Relationship between liver function tests & cardiovascular risk factors in stage 3-5 pre-dialysis chronic kidney disease

Tamer Selen¹, Hadim Akoglu² & Kemal Agbaht³

¹Department of Nephrology, Health Sciences University Ankara Dışkapı Yıldırım Beyazıt Education & Research Hospital, ²Department of Nephrology, Health Sciences University Ankara Gülhane Education & Research Hospital, Ankara & ³Department of Endocrinology & Metabolic Diseases, Private Define Hospital, Hatay, Turkey

Received October 14, 2019

Background & objectives: Cardiovascular disease (CVD) remains the leading cause of mortality among patients with chronic kidney disease (CKD). Liver function tests (LFTs) have emerged as markers of CVD risk in some population-based studies. Hence, in the present study the relation between LFTs and biochemical cardiovascular risk factors (CRFs) were evaluated in CKD patients.

Methods: A total of 246 patients with stage 3-5 pre-dialysis CKD were enrolled. Demographics, LFTs [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT)] and biochemical CRFs were recorded retrospectively. Glomerular filtration rate (GFR) was calculated using CKD-EPI equation.

Results: ALT was positively correlated with GFR, albumin, triglyceride and 25-hydroxyvitamin D and negatively correlated with CRP and intact parathyroid hormone (iPTH); AST was positively correlated with GFR, albumin, high-density lipoprotein cholesterol (HDL-C) and 25-hydroxyvitamin D and negatively correlated with CRP and iPTH; GGT was positively correlated with GFR, CRP and triglyceride and negatively correlated with HDL-C. In diabetic patients, ALT correlated positively with GFR; AST correlated positively with GFR and HDL-C, but correlated negatively with iPTH. In the correlation analysis between GFR and CRF, GFR was positively correlated with albumin, triglyceride and 25-hydroxyvitamin D and negatively correlated with CRP, iPTH and albuminuria in both total study population and diabetic group. A partial correlation analysis revealed no correlation between LFTs and CRFs after being controlled for GFR.

Interpretation & conclusions: The results of the present study suggest that the relationship between LFTs and biochemical CRFs seems to be a function of impaired GFR.

Key words Alanine aminotransferase - aspartate aminotransferase - cardiovascular disease - gamma-glutamyltransferase - kidney disease - risk factors

Chronic kidney disease (CKD) is characterized by a progressive and an irreversible deterioration of the renal function which is associated with increased morbidity as well as mortality particularly from cardiovascular disease (CVD)¹. CVD events increase rapidly through CKD stage 3 by 43 per cent and stage 5
by 343 per cent. Although the mechanism underlying the increased CVD risk is not well established, the presence of multiple classical and uraemia-related risk factors in CKD patients leads to premature cardiovascular mortality. A number of uraemia-related biomarkers are identified as predictors of cardiac outcomes, however, there is limited utility of these diagnostic modalities in patients with CKD.

Over the past decade, liver function tests (LFTs) including gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) have emerged as markers of CVD risk in both population-based studies and patients with coronary artery disease independent of their relationship with metabolic syndrome or non-alcoholic steatohepatitis (NASH). The association of LFTs with CVD risk, irrespective of metabolic syndrome or NASH, has been linked to various mechanisms including direct or indirect contribution of GGT activity within atherosclerotic lesions, vascular calcification, pro-inflammatory activities, endothelial dysfunction, oxidative stress and impaired haemostasis. Although LFTs have been proposed as independent risk markers for all-cause and cardiovascular death in various patient populations, the association between LFTs and CVD risk has not been extensively evaluated in patients with CKD. In the present study, the relationship between LFTs and biochemical cardiovascular risk factors (CRFs) were evaluated in stage 3-5 pre-dialysis CKD patients.

**Material & Methods**

Adult patients with stage 3 to 5 CKD without dialysis treatment presenting to outpatient nephrology clinic for follow up care between January 2014 and December 2017 were retrospectively considered for the study at Dişkapı Yıldırım Beyazıt Education and Research Hospital, Ankara, Turkey. Patients with active viral hepatitis B or C diagnosed by serological tests including HBsAg, anti-HBs, IgM/IgG anti-HBc, anti-HCV, HCV RNA (n=1), NASH (n=5), autoimmune hepatitis (n=1), chronic liver disease (n=4), cirrhosis (n=2), patients with acute/chronic inflammatory/autoimmune diseases (n=8), patients with malign disease (n=2) or history of malignancy (n=2) and CKD patients due to kidney transplant dysfunction (n=9) were excluded. The diagnosis of the liver diseases was based on medical history and biochemical and radiological findings without a liver biopsy. Thus, a total of 246 patients were enrolled for the study. The study protocol was approved by the local Ethics Committee in Ankara, Turkey.

The demographic information of all patients was collected from patients’ charts. LFTs (ALT, AST and GGT) and biochemical inflammatory and cardiovascular risk parameters were also recorded. All demographic and biochemical parameters were entered into a database for further analysis. Glomerular filtration rate (GFR) was estimated using the CKD-EPI equation (eGFR). Patients were assigned to eGFR stages as follows: stage 3 (eGFR 30-59 ml/min per 1.73 m²), stage 4 (eGFR 15-29 ml/min per 1.73 m²) and stage 5 (eGFR <15 ml/min per 1.73 m²).

**Statistical analysis:** Kolmogorov–Smirnov test was employed to check for the distribution of variables. Continuous variables with normally distributed data were presented as mean ± standard deviation. Unequal distributed variables were expressed as medians (interquartile ranges: Q1-Q3). Categorical variables were expressed as numbers and percentages. Spearman’s correlation coefficient was used to evaluate the relationship between variables since some variables were unequally distributed. A partial correlation analysis was used to remove the effect of GFR on the association between variables. Significance was considered at a two-tailed value of \( P < 0.05 \). SPSS software (Statistical Package for the Social Sciences, version 20.0; SPSS Inc., Chicago, IL, USA) was used for carrying out all the statistical analysis in this study.

**Results & Discussion**

A total of 246 patients were included in the study. Baseline clinical and biochemical characteristics are shown in Table I. A correlation analysis was performed to explore the association between LFTs and biochemical CRFs. ALT was positively correlated with GFR, serum albumin, triglyceride and 25(OH)D. AST was positively correlated with GFR, serum albumin, high-density lipoprotein cholesterol (HDL-C) and 25(OH)D. Both ALT and AST correlated negatively with CRP and intact parathyroid hormone (iPTH). GGT was positively correlated with GFR, CRP and triglyceride and negatively correlated with HDL-C. When the analysis was performed on diabetic patients only, ALT was positively correlated with GFR; AST was positively correlated with GFR and HDL-C and negatively correlated with iPTH; GGT had no correlation (Table II). Since ALT, AST and GGT were all positively correlated with GFR in the entire study population, we further analyzed to see
whether biochemical cardiovascular risk parameters which correlated with LFTs, were also affected by GFR. In the correlation analysis, GFR was positively correlated with serum albumin, triglyceride and 25(OH)D and negatively correlated with CRP, iPTH and albuminuria in both the overall study population and in the diabetic group. In partial correlation analysis performed to remove the effect of GFR on parameters tested, no significant correlation was found between LFTs and cardiovascular risk parameters. Likewise, no significant correlation existed between liver functions and CRFs after controlled for body mass index (BMI), GFR and presence of diabetes (Table III).

Serum aminotransferase levels have been previously found to be close to the lower limit of the normal range in patients with chronic dialysis\(^8\). Although the reduction in aminotransferase values in pre-dialysis CKD patients was reported in small patient groups, only AST level was analyzed\(^9\). The findings of the present study confirm that both ALT and AST levels are depressed in pre-dialysis CKD patients. High levels of uraemic toxins leading to suppression of aminotransferase synthesis and release from the hepatocyte into the bloodstream have been suggested to have a role in low serum aminotransferase levels\(^8,9\). Besides being an independent predictive marker of CVD in general population, GGT has also been suggested as a strong, independent risk factor for all-cause and cardiovascular death in haemodialysis patients\(^10\). However, only two studies have evaluated the association of GGT levels with CVD risk in pre-dialysis CKD patients. The first study retrospectively found an independent association between GGT and mortality. GFR values were not different between patients with high or normal serum GGT levels\(^11\). The second study demonstrated a significant association between circulating GGT and endothelial dysfunction in CKD stage 3-5 patients. Serum GGT levels were found to be inversely related to GFR\(^12\). Contrary to this, in our study, GGT levels decreased in parallel with the severity of CKD. This inconsistency may be due to the fact that those studies included CKD patients with a narrow range of GFR and the proportion of CKD patients did not adequately represent the actual distribution of patients due to CKD stages. In the present study, distribution of patients by stage of CKD was more appropriate than previous reports which allows for generalization of the findings across stage 3-5 pre-dialysis CKD population.

In the present study, all biochemical CRFs in correlation with liver enzymes also correlated with

| Table I. Clinical and biochemical characteristics of the patients* |
|------------------|------------------|
| Parameters       | Patient population (n=246) |
| Clinical characteristics |
| Age (yr)         | 65.3±13.5         |
| Male, n (%)      | 111 (45.1)        |
| BMI (kg/m²)      | 26.2±1.9          |
| DM, n (%)        | 72 (29.3)         |
| Hypertension, n (%) | 180 (73.2)      |
| CKD stage, n (%) |
| Stage III        | 162 (65.9)        |
| Stage IV         | 60 (24.4)         |
| Stage V          | 24 (9.7)          |
| Biochemical characteristics |
| Haemoglobin (g/dl) | 12.5±2.0        |
| Leucocyte count (×1000/mm³) | 7.80±2.20 |
| Serum creatinine (mg/dl) | 1.63 (1.35-2.37) |
| eGFR (ml/dk/1.73 m²) | 37 (24-46)        |
| AST (U/l)        | 18 (15-23)        |
| ALT (U/l)        | 15 (11-21)        |
| GGT (U/l)        | 23 (17-32)        |
| ALP (U/l)        | 91 (74-114)       |
| Serum total calcium (mg/dl) | 9.2±0.7 |
| Serum phosphorus (mg/dl) | 3.7±0.8 |
| Ca×P (mg/dl)²   | 32.9 (29.2-37.9) |
| iPTH (ng/l)      | 100 (55-191)      |
| 25-OH vit D (ng/ml) | 13.3 (8.8-23.1) |
| Serum albumin (g/dl) | 4.10±0.45       |
| Serum uric acid (mg/dl) | 7.3±1.7 |
| Serum ferritin (ng/ml) | 52 (26-83) |
| Triglycerides (mg/dl) | 150 (103.8-219.5) |
| HDL-C (mg/dl)    | 44 (37-52)        |
| LDL-C (mg/dl)    | 139 (110-168)     |
| Total cholesterol (mg/dl) | 198 (166-240) |
| CRP (mg/dl)      | 5 (3-10)          |
| Albuminuria (mg/day) | 273 (38-1206)    |

*Continuous variables are reported as mean±SD for normally distributed data or median (IQR) for asymmetrically distributed data. Categorical variables are reported as n (%). IQR, interquartile range; BMI, body mass index; CKD, chronic kidney disease; CRP, C-reactive protein; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine transaminase; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; Ca, calcium; P, phosphorous; iPTH, intact parathyroid hormone; HDL-C, high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SD, standard deviation
## Table II. Spearman correlation analysis between liver function tests and biochemical cardiovascular disease risk factors in both the overall study population and in diabetics as a separate subgroup

| Parameters          | Overall study population (n=246) | Diabetics (n=72) | Overall study population (n=246) | Diabetics (n=72) | Overall study population (n=246) | Diabetics (n=72) |
|---------------------|----------------------------------|------------------|----------------------------------|------------------|----------------------------------|------------------|
|                     | ALT                              | AST              | GGT                              |                  | ALT                              | AST              |
|                     | $r_s$  | $P$  | $r_s$  | $P$  | $r_s$  | $P$  | $r_s$  | $P$  | $r_s$  | $P$  |
| GFR                 | 0.307  | <0.001** | 0.324  | 0.006** | 0.309  | <0.001** | 0.296  | 0.013** | 0.169  | 0.021** |
| Serum creatinine    | −0.191 | 0.003** | −0.180 | 0.130 | −0.285 | <0.001** | −0.182 | 0.132 | −0.116 | 0.113 |
| CRP                 | −0.209 | 0.004** | −0.240 | 0.075 | −0.171 | 0.021** | −0.240 | 0.078 | 0.232  | 0.005** |
| Serum albumin       | 0.214  | 0.001** | 0.194  | 0.104 | 0.228  | <0.001** | 0.180  | 0.139 | 0.001  | 0.994 |
| Total cholesterol   | 0.048  | 0.478 | −0.109 | 0.387 | 0.123  | 0.072 | 0.107  | 0.398 | −0.107 | 0.161 |
| Triglycerides       | 0.177  | 0.006** | 0.031  | 0.798 | 0.001  | 0.987 | −0.086 | 0.484 | 0.162  | 0.027** |
| HDL-C               | −0.024 | 0.716 | −0.040 | 0.754 | 0.218  | 0.001** | 0.331  | 0.008** | −0.237 | 0.002** |
| LDL-C               | 0.049  | 0.449 | −0.098 | 0.412 | 0.101  | 0.125 | 0.106  | 0.383 | −0.050 | 0.502 |
| iPTH                | −0.234 | <0.001** | −0.103 | 0.404 | −0.258 | <0.001** | −0.252 | 0.041** | −0.134 | 0.075 |
| Ca×P                | −0.039 | 0.559 | −0.189 | 0.314 | −0.039 | 0.565 | 0.211  | 0.089 | 0.015  | 0.846 |
| Serum uric acid     | −0.050 | 0.443 | 0.025  | 0.838 | −0.031 | 0.642 | 0.030  | 0.810 | 0.103  | 0.164 |
| 25-OH vit D         | 0.177  | 0.011** | 0.020  | 0.873 | 0.245  | <0.001** | 0.128  | 0.321 | 0.010  | 0.898 |
| Albuminuria         | −0.044 | 0.549 | 0.036  | 0.784 | −0.146 | 0.051 | −0.104 | 0.442 | 0.039  | 0.637 |
| BMI                 | 0.049  | 0.458 | −0.016 | 0.902 | 0.069  | 0.309 | −0.031 | 0.807 | −0.111 | 0.140 |

"Correlation is significant at the 0.05 level (two-tailed)
GFR. This finding is not surprising since traditional and non-traditional risk factors such as inflammation, malnutrition, endothelial dysfunction, oxidative stress, vascular calcifications, vitamin D deficiency and abnormal calcium-phosphate metabolism deteriorate with progressive decline in renal function. Albuminuria has also been found to be associated with the progression of kidney disease. After negating the effect of GFR, liver enzymes were no longer found to be related to biochemical CRF. This finding suggests that LFTs may not reflect the actual CVD risk in pre-dialysis CKD patients. Rather, the potential link between LFTs and biochemical CRFs may be secondary to the effect of decreased GFR on both circulating markers of CRF and LFTs.

Diabetes mellitus, BMI and NASH (non-alcoholic steatohepatitis) have been implicated with increased serum levels of ALT and GGT, are also related to CVD risk in the general population. This correlation may be extended to the findings of the present study. CKD patients with NASH in this study may have more elevated levels of liver enzymes than CKD patients without NASH, and this difference could have potentially influenced our analysis. Keeping this in mind, in order to reduce this effect, patients with NASH were excluded. Furthermore, all correlation analyses were repeated for our diabetic subjects separately, and the results were similar to those obtained for the entire study population. Interestingly, there was no association between BMI and liver enzymes in our study population. Although this finding is seemingly unexpected, we believe that this point is the novelty of this study. That is, diabetes, NASH, BMI and CVD risk factors are traditionally well established to associate with each other in the general population. However, with a progressive decrease in kidney function, such associations become indistinct, and the kidney function takes an active role in those associations.

Although the present study included a relatively small number of patients for analysis, the distribution of the patient population was representative for CKD stages. In addition, since stage 3-5 CKD patients typically had a substantially higher risk for CVD than patients with stage 1-2 CKD, and patients on haemodialysis are affected by many other factors for CVD risk which arise from haemodialysis treatment itself, in the present study, patients with stage 3-5 pre-dialysis CKD represent a relatively homogeneous CKD population for potentially increased CVD risk factors. Therefore, our findings, although, obtained from a relatively small number of patients, provide valuable information and may be generalized to stage 3-5 pre-dialysis CKD population and even though subgroup analysis could not be performed in each CKD stage. Since the retrospective cross-sectional design of our study was not amenable to observe the relation between CRFs, LFTs and GFR in a longitudinal manner, prospective studies with larger sample sizes are needed to confirm our findings.

**Financial support & sponsorship:** None.

**Conflicts of Interest:** None.

**References**

1. Go AS, Chertow GM, Fan D, Mcculloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; 351 : 1296-305.

2. Mccullough PA, Li S, Jurkovitz CT, Stevens L, Collins AJ, Chen SC, et al. Chronic kidney disease, prevalence of premature cardiovascular disease, and relationship to short-term mortality. *Am Heart J* 2008; 156 : 277-83.
3. Anavekar NS, Pfeffer MA. Cardiovascular risk in chronic kidney disease. *Kidney Int* 2004; 66: 11-5.

4. Franzini M, Corti A, Martinelli B, Del Corso A, Emdin M, Parenti GF, et al. Gamma-glutamyltransferase activity in human atherosclerotic plaques – Biochemical similarities with the circulating enzyme. *Atherosclerosis* 2009; 202: 119-27.

5. Kunutsor SK, Apekey TA, Khan H. Liver enzymes and risk of cardiovascular disease in the general population: A meta-analysis of prospective cohort studies. *Atherosclerosis* 2014; 236: 7-17.

6. Liu Z, Ning H, Que S, Wang L, Qin X, Peng T. Complex association between alanine aminotransferase activity and mortality in general population: A systematic review and meta-analysis of prospective studies. *PLoS One* 2014; 9: e91410.

7. Yamada J, Tomiyama H, Yambe M, Koji Y, Motobe K, Shiina K, et al. Elevated serum levels of alanine aminotransferase and gamma glutamyltransferase are markers of inflammation and oxidative stress independent of the metabolic syndrome. *Atherosclerosis* 2006; 189: 198-205.

8. Guh JY, Lai YH, Yang CY, Chen SC, Chuang WL, Hsu TC, et al. Impact of decreased serum transaminase levels on the evaluation of viral hepatitis in hemodialysis patients. *Nephron* 1995; 69: 459-65.

9. Fabrizi F, Lunghi G, Finazzi S, Colucci P, Pagano A, Ponticelli C, et al. Decreased serum aminotransferase activity in patients with chronic renal failure: Impact on the detection of viral hepatitis. *Am J Kidney Dis* 2001; 38: 1009-15.

10. Postorino M, Marino C, Tripepi G, Zoccali C. Gamma-glutamyltransferase in ESRD as a predictor of all-cause and cardiovascular mortality: Another facet of oxidative stress burden. *Kidney Int Suppl* 2008; 111: S64-6.

11. Caravaca-Fontán F, Azevedo L, Bayo MÁ, Gonzales-Candia B, Luna E, Caravaca F. High levels of both serum gamma-glutamyl transferase and alkaline phosphatase are independent predictors of mortality in patients with stage 4-5 chronic kidney disease. *Nefrologia* 2017; 37: 267-75.

12. Yilmaz MI, Turgut F, Kanbay M, Saglam M, Sonmez A, Yaman H, et al. Serum gamma-glutamyltransferase levels are inversely related to endothelial function in chronic kidney disease. *Int Urol Nephrol* 2013; 45: 1071-8.

13. Tonelli M, Pfeffer MA. Kidney disease and cardiovascular risk. *Annu Rev Med* 2007; 58: 123-39.

14. Levey AS, De Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, et al. The definition, classification, and prognosis of chronic kidney disease: A KDIGO controversies conference report. *Kidney Int* 2011; 80: 17-28.

15. Choudhary NS, Duseja A. Screening of cardiovascular disease in nonalcoholic fatty liver disease: Whom and how? *J Clin Exp Hepatol* 2019; 9: 506-14.

For correspondence: Dr Tamer Selen, Department of Nephrology, Health Sciences University, Ankara Dışkapı Yıldırım Beyazıt Education & Research Hospital, Ziraat Mah, Şehit Ömer Halisdemir Cad. No: 20 Dışkapı, Ankara 06110, Turkey
e-mail: tamer_selen_@hotmail.com