

Evaluation of gamma glutamyl transferase activity in sera of patients with Alzheimer's disease

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تقييم نشاط انزيم كاما كلوتامايل ترانسفيريز في مصول المرضى المصابين بالزهايمر

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المستخلص

كاما كلوتاميل ترانسفيراز (GGT) يلعب دورا في امتصاص الكلوتاثيون الخلوية، والذي يشكل عنصرا هاما من الآليات المضادة للأكسدة. و أن الزيادة في GGT مصل الدم ليكون علامة مبكرة وحساسة من الاجهاد التأكسدي. الاجهاد التأكسدي لها دور في النشوء مرض الزهايمر. وان الهدف من الدراسة الحالية هي التحقيق من مستويات (GGT) في عينات مصل مرضى الزهايمر. تضمنت الدراسة 67 مريضا مصابا بالزهايمر و 70 فردا من الاصحاء (السيطرة)، حيث كشفت الدراسة أن النشاط S.GGT كان أعلى بكثير في المرضى (الزهايمر 67 ± 2.1 IU/L / 2.1IU لتر) مما كان عليه في السيطرة (الاصحاء). (27 \pm 2.1IU/L) وقد أجريت هذه الدراسة في الرقم الهيدروجيني الأمثل (8.2) ودرجة الحرارة المثلى 37 $^{\circ}$ C. أظهرت النتائج أن هناك علاقة ايجابية كبيرة بين العمر ونشاط (GGT) في مصل الذكور ($R=0.75$) والإناث ($r=0.72$) وان زيادة في العمر يرتبط بشكل كبير مع الزيادة في مستويات GGT التي أثرت في مجموعة السيطرة ومجموعة من المرضى. وان معدل بروتين سي التفاعلي (CRP) المصل كانت ($P<0.001$) أكبر لدى مجموعة مرضى (33.9 ± 2.3) بالمقارنة مع مجموعة السيطرة (4.8 ± 0.2).

Abstract

Gamma glutamyl transferase (GGT) plays a role in cellular glutathione uptake, which is an important element of antioxidant mechanisms. An increase in serum GGT is thought to be an early and sensitive marker of oxidative stress. Oxidative stress has a role in the pathogenesis of Alzheimer's disease (AD). The aim of present study was to investigate the levels of serum Gamma glutamyl transferase (S.GGT) in samples from patient with Alzheimer's disease. The study included 67 AD patients and 70 individuals as normal controls. Serum Gamma glutamyl transferase GGT and C-reactive protein (CRP) were determined. The study revealed that the S.GGT activity was significantly higher in patients with Alzheimer's disease (75 ± 2.1 IU/L) than in control (healthy individuals) (25 ± 2.1 IU/L). The study was carried out in optimum pH (8.2) and optimum temperature 37 $^{\circ}$ C. The results showed that There was a significant positive correlation between age and S.GGT activity in males ($r=0.75$); and females ($r=0.72$). Increase in age was significantly associated with raised GGT levels in control group and patients group. The mean serum C-reactive protein (CRP) levels

were significantly ($p < 0.001$) greater in patients group ((33.9 ± 2.3)) when compared with control group (4.8 ± 0.2). The high serum GGT concentration may play a critical role in the oxidative stress present in AD brain and, consequently, may play a central role in the pathogenesis of the disease.

Key words: Alzheimer's disease, gamma glutamyltransferase

Introduction

Alzheimer's disease (AD) is progressive, degenerative, neurological disorder that results in memory impairment and deterioration in cognitive function, reasoning, and behavior of the individual. Alzheimer's disease is the most common form of dementia - accounting for more than 60 percent of late life disorders of cognitive dysfunction [1]. The loss of intellectual function initially interferes with daily life, and after a disease course that may last many years, eventually results in death[2]. Death is usually due to factors such as compromised nutrition, complications of the immune system (pneumonia, sepsis, and other infections), trauma, or aspiration. AD characterized by progressive loss of memory and cognition, is an age-related neurodegenerative disorder currently affecting more than 4 million persons in the United States. The 40–42 amino acid peptide, amyloid β -peptide (Ab), is produced in excess in AD brain, and many researchers opine that Ab is central to the pathogenesis of this disorder. Moreover, the AD brain is under extensive oxidative stress, manifested by, among other indices, lipid peroxidation, protein oxidation, free radical formation, DNA/RNA oxidation, protein-bound 3-nitrotyrosine, and advanced glycation endproducts [3]. Serum gamma-glutamyl transferase (EC 2.3.2.2) is an ectoplasmic enzyme responsible for the extracellular catabolism of glutathione, which is synthesized in epithelial cells of the intrahepatic duct. It distributes in different cells with various secretory or absorptive activities. GGT has an important role in glutathione homeostasis by initiating the breakdown of extracellular glutathione and turnover of vascular glutathione. Considering the antioxidant activity of glutathione, increased level of GGT may be linked to greater oxidative stress. Gamma glutamyltransferase plays a role in cellular glutathione uptake and extracellular catabolism of glutathione [4]. These mechanisms are important elements of intracellular protective antioxidant mechanisms. Glutathione is an important element in antioxidant mechanisms, and GGT is therefore thought to have a role in oxidative mechanisms and is regarded as an early and sensitive marker of oxidative stress [5]. (6). have stated that modest increases in serum GGT activity within normal range may be an early marker of cellular oxidative stress. Oxidative stress is one of the mechanisms which are thought to be involved in the pathogenesis of Alzheimer's disease. Accumulation of free radicals and oxidative stress may have a role in the pathology of AD by leading to lipid peroxidation and neuronal degeneration in the brain. Therefore, we hypothesized that as GGT is a marker for oxidative stress it may also be a marker for AD. Glutathione (glutamyl-cysteinyl-glycine, GSH) is the most abundant nonprotein thiol in most cells[6]. As a substrate for the glutathione

peroxidases (GPx) and glutathione S-transferases (GSTs), GSH plays key roles in protection against oxidative stress and in detoxification/metabolism of endogenous and exogenous compounds, including carcinogens and drugs. In addition, GSH also plays roles in cell cycle regulation, cell signaling, and apoptosis [7,8]. The aim of this study was to investigate the GGT levels in AD and control groups as a possible marker of oxidative stress. C-reactive protein (CRP) is protein found in the blood, the levels of which rise in response to inflammation (i.e. C-reactive protein is an acute-phase protein). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells.

Materials and Methods

Materials (Subjects)

The study was performed on Alzheimer's disease (AD) patients and healthy non AD control. A total of 137 sera samples were enrolled in the present study during their attendance to clinics and used for determination of serum gamma-glutamyl transferase activity. 67 of these samples were of AD patients (37 males and 30 females), their age range from (30-59) years as diagnosed by physician, the control group involved 70 cases (40 males and 30 females with no reported family history of AD).

Collection of samples

Blood sample analysis is usually done on venous or capillary blood. Six milliliters of blood has been collected and allowed standing at room temperature until it has clotted. Restriction of clot may be assisted by gently losing it from the walls of the container. The separated serum, about 2-3 mL is centrifuged at 3000 rpm using T-centrifuge for removal of any suspended cells. Sera were separated and stored at -20°C until analysis.

Methods

Estimation of serum Gamma-Glutamyl-transferase activity:

The protocol of Szasz, [9] was adopted for determination of GGT activity. The principles depend on hydrolysis of γ -glutamyl p-nitroanilide in the presence of the acceptor glycylglycine. Standard assay included final reagent concentrations 4 mM of γ -glutamyl p-nitroanilide, 40 mM of glycylglycine and 185 mM of Tris-HCl, pH 8.2. The rate of p-nitroaniline formation was measured at 405 nm by using spectrophotometer. Serum GGT activity was expressed as U/L. One Unit of enzyme represents the amount of enzyme that catalyzes the release of 1mmol of nitroaniline/min.

Determination of optimum temperature

The serum GGT was incubated at 56°C at different periods (5- 30 min) keeping all other variables constant for the thermal stability determination. For optimum temperature detection, the GGT activity was determined after incubation for 1 min and 10 min at different temperatures (25-56°C). The reaction mixture contained 4 mmol of γ -glutamyl p-nitroanilide per L and 40 mM of glycylglycine per L [9].

Determination of optimum pH

Wide range of pH(5-12) has been used to determine the highest serum GGT activity at optimum pH value using Tris-HCl buffer (pH 5-7) and phosphate buffer (pH 8-9) containing 4 mmol of γ -glutamyl p- nitroanilide per L and 40 mmol of glycylglycine per L[9].

Determination of C-reactive protein (CRP)

C-reactive protein levels were measured on a BioB (Roche Diagnostics, Mannheim, Germany) by means of turbidimetry. Normal serum level of CRP was 0–7 mg/dl.

Statistical analysis

The statistical analysis was performed using SPSS for Windows. Categorical data were presented as absolute values and percentages, whereas continuous data were summarized as mean values \pm SD. Chi-square and Fisher's exact tests were used for comparison of categorical variables, as appropriate. Comparison of continuous variables was performed by means of Student's t-test, as appropriate. $P < 0.05$ was considered statistically significant.

Results and Discussion

The activity of serum Gamma glutamyltransferase in Alzheimer's disease was estimated quantitatively and compared with that of control groups using developed calorimetric method .Data obtained revealed specific elevation of GGT activity(75.9 ± 2.15 IU/L)in case of Alzheimer's disease (AD)(Table1,Figure1) comparing with normal individuals($25. \pm 1.11$ IU/L). This increment may be a marker of oxidative stress in AD.

Table (1): Mean \pm SE of serum GGT levels in Alzheimer's disease (AD)and Control subjects , according to age and sex.

Groups	Age (years)	Sex	GGT mg/dL) Mean \pm SE	P value
Alzheimer's disease (AD)	30-40	Females	63.83 ± 1.01	0.001
		Males	62.72 ± 1.03	
		Both	63.5 ± 1.42	
	40-59	Females	76.4 ± 2.0	0.002
		Males	78 ± 1.91	
		Both	78 ± 2.02	
Control subjects	30-40	Females	24.1 ± 0.8	0.001
		Males	25.0 ± 0.15	
		Both	24.8 ± 0.1	
	40-59	Females	32.5 ± 1.022	0.001
		Males	41.7 ± 1.06	
		Both	38 ± 2.06	

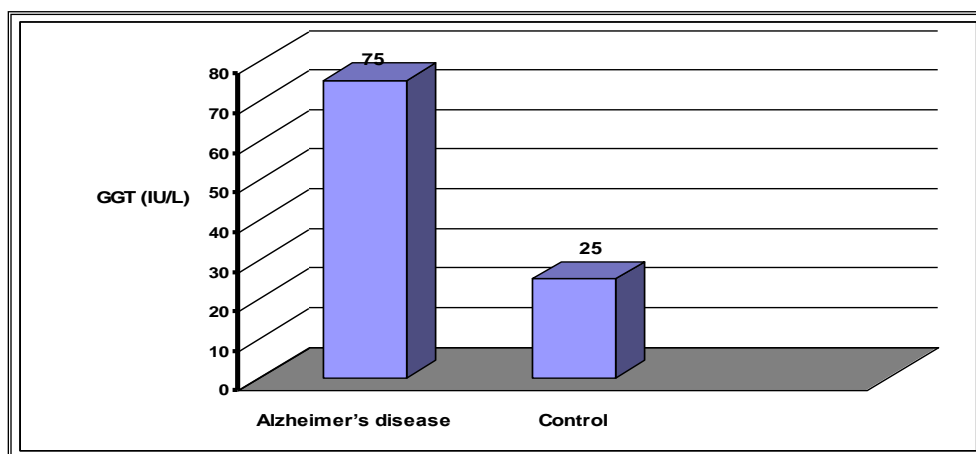


Figure (1): Serum gamma-Glutamyltransferase activity in Alzheimer's disease and control

Table 2 demonstrates results of the activity of serum Gamma glutamyltransferase (SGGT) in Alzheimer's disease (AD) and control groups. Data obtained revealed specific elevation of GGT activity in case of Alzheimer's disease (AD, comparing with normal individuals). This increment may be a marker of oxidative stress in AD [10]. Oxidative stress is believed to play an important role in neuronal dysfunction and ultimately cell death. AD has been hypothesized to be associated with oxidative stress. GGT has an important role in antioxidant defense systems. The primary function of GGT is to supply intracellular support for the precursors of GSH, which is an important antioxidant. Several studies demonstrated that an increase in serum GGT activity can be used as a marker for increased oxidative stress in humans. It has been shown that GGT activity is directly related to the oxidative events. Serum GGT is proposed as an early and sensitive marker for oxidative stress [11, 12,13]. It has an important role in maintaining intracellular glutathione transport into cells, thus mediating intracellular protective antioxidant mechanisms [14,15]. As it has a role in increasing glutathione transport into the cell, an increase in GGT levels was thought to be a response to oxidative stress [16]. Increased levels of oxidative stress have been demonstrated in the brain of AD patients [17] and markers for oxidative stress such as advanced glycation end products (AGE), glycoxidative end products and lipid peroxidation adduction products were detected in neurofibrillary tangles, amyloid plaques, and neurons of patients with AD [18,19]. As well as these cerebral changes, a systemic increase in oxidative stress markers may also occur in AD. A decrease in antioxidant levels and alterations in antioxidant enzyme activities are reported in AD [20, 21,22]. These changes suggest a systemic imbalance in oxidative defense mechanism in AD patients [23]. Markers of oxidative damage such as heme oxygenase-1 and 8-hydroxyguanosine were found to be increased in the AD brain [24]. Based on this evidence it can be argued that GGT may serve as a simple and feasible marker for oxidative stress in AD. Oxidative stress can also play a role in the pathophysiology of cardiovascular diseases. The vascular endothelium regulates the passage of macromolecules and circulating cells from blood to tissue. It is thus a major target of oxidative stress and plays a critical role in the pathophysiology of

several vascular diseases [25, 26]. Atherosclerosis may be a consequence of oxidative stress and inflammation [27], [28] identified Alanine transferase (ALT) and GGT as independent markers of cardiovascular risk associated with systemic inflammation and oxidative stress (Both cardiovascular factors and oxidative stress are the possible mechanisms in the pathogenesis of AD [29]) stated that GGT may contribute actively to atherothrombogenesis. Much epidemiological and clinical data suggest that cardiovascular risk factors are involved in the pathogenesis of AD.

Figures (2,3) show the effect of pH and temperature on serum GGT activity respectively. Highest activity of Serum gamma-Glutamyltransferase at pH 8.2 was found in both Alzheimer's disease and control serum. Control and Alzheimer's gamma-Glutamyltransferase enzyme has an optimum temperature as about 37°C.

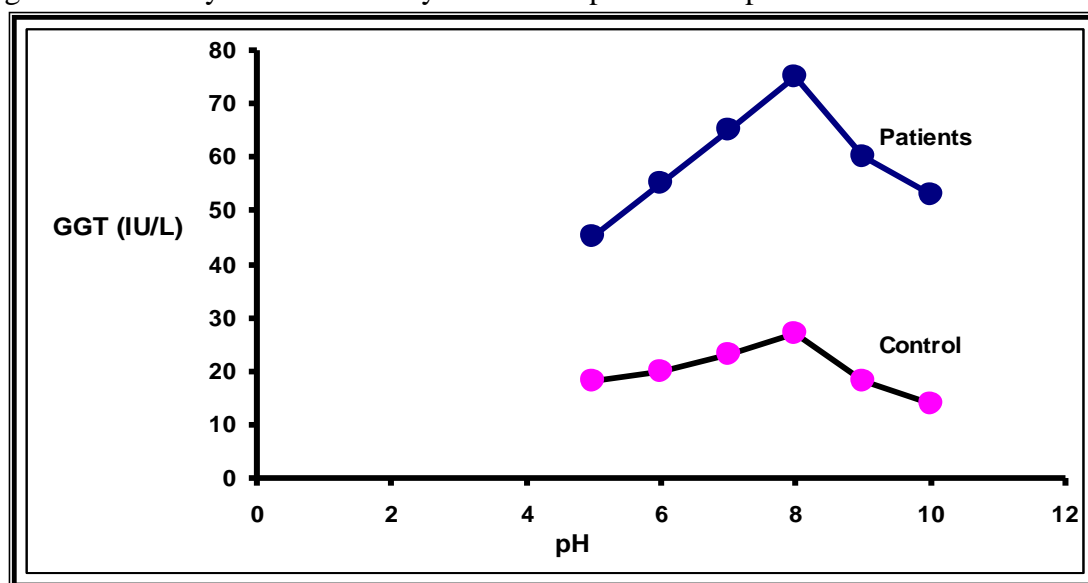


Figure (2): Effect of pH on serum GGT activity.

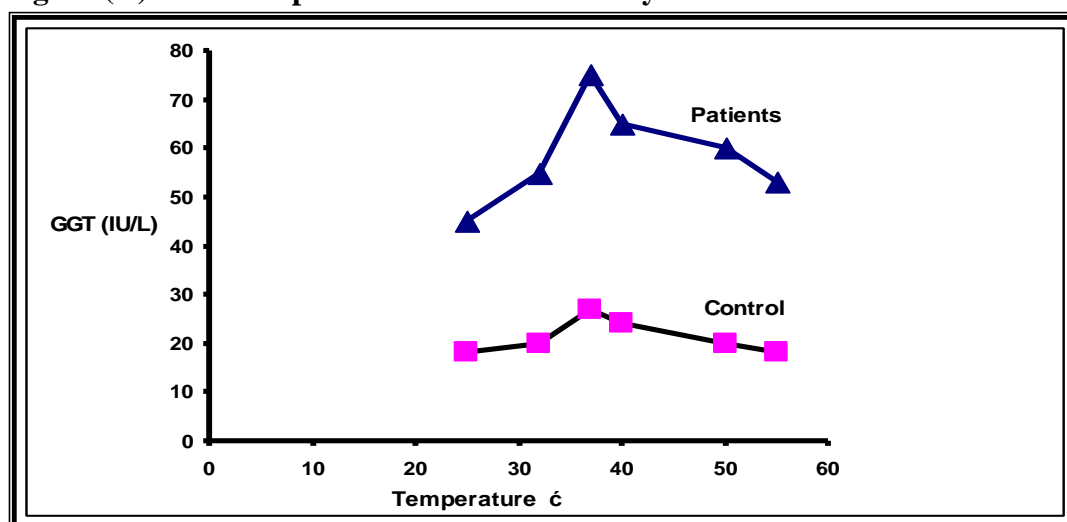


Figure (3): Effect of temperature on serum GGT activity

Highest activity of serum gamma-Glutamyltransferase at pH 8.2 was found in both Alzheimer's disease control serum figure(2). This can be explained to be due to alteration in the protein nature of the enzyme and exactly its active site, i.e. amino acid content and their ionic state. Hence alteration will occur in the enzyme free form or I

the enzyme-Glutamyl complex of the transition state [30]. However from fig.3 we note that above and below optimum temperature Alzheimer's gamma-Glutamyltransferase enzyme is more stable than control gamma-Glutamyltransferase enzyme. This may be due to the differences in the ratio of isoenzyme in the serum of Alzheimer's patients because isoenzymes have different stability to heat [30].

In the present study GGT levels rise with age in both sexes. Figures 4;5 showed the relationship between age and serum GGT activity in AD patients. The serum Gamma glutamyltransferase activity in the age group of 30-39 years was found significantly lower than those of the age group 40-49 ($p < 0.05$). The serum GGT activity was found significantly higher in the age group of 50-59 years than that of the age group of 40-49, 30-39 years ($p < 0.05$). The study indicated that as the age increased it is possible to get higher values of GGT. More studies are however needed to know the exact mechanism associated with this correlation.

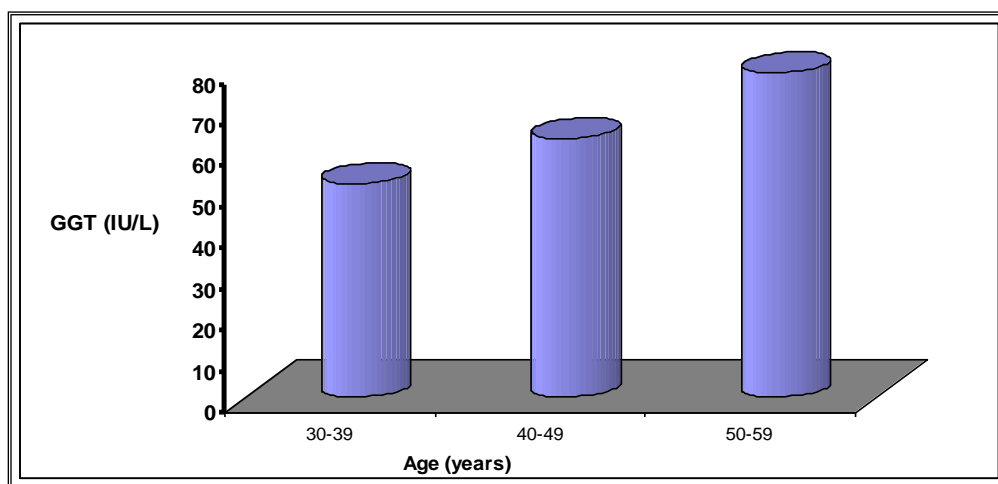


Figure (4): Serum GGT levels in male Alzheimer's patients according to age

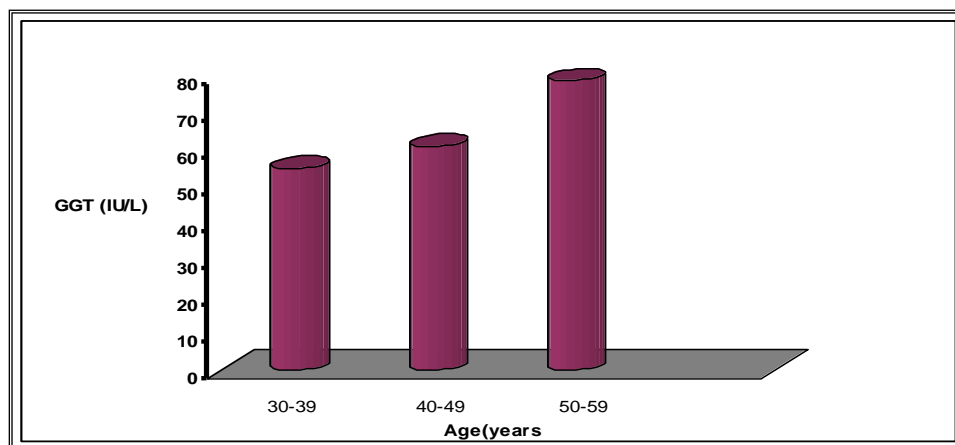


Figure (5): Serum GGT levels in female Alzheimer's patients according to age

Figures (6,7), showed the Correlation of serum GGT levels with age in females and males of AD patients. Men and women showed a significant ($P < 0.001$) increase in serum GGT levels with advancing age. There was a significant positive correlation between age and serum GGT levels in males ($r = 0.75$,

$P < 0.03$) and females ($r = 0.72$), ($P < 0.02$)

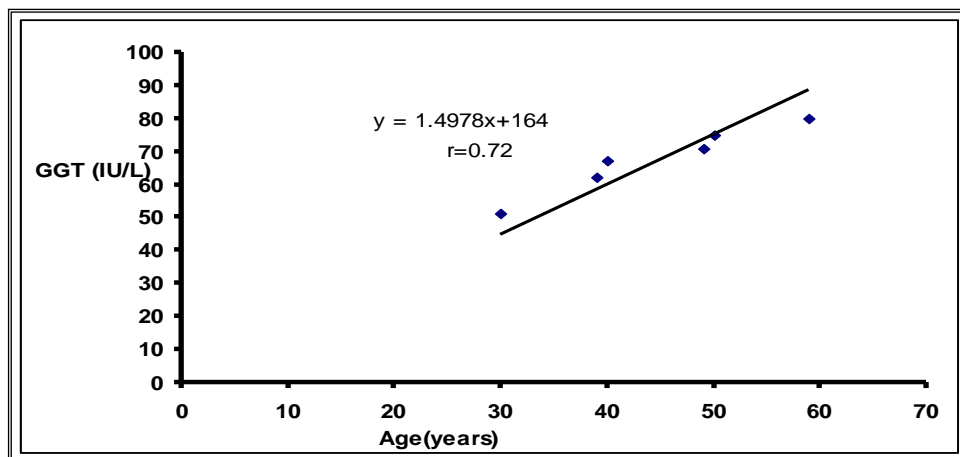


Figure (6): Correlation of serum GGT levels with age in female Alzheimer's patients

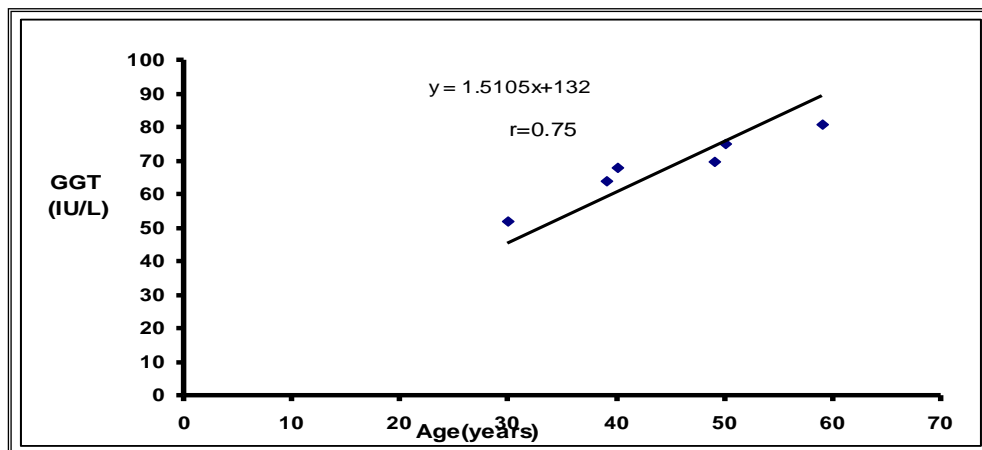


Figure (7): Correlation of serum GGT levels with age in male Alzheimer's patients

It is clear from the results that a significant increase ($p < 0.001$) of C - reactive protein (CRP) concentration in Alzheimer's disease (AD) (33.9 ± 2.3 mg/dL) compared with controls (4.8 ± 2), table2. The results of this study Confirm previous observation that there is high serum c-reactive protein (CRP) level in Alzheimer's disease [31,32]. Table (2) summarizes data on serum concentrations of C - reactive protein (CRP) levels in Alzheimer's disease (AD) and in the control.

Table (2): The mean \pm SE of serum levels of C - reactive protein (CRP) In Alzheimer's disease (AD) and normal subjects

Parameters	Alzheimer's disease (AD)	normal subjects
c-reactive protein (CRP) mg/dL	(33.9 \pm 2.3)	(4.8 \pm 0.2)

It is clear from the results that a significant increase ($p < 0.001$) of c-reactive protein (CRP) concentration in Alzheimer's disease (AD) (33.9 \pm 2.3 mg/dL) compared with controls (4.8 \pm 0.2mg/dL) figure (8)

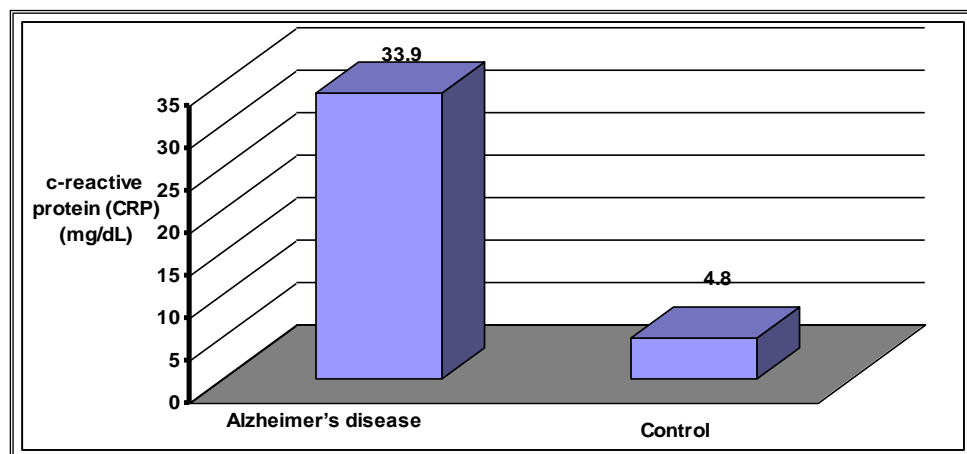


Figure (8): Serum C - reactive protein (CRP) levels in control and Alzheimer's disease (AD)

Fig.9 shows a significant positive correlation was found between serum GGT activity and serum CRP levels.

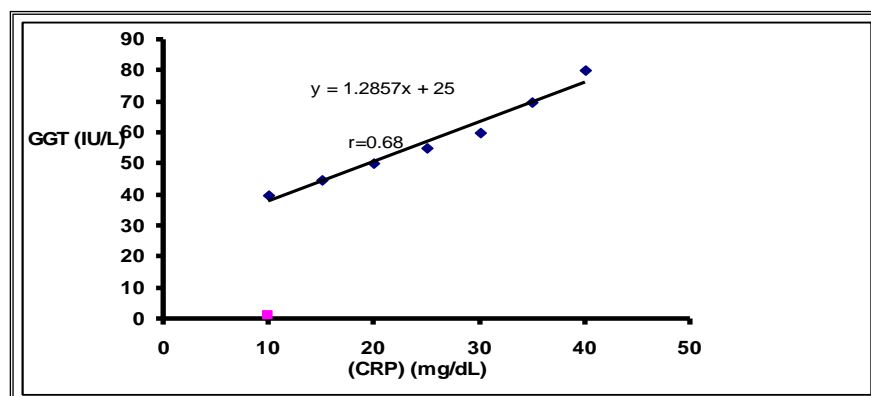


Figure (9): Correlation between serum GGT and CRP levels in Alzheimer's patients.

.CRP, an acute-phase reactant of hepatic origin and a sensitive marker for systemic inflammation, in our study, similar to GGT activity, CRP levels were significantly

different between patients and healthy individuals. In addition, a significant positive correlation was found between serum GGT activity and serum CRP levels. Previous studies have found associations between GGT and CRP or other inflammatory parameters, suggesting that this enzyme represents the expression of subclinical inflammation, and has a role in cellular Oxidative stress processes might have an implication in chronic inflammation, it has been hypothesized that elevation in GGT might occur before an elevation in CRP, and the related oxidative stress would give rise to a subsequent inflammatory response [33].

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