Original Article

Serum Gamma GlutamylTransferase, Amylase and Alkaline Phosphatase activities in kidney diseases

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Summary:

Background: The study enrolled a total of 78 patients with acute and chronic kidney diseases for evaluation of the activity of enzymes, gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) and amylase and compared with 42 normal healthy groups with normal albumin, creatinine and other kidney functions test and the samples were obtained from different hospitals in Baghdad.

Patients and methods: Three groups of patients were included. The first group consists of 15 patients with acute kidney diseases that occurs over days to weeks in which measurement of GGT, ALP and amylase were performed. Second group consists of 38 patients suffering from acute kidney diseases for 2-3 months in which serum GGT was evaluated .Third group consist of 25 patients with chronic kidney diseases used for estimation of GGT, ALP and amylase and other biochemical parameters, total cholesterol, urea, creatinine, uric acid and total protein.

Results: There was statistically highly significant difference in the activity of enzymes GGT, ALP and amylase $P< 0.001$ for three groups of patients with acute kidney diseases, and there was a remarkable difference in GGT activity with chronic kidney diseases ($P < 0.001$).

Conclusion: Acute and chronic kidney diseases in human was diagnosed by measurement of enzyme GGT, ALP, and amylase activities

Keyword: Chronic kidney disease, acute kidney disease, GGT, ALP, Amylase.

Introduction:

The number of patients with end-stage renal disease (ESRD) is increasing worldwide (1). Acute renal failure occurs suddenly and is usually initiated by underlying causes as dehydration, infection, serious injury to the kidney, or chronic use of medication, it is often reversible with no lasting damage. The early stage of chronic kidney disease (CKD) define by the presence of proteinuria or glomerular filtration rate (GFR) of less than 60ml/ min, or the presence of kidney damage regardless of the cause for three or more months (2). There is no single cause and the damage is usually irreversible and can lead to ill health. In some cases dialysis or transplantation may become necessary for kidney failure. Thus the identification of the precursors of CKD is very important (3). Gamma glutamyltransferase (GGT) (EC2.3.2.3) present on the surface of most cells and in serum, also in various body compartment and tissues of human body (4).GGT as a microsomal enzyme, plays a role in external secretary and absorptive events in the hepatobiliary system and pancreas (5).However, it has also been detected in the kidney, spleen, brain and seminal vesicles (6, 7). GGT activity is localized in the brush border of the proximal convoluted tubules of the kidney and also in the loop of henle. This pattern of distribution suggested the possibility that the enzyme may play a role in kidney function, as in the tubular reabsorption of amino acids (8).

GGT is involved with glutathione synthase in the gamma glutamyl cycle, which allows absorption of amino acid from glomerular filtrate and the intestinal lumen .It catalyzes the hydrolytic cleavage of peptide to form amino acid or smaller peptide or both (7, 8). It is involved in the glutathione metabolism by transferring the glutamyl moiety to a variety of acceptor molecule including water, certain L-amino acid and peptides a cross the cellular membrane (9). GGT is the enzyme responsible for the extracellular catabolism of glutathione (GSH-gamma glutamyl-cysteinyl-glycine), the main thiol Intracellular antioxidant agent (7, 9); it is present linked through a small lipophilic sequence of its larger subunit, on the cell surface membrane of the most cell type.

Amylase (EC3.2.1.1) is found in all types of organs and tissues, higher concentration may reflected one of the several medical condition, including acute inflammation of pancreas ,peptic ulcer and mumps (10).other causes of hyperamylasemia include intestinal obstruction and infarction ,afferent loop syndrome, peritonitis, cerebral trauma, burns and traumatic shock(11).However, elevation of ?-amylase activity in serum is also associated with abdominal disorders, diabetic ketoacidosis, sever glomerular dysfunction (12). Serum amylase is cleared by renal excretion and increase one to two times of upper limit of normal in renal failure without diagnostic significance(11,12).Alkaline phosphates (ALP) (EC3.1.3.1) are a group of relatively non specific enzyme, it is found in all tissues of the body. It is

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present in the sera of normal adult originate mainly in the liver (or biliary tract) and bone. Minor contribution also comes from intestine and rarely renal tissue, ALP is a protein found in cell membrane, it is needed in small amount to trigger specific chemical reaction .It occurs a particularly in highly level in intestinal epithelium, kidney tubules (13,14)in the proximal convoluted tubules and placenta. The level of ALP increasing in pain of upper abdominal area and acute tissue damage in the heart or lungs.ALP accumulates and eventually escapes into the blood stream. ALP from intestine is increased in a person with inflammatory bowel disease, such as ulcerative colitis (14). There is very little information on ALP, and amylase activity in kidney disease. So the goal of the present study aimed to investigate the elevation of GGT, ALP& amylase in acute and chronic kidney disease and to evaluate the correlation between these enzymes in kidney diseases.

Materials and Methods:

This study included three groups. The first group was of 15 patients comprised 10 males and 5 females with mean ±SD (46.2 ± 11.35) years with acute kidney diseases the primary diseases occurs over days to weeks and they were tested for three different enzymes activities, GGT, ALP & amylase and they were compared with normal groups derived from 15 healthy individuals with mean ±SD (48 ± 10.98) years including 7 males and 8 females. The second group consists of 38 patients with acute kidney diseases for 2-3 months occurs suddenly due to infection or serious injury to kidney, with mean (50 ± 13.1) years including 15 males and 23 females, this group of patients were tested only for the activity of GGT and compared with 14 normal healthy individual with mean ±SD (39.42 ± 9) years of 7 males and 7 females. Third group was consisted of 25 patients including 13 males and 12 females with mean ±SD (55 ± 13) years they were suffered from chronic kidney disease (CKD) with high level of creatinine, proteinuria and abnormal other kidney function tests. The blood samples were investigated for the activity of the three enzymes. GGT, ALP, amylase and other different biochemical parameters including urea, creatinine, uric acid, total cholesterol,and total protein,third patient group were compared with normal group including 13 healthy subjects with mean ±SD (41± 8.5) of 7 males and 8 females. All patient groups have been identified through appropriate history and physical examination and selected laboratory test and they were of abnormal kidney function tests and no any other diseases, normal liver And no alcohol intake. All control groups of healthy individuals were judged by clinical examination and were normal for testing of kidney function and have no proteinuria and normal creatinine. The activity of GGT was determined by kinetic methods using a special kit Biolabo SA Reagents, 021602, maziy France (15). Assay of ALP activity is estimated by kit (Biomerieux, 61511, France (16) Quantitative determination of amylase activity in human serum by amylase2-chloro-4-nitrophenyol malto trioside CNPG3 using kit (Biolabo SA, Reagents, 02160, maziy, France (17). Urea was measured by kit urea Berthelot CE, by urease \ salycliate enzymatic method. Creatinine measured by deproteinisation procedure by kit SYRBIO, diagnostic reagents for laboratories under license of EUROBIO laboratories Paris France. Uric acid measured by kit: CE uric acid, uricase-POD. Enzymatic method, Spinreact,S.A.Spain. Total cholesterol and total protein were measured in serum by enzymatic colorimetric method using kit cholesterol MR CE. Total protein CE, Ccromatet, liner chemical. Total protein was measured by biuret reaction.

Statistical analysis:

Statistical analysis selected were mean and standard deviation (Meam ± SD). Also unpaired student t test, coefficient of variation and correlation and regression (18).

Results:

Results are illustrated in table 1 to 4, a total of 78 patients of acute and chronic kidney disease were included in this study they were 38 male and 40 females and compared with 42 normal healthy individuals 21 males and 21 females with no proteinuria and normal kidney function tests.. Table (1) represents the serum enzyme activities of GGT, ALP and amylase in 15 patients of acute kidney disease with primary disease occurs over days to weeks. There were Very highly significant increases in the enzyme activities of patients in comparison with normal population group ($p<0.001$). Table 2 summarizes the data of 38 patients of acute kidney disease occurs for few months due to infection or injury to kidney and show very highly significant rising in serum GGT activity in comparison with normal population ($p<0.001$).Table 3 demonstrates the measurements of different biochemical parameters GGT, ALP Amylase, urea, creatinine, uric acid, total cholesterol and total protein in patients with chronic kidney disease. The results for all enzyme activities show very highly significant increase in comparison with normal individual $P<0.001$. A change in concentration of urea, creatinine, uric acid and total cholesterol were show very highly significant increase in comparison with normal $P< 0.001$, but there is very highly significant decrease in total protein concentration $P<0.001$ table 3. Table 4 A represent that there was statistically highly significant correlation between the activities of GGT, ALP and amylase $P<0.001$ in 15 patient of acute kidney disease. Table 4 B demonstrate that there was significant correlation between the activities of the enzymes GGT, ALP and amylase in normal population.

Table (1): Mean (±SD) values of serum enzymes activities of GGT, ALP and Amylase in patients with acute kidney diseases and in normal individuals group

Case	Mean ± SD			
	No. of Sample	GGT Activity (U/L)	ALP Activity (U/L)	Amylase Activity (U/L)
Normal	15	24.36± 3.11 CV %12.76	35.18±2.62 CV %7.44	65.88±2.14 CV %3.24
Acute kidney disease	15	115.73±3.36 CV%2.90 t=13.88 P<0.001	136.37±3.35 CV% 2.45 t =7.12 P<0.001	165.83±3.81 CV % 2.30 t = 11.11 P<0.001

Table (2): Mean (±SD) values of Serum enzyme GGT activity in patients with acute kidney diseases in comparison with normal individuals.

Case	Mean ± SD	
	No. of Sample	GGT Activity (U/L)
Normal	14	20.71 ± 5.91 CV % 28.53
Acute kidney disease	38	102.59 ± 19.71 CV% 19.21 t = 15.23 P<0.001

Table (3): Mean (±SD) values of serum biochemical parameters in patients with chronic kidney diseases and normal individuals.

parameter	GGT (U\L)	ALP (U\L)	Amylase (U\L)	Urea Mg/dl	Creatinine Mg/dl	Uric acid Mg/dl	Cholesterol Mg/dl	Total protein g/dl
Normal n=13	24.2 ±4.9 Cv=20.2	23.5±4.5 Cv=19.4	63.2± 19.8 Cv=31.32	23.96± 3.34 Cv=13.93	1.06±0.19 Cv=17.92	3.69±0.49 Cv=13.27	172.37±23.8 Cv=13.5	6.91±1.23 Cv=17.89
Chronic kidney Disease n=25	103.20±25.5 CV% = 24.7 t = 12.8 p<0.001	153±15.9 CV%= 10.39 t= 12.3 p<0.001	334.05±39.6 CV%=11.85 t= 23 P<0.001	100.54±31 CV%=30.83 t=20.75 P<0.001	68.4±12 CV%=17.54 t=17.15 P<0.001	9.09±2.12 CV%=23.32 t=14.63 P<0.001	370±59.2 CV%=167 t=25.76 P<0.001	3.1±0.39 CV%=12.58 t=7.94 P<0.001

Table (4 A): Correlation of GGT, ALP and Amylase in patients with acute kidney disease.

correlation	No. of sample	r-value	t-value	Degree of correlation
1.GGT& ALP	15	0.829	5.33	P<0.001
2.GGT& Amylase	15	0.346	10.32	P<0.001
3.ALP & Amylase	15	0.979	17.22	P<0.001

Table (4 B): Correlation of GGT, ALP and Amylase in normal population group.

correlation	No. of sample	r-value	t-value	Degree of correlation
1.GGT & ALP	15	0.726	3.66	P<0.01
2.GGT & Amylase	15	0.912	7.48	P<0.001
3.ALP & Amylase	15	0.233	5.16	P<0.001

Discussion:

Chronic kidney disease CKD is an increasingly common and important condition, it is more serious than acute kidney disease. Because symptoms may not appear until the kidney are extremely damaged. CKD can be caused by other long term diseases such as diabetes and high blood pressure (2, 3). The present result was performed on a patients suffering from different kidney disease consisting of both acute and chronic kidney disease. First group of patients with acute kidney disease were used for evaluation of serum enzyme activities, GGT, ALP & amylase a significant difference in their activities were shown in table 1.

The results were shown a significant difference in serum GGT activity in patients with acute kidney disease in comparison with normal population.Our result of GGT was in agreement with Ryu et al (19) to the best of our knowledge little research has been done to examine wether serum GGT and amylase are associated with prospective develop of kidney disease, while there is no information on ALP activity in kidney disease, therefor it is impossible for comparison of our result with other studies. The values for the activities of serum GGT, ALP and amylase in 15 patients with acute kidney disease were 4.75, 3.87, 2.5 times above the normal limit respectively.

The result of 38 patients of acute kidney disease for serum GGT activity was 4.9 times the upper normal range. Defining the contributing role of GGT in pathogenesis of chronic kidney disease will require more invesigations. This suggest that GGT may be a marker of the even a contributor, but in either case, GGT is a novel predicter of chronic and acute kidney disease that contribute to early intervention and improved outcome (20). GGT may also be released into circulation from the kidneys and prostate e.g. patients with renal infarct or prostate cancer (4-6). A marked increase of GGT is associated with cardiovascular and kidney disease(7,20).this is due to functional role of the enzyme which is involved in the transport of amino acid and peptide into cells of renal tissues as well as glutathione metabolism(21). In addition GGT is leaked into the serum, possibly as a result of normal cell turnover (8, 9, and 21).
The result in a group of 25 patients with chronic kidney disease of the present study was show a statistically marked rising in GGT, ALP and amylase activities. The activity was 4.26, 6.5, and 5.28 times above the normal limit for the GGT, ALP and amylase respectively.High serum GGT correlates with different types of renal diseases (20) because chronic kidney disease have a vascular component, the evidence that GGT predicts vascular disease including myocardial infarction (22).
GGT contribute to amino acid transport in the proximal renal tubule and on the tubule epithelial membrane and also transfer gamma glutamyl moiety into the glomerular filtrate to an acceptor amino acid (7, 8, 9, and 21). As concerns the possible association between GGT and inflammatory process, it should also be considered that GGT is the only enzyme responsible for glutathione catabolism by hydrolysis of gamma-glutamyl bond between glutamate and cysteine. The physiological function of GGT enzyme as a source of peptide precursors for intracellular (7, 23). The present result demonstrated that there was very highly significant increase in amylase activity in acute and chronic kidney diseases in comparison with normal population. The activity was 2.5, 5.28 times above the normal limit in case of acute and chronic kidney diseases respectively. Chronic kidney diseases may cause high level of amylase activity when the kidney is no longer able to remove amylase from the blood. Serum amylase used for medical diagnosis of acute inflammation of the pancreas, macroamylasemia, perforated peptic ulcer and mumps (12) but there is very little references on amylase with kidney diseases. Our result was in agreement with Bahar et al (24). increase serum amylase result from escape of enzyme into the interstitial tissue and Peritoneal cavity with increased absorption through the lymphatic vein (10, 24). Amylase is involved in anti-inflammatory reaction such as those caused by release of histamine and related substance Increased plasma amylase level, with normal to low urine amylase levels may indicate decreased kidney function or the presence of macroamylase, benign complex of amylase and other proteins that accumulate in the blood (12,24).The present study demonstrated that the measurements of serum ALP was abnormal in both acute and chronic kidney diseases and there was a significance increase in the activity this may be attributed to tissue damage of kidney tubule may cause a release of the enzyme into the blood depending on the severity of the renal failure.Also the abnormality of ALP may be attributed to congestive heart failure and bacterial infection .ALP enzyme accelerate and initiate chemical reaction essential for life and one of the hydrophilic enzyme which is responsible for breakdown of phosphate ester (25).

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