Biochemical Parameters as Predictors of Underlying Liver Disease in Patients with Chronic Kidney Disorders

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is an increasingly common cause of chronic liver disease and is becoming a major public health problem. NAFLD has been recognized as a hepatic manifestation of metabolic syndrome, associated with systemic diseases such as cardiovascular disease (CVD) and chronic kidney disease (CKD). Objective: The aim of this study was to examine the role of serum LFT parameters and renal function parameters as predictors of unmanifested liver disease. Methods: In this study, the presence of possible liver disease detected by biochemical parameters and confirmed by Transient Liver Elastography (TE) in a group of patients with different stages of chronic kidney disease (CKD) was investigated. Patients with various stages of CKD were divided into five subgroups regarding etiology: nephroangiosclerosis, diabetic nephropathy, glomerulonephritis and pyelonephritis, autoimmune kidney disease, and polycystic and another morphological kidney disease. Liver stiffness was used to quantify liver fibrosis while Controlled attenuation parameter (CAP) was used to quantify liver steatosis. Functional liver tests and biochemical parameters of kidney function were measured in all patients. Results: Statistical analysis used in this study was a decision tree as a predictive model to map observed variables resulting in the conclusion about outcomes. The application of existing laboratory parameters, in combination with other parameters in presence of the defined etiological factors of kidney diseases, indicate development of hepatic diseases. Higher values of phosphorus and low values of ferritin in patients with autoimmune kidney disease, and polycystic and another morphological kidney disease, expresses steatosis of the hepatic parenchyma. Conclusion: In contrary, low values of phosphorus and higher values of ferritin in patients with nephroangiosclerosis, diabetic nephropathy, glomerulonephritis and pyelonephritis, are in a favour steatosis of the hepatic parenchyma. Serum values of phosphorus and ferritin are valuable predictors of the liver disease in patients with end-stage kidney diseases of different etiology. Keywords: Chronic Kidney Disorders, Liver disease, Biochemical parameters.

1. BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a cause of a liver disease, which is significantly increasing, with a tendency to become the main public health problem and the most common cause for the liver transplantation (1). NAFLD is actually a liver manifestation of the metabolic syndrome associated with insulin resistance. In addition, NAFLD is associated with diseases of other organ systems such as cardiovascular disease, chronic kidney disease, type 2 diabetes mellitus, and obesity and certainly as the basis of these conditions—metabolic syndrome. The most common cause of NAFLD death is cardiovascular disease, where mortality is greater in patients with advanced liver disease, NASH (non-alcoholic steatohepatitis) compared with only evident liver steatosis. Therefore, NAFLD can be considered not only as a liver metabolic syndrome, but also a disease mediator in various organ systems that are also affected by the metabolic syndrome (2). NAFLD is considered as an independent risk factor for development of cardiovascular disease, and there is an increasing number of recent ev-
idence linking NAFLD and the onset and progression of chronic kidney disease (3).

In several recently published studies, it was clearly specified that metabolic syndrome and insulin resistance were associated with an increased incidence of microalbuminuria and chronic renal disease (4-9). The same panel of cardiometabolic risk factors including metabolic syndrome and its individual components links chronic kidney disease and NAFLD. The relationship between NAFLD, insulin resistance and metabolic syndrome suggests that NAFLD can predict the development and progression of chronic kidney disease. Taking into account the heterogeneity of the population groups analysed, as well as the differences in NAFLD diagnostic modalities, the reported incidence and prevalence of chronic kidney diseases are significantly different in the available studies. However, a recently published study on a large number of subjects showed that the prevalence of chronic kidney disease is significantly higher in NAFLD patients (10). In addition, according to recent meta-analysis results, NAFLD is associated with increased incidence and prevalence of chronic kidney disease, with NASH compared to liver steatosis showing a significant increase in the incidence of chronic kidney disease, independent of associated factors such as age, smoking, thickness, hypertension, and components of the metabolic syndrome (11).

While there are numerous retrospective studies, there are still very few prospective data linking NAFLD and chronic kidney disease. Covert mechanisms associated with inflammation, oxidative stress, and fibrogenesis are in the research phase due to clarification of the development of renal disease in the presence of fatty liver disease (12). The use of serum NAFLD tests has been studied in multiple ways that would be ideal for diagnosis, monitoring, progression, therapeutic response and predicting disease prognosis. Mildly elevated aminotransferase values are primarily observed in NAFLD patients, while at the same time LFTs (liver function tests) can be normal in as much as 78% of cases with NAFLD (13). According to the results of the same study, functional liver tests correlate with liver steatosis and fibrosis confirmed with Transient Elastography (TE) only in the group of patients with coronary arterial disease (CAD), and therefore there are no adequate markers for detection of NAFLD in the group of patients with renal disease (13).

Other serum markers that could serve in prediction of liver disease in patients on haemodialysis treatment for end-stage kidney disease have not been investigated. In this study, in addition to parameters of impaired liver function, the association of abnormal iron metabolism (ferritin) and phosphorus in the serum of the subjects was observed. The inability of the kidneys to excrete phosphorus from the body leads to its increased serum concentration, which consequently results in the deposition of phosphorus and calcium in the blood vessel wall and parenchymal organs. At the same time, patients with end-stage kidney disease have significant anaemia with low values of serum iron and ferritin. Exceptions are patients who receive blood transfusions for correcting anaemia, in which ferritin values are elevated. Ferritin has also been previously confirmed as a serum marker of deposited iron in the liver parenchyma that exhibits a significant fibrogenic effect.

In the available literature we did not find mutual relationship of these serum parameters, as well as their influence on changes in the liver parenchyma detected by TE parameters.

2. OBJECTIVE

The aim of this study was to determine the presence of NAFLD, detected by the Functional Liver Tests (LFTs) and Transient Elastography (TE) in the group of patients with end-stage renal disease on haemodialysis treatment, also to examine the role of serum LFT parameters and renal function parameters as predictors of unmanifested liver disease.

3. MATERIAL AND METHODS

Patients

In this study, data of all patients were obtained from Institute for Gastroenteropathology, University Hospital Sarajevo, Bosnia and Herzegovina. A group of 80 patients with end-stage kidney diseases, both sexes, were divided into groups according to kidney diseases aetiology: nephroangiosclerosis, diabetic nephropathy, glomerulonephritis and pyelonephritis, autoimmune kidney disease, polycystic kidney disease. Depending on the length of dialysis treatment, patients were divided into five groups according to the shortest interval of 12 months and the longest interval of 190 months.

This study was approved by Ethic committee, Clinical Centre University of Sarajevo (KCUS), Bosnia and Herzegovina, and Inform Consent Forms (ICFs) were signed by the patients.

Biochemical parameters of metabolic syndrome and methods

All patients were evaluated for functional liver tests and laboratory parameters of the metabolic syndrome (triglyceride cholesterol values, BMI, glycaemic values) and laboratory parameters of renal function (phosphorus) and parameters of anaemia (Fe, ferritin). In addition, co-morbidities from the metabolic syndrome domain were detected as well as therapies that patients have taken to regulate these disorders (statins, insulin, oral antidiabetic, diuretic, calcium channel blocker, beta-blocker, ACE inhibitor, angiotensin II receptor blocker). Body Mass Index (BMI) is calculated by dividing weight in kilograms by height in m². The waist circumference was measured at the umbilical level.

Lever stiffness was used in the assessment of liver fibrosis and the controlled attenuation parameter (CAP) was used in the detection and quantification of liver steatosis using transient elastography, TE (Fibroscan, Echosense, Paris, France). By implementing CAP in TE, it is possible simultaneously to evaluate steatosis and fibrosis. According to the evaluation parameters, NAFLD is defined as the presence of steatosis at a value of CAP ≥ 215 dB/m, regardless of the presence or absence of any stage of fibrosis. The presence of fibrosis was expressed with
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Serum liver enzymes (aspartate aminotransferase AST, alanine aminotransferase ALT, alkaline phosphatase AP, and gamma-glutamyl transferase GGT) were determined by the spectrophotometry method (Architect ci8200). Biochemical serum parameters: haemoglobin-Hgb, iron, ferritin, C reactive protein-CRP, calcium, phosphorus, parathyroid hormone-PTH were determined by standard laboratory procedures (DAX 96; Bayer Diagnostic).

Serum creatinine level was measured using Jaffe reaction (using alkaline picrate). Normal values for aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl-transferase were 10-35 U/L for women and 10-50 U/L for men. All patients had a negative serology on viral hepatitis B and C. Insulin resistance was assessed based on the HOMA-IR score.

**Statistical analysis**

The presence of underlying liver diseases within different etiological kidney diseases, detected by CAP and liver stiffness, in regard to activity of serum aminotransferases, ALT and AST were assessed. Average value for the group was tested against the average value of all data and estimated by expected value of numerical variable. This data was analysed by 2-tails, paired T test and was performed in Excel, Microsoft Office 2016.

The relation between phosphorus and liver diseases, and relation between kidney diseases and liver stiffness were analysed by Decision tree. The Decision tree as a classification and predictive model to map observed variables resulting in the conclusion about outcomes. It is very useful to handle nominal and numeric data and represents any discrete-value classifier. In addition, this statistical analysis is non-parametric method on which the space distribution and the classifier structure have no input (BigML, USA).

### 4. RESULTS

Results are presented as metabolic syndrome data, relation between phosphorus and liver stiffness and relation between kidney diseases and liver stiffness.

#### Metabolic syndrome data

The observed average CAP values with regard to etiology of kidney disease did not significantly differ from the overall average and distribution, P-value was 0.848.
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In addition, the observed average values of Liver Stiffness with regard to etiology of kidney disease did not significantly differ from the overall average and distribution, $P$-value was 0.757. Therefore, even though neither CAP or Liver Stiffness showed significant differences still both could indicate the presence of underlying liver diseases (steatosis of liver parenchyma) in different aetiological groups of kidney diseases (Table 1).

The observed average AST values with regard to etiology of kidney disease did not significantly differ from the overall average and distribution, $P$-value was 0.641. The observed average ALT values with regard to etiology of kidney disease did not significantly differ from the overall average and distribution, $P$-value was 0.730. Both hepatic aminotransferases, AST or ALT did not show significant difference, indicating that their significant activity was not detected in any group of kidneys diseases. Despite that, their slight activity still can indicate the presence of underlying liver diseases in different aetiological groups of kidney diseases (Table 1).

Relation between phosphorus and liver stiffness

The relation between phosphorus and liver stiffness data in this study was presented by the Decision tree. If $P \leq 1.77$ µmol/L and kidney disease $< 3$ (group 1 nephroangiosclerosis and group 2 diabetic nephropathy) or $\geq 3$ (glomerulonephritis and pyelonephritis, autoimmune kidney disease, and polycystic and another morphological kidney disease), in either case, the liver stiffness was No with confidence of 51.01% and 86.68%, respectively. However, if $P > 1.77$ µmol/L, in combination with 1M thick (Intima-media thickness) $\leq 1.05$, PTH $\leq 383$ and gender (male and female), than results in liver stiffness Yes with different percentage of confidence. For female, the liver stiffness is Yes with 56.55% of confidence in combination with all three mentioned predictors. Interestingly, for male, additional three predictors are necessary for the liver stiffness Yes with confidence of 56.55% and these three additional predictors are: Diur (Diuretics used) Yes, PTH $> 5$ and CRP $> 1.3$. Observing all other predictors (CAP and weight) liver stiffness results in No with different percentage of confidence.

Relation between kidney diseases and liver stiffness

The relation between kidney diseases and liver stiffness data in this study was also presented by the Decision tree. However, the relation between kidney diseases and liver stiffness was observed using phosphorus and ferritin. If $P > 3$ µmol/L and ferritin $\leq 323.45$ ng/mL, then liver stiffness is Yes with 56.55% of confidence and in combination they were in the relation with kidney diseases 4 and 5 (autoimmune kidney disease, and polycystic and another morphological kidney disease, respectively). In other hand, if $P \leq 3$ µmol/L, AST $> 13$ U/l and ferritin $> 484.68$ ng/mL then liver stiffness is Yes with 56.55% of confidence and in combination they were in the relation with kidney diseases 1, 2 and 3 (nephroangiosclerosis, diabetic nephropathy, glomerulonephritis and pyelonephritis, respectively). Interestingly, not all other predictors (haemoglobin and CRP) were in relation with kidney diseases and liver stiffness.

5. DISCUSSION

The precise mechanism and pathogenesis of NAFLD in relation to other affected organ systems has not yet been fully clarified. One of the reasons for the lack of data is the need for long-term monitoring of liver damage by invasive methods (liver biopsy) which is an obstacle in terms of patient collaboration. Therefore, the introduction of new non-invasive methods for assessing the stage
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of fibrosis and quantifying the underlying liver steatosis opened new possibilities in analysing the parameters of liver damage and associated damage to other organic systems, primarily chronic kidney diseases.

In addition to the multiple-confirmed method of determining the stages of liver damage by transient elastography, the introduction or recognition of other, simpler parameters that would indicate the presence of liver disease in patients with end-stage renal disease, is gaining in importance.

It is interesting that analysing the mean CAP value and Liver Stiffness, as well as the liver enzymes AST and ALT, did not show significant results for the presence of underlying liver disease in etiological groups of various kidney diseases. However, taking into account the application of existing laboratory parameters, which in combination with other parameters and in the presence of certain etiological factors of renal disease, indicate the development of liver disease. Therefore, in this study, the serum phosphorus and ferritin values were highlighted.

Namely, in patients with phosphorus values \( P > 1.77 \), with intima-media thickness \( \leq 1.05 \), and \( \text{PTH} \leq 383 \), depending on the sex of patients, hepatic parenchyma fibrosis was registered. Thus, in the case of female patients in the presence of all three parameters, fibrosis of liver parenchyma was detected in a different proportion.

At the same time, in the male sex, two additional predictors are needed: with \( \text{PTH} \), the value of CRP and previous diuretic consumption to detect the presence of liver fibrosis.

We also monitored the relationship between kidney disease and liver damage stages through the values of phosphorus and ferritin. Thus, if phosphorus \( > 3 \ \mu\text{mol/L} \) and ferritin \( \leq 323.45 \ \text{ng/mL} \), hepatic parenchyma fibrosis was detected with 56.55% confidence in the group of patients with autoimmune diseases of the kidneys, and polycystic and other morphological diseases.

It follows from the above that the higher values of phosphorus but a lower level of ferritin are necessary to prove a certain stage of liver fibrosis in previously confirmed autoimmune and morphological diseases of the kidney. On the other hand, if phosphorus \( \leq 3 \ \mu\text{mol/L} \) and ferritin \( > 484.68 \ \text{ng/mL} \), hepatic parenchyma fibrosis was detected in the group of patients with kidney disease nephroangiosclerosis, diabetic nephropathy and glomerulo- and pyelonephritis. Thus, the lower phosphorus values with higher ferritin values in inflammatory and degenerative kidney diseases favor the occurrence of hepatic parenchyma fibrosis, which is consistent with the results of previous studies that confirmed the fibrogenic properties of ferritin.

In accordance with the results of our study, Baqurezo et al. established a better prognosis of acute liver failure in patients with lower serum phosphorus (less than 2.5 mg/dL) versus mean (2.5 to 5 mg/dL) and elevated values (greater than 5 mg/dL). Hypophosphatemia was associated with a good prognosis of acute liver disease, while hyperphosphatemia was a predictor of severe hepatic impairment (14).

Haemodialysis patients who usually have hypophosphatemia may develop iatrogenic hypophosphatemia caused by haemodialysis treatment. Pradeep et al. results confirm, however, that significant hypophosphataemia can be found in acute and chronic liver disease (15). Therefore, further research is needed to establish whether regular monitoring of serum phosphorus levels and early treatment of severe hypophosphatemia would improve the prognosis in patients with acute and chronic liver disease.

Based on the results of our research, determination of serum phosphorus and ferritin values in patients with kidney disease would eventually indicate groups of patients in whom hepatic parenchyma damage could be present. So defined predictors could show which patients would benefit from TE.

6. CONCLUSION

According to the results obtained and the results of several ongoing studies, there is sufficient evidence linking the development of chronic kidney disease to NAFLD. Using simple parameters such as serum phosphorus and ferritin values that may indicate the presence of liver disease in confirmed renal disease opens the possibility of simple and cost-effective screening. This would create the possibility of prevention and early detection of NAFLD in patients with chronic kidney disease, leading to more favourable outcomes in patients with hepatic steatosis and liver fibrosis, which at the end ultimately require organ transplantation.

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