية الحنطة، الانزيمات الناقلة، الاشباه الجزئية .