The effect of hemodialysis on the liver enzymes (AST & ALT) in patients with renal failure

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Abstract:
This study was carried out to measure serum levels of the liver enzymes; aspartate transaminase and alanine transaminase in patients of renal failure under hemodialysis. Eighty samples were collected from patients in period between January to March 2015, chosen randomly from ALnaw teaching hospital and Alrebat teaching hospitals, and forty apparently, healthy individuals as controls, to assess the effect of hemodialysis on liver transaminases enzymes level.

Measure serum aspartate transaminase and alanine transaminase by using automation, mindray BS 200, and results were analyzed using statistical of package social science (SPSS), computer program.

The study showed that the serum levels of aspartate transaminase" AST", and alanine transaminase "ALT" were
significantly decreased, (p-value = 0.00) in the patients under hemodialysis group.

Mean±SD controls versus cases.
(34±7 versus 15±5 u/l) for AST.
(24±7 versus 10±4 u/l) for ALT.

Also the serum levels of liver transaminase enzymes were measured in 20 cases with renal failure under hemodialysis, compare the levels of them before dialysis and post dialysis, showed (AST&ALT) were significantly decreased (p-value=0.00) in cases before dialysis when compared to the levels of them after dialysis.

Mean±SD for pre dialysis versus post dialysis.
(13±4 versus 22±4 u/l) for AST.
(8±4 versus 17±3 u/l) for ALT.

The study indicates that there was no significant difference between the levels of liver transaminase enzymes according to gender "males or females". The results as follow;

Mean±SD for male versus female
(10±4 versus 9±4 u/l, p-value ≤0.42) for ALT.
(15±6 versus 15±5 u/l, p-value ≤0.96) for AST.

According to causes of chronic kidney disease the results of this study showed that; hypertension, diabetes and family history are the most common causes in Sudan, and hepatitis is the common risk disease in patients with renal failure under hemodialysis. Also showed that increasing of body mass index is significant related to renal failure disease. (p-value ≤0.02).

It is concluded that the levels of transaminase (AST&ALT) are significantly decreased in patients with renal failure under hemodialysis, and significantly decreased when compared levels of them before dialysis to levels after dialysis.

Key words: hemodialysis, liver enzymes (AST, ALT), renal failure

Introduction

Kidneys are the organs that help filter waste products from the blood. They are also involved in regulating blood pressure,
electrolyte balance, and red blood cell production in the body. Kidneys are located just below the rib cage, one on each side of the spine. Renal failure is a condition in which the kidneys fail to remove metabolic end-products from the blood and regulate the fluid, electrolyte, and pH balance of the extracellular fluids. Renal failure can occur as an acute or a chronic disorder.\(^1\)

End stage renal failure (ESRD) is when the kidneys stop working well enough without dialysis or a transplant. This kind of kidney failure is permanent.

There are different types of kidney dialysis, including:

Hemodialysis is most commonly used to treat people with end-stage kidney disease, Blood is filtered using a dialyzer and dialysis machine. During a hemodialysis session, your blood flows a little bit at a time through a special filter inside the machine. The filter removes wastes and extra fluids from your blood, but retains the proper balance of minerals such as potassium and sodium. Once the blood is cleaned, it is returned to the body.

Peritoneal dialysis is filtering blood inside the body after the abdomen is filled with a special cleaning solution. This method allows your blood to be cleaned while you sleep, while you work, or while you perform your everyday activities.\(^2\)

Liver enzyme tests are a group of blood tests that detect inflammation and damage to the liver. They can also check how well the liver is working. Liver enzyme testing includes ALT, AST, and alkaline phosphatase.

Aminotransferases are enzymes (proteins that help speed up chemical reactions in the body) that are found mainly in the liver, but also in other tissues, such as muscles. They are a part of the normal metabolic processes in the liver and are responsible for transferring amino acids (components that build proteins) from one molecule to another. ALT was formerly known as serum glutamic-pyruvic transaminase (SGPT) and AST as serum glutamic-oxaloacetic transaminase (SGOT).
Elevated levels of liver enzymes in general signify some form of liver (or hepatic) damage or injury.

Some studies have shown that patients with chronic kidney disease (CKD) on hemodialysis may have lower serum levels of liver enzymes than those with normal renal function for reasons may be lower because of a deficiency in vitamin B6, which is a coenzyme of ALT& AST or hemodilution, which occurs because of water retention in patients with renal failure before an hemodialysis session. This profile may adversely affect the diagnosis, clinical management, and treatment of liver disease in these patients.\(^{(3)}\)

The prevalence of hepatitis C virus (HCV) infection is significantly higher in hemodialysis patients than in the general population.

Studies have revealed that Patients with chronic kidney disease (CKD) who are undergoing hemodialysis and are infected with hepatitis C virus (HCV) have been shown to present with lower serum levels of alanine aminotransferase (ALT) and(AST) than HCV-infected patients with normal renal function, even in non infected HCV patients, ALT serum levels are lower in patients with renal failure undergoing hemodialysis compared with the population that has normal renal function. (ALT&AST) levels may be lower due to hemodilution, which occurs because of water retention in patients with chronic kidney disease (CKD) before an hemodialysis HD session.\(^{(4)}\)

**MATERIAL AND METHODS**

**Study approach**
Quantitative methods were used to measure Aspartate transaminase and Alanine transaminase (AST&ALT) activity in Sudanese patients with renal failure in Khartoum state, during the period from January to March 2015.
Study area:
This study was conducted in Alnaw hospital, and Alrebat hospital, in Khartoum state, (Capital of Sudan).

Target population:
The study included patients with renal failure (males and females) under hemodialysis.

Sample size:
A total of 80 patients with renal failure were enrolled in this study, plus (40) non patients apparently healthy volunteers' (age and sex matched with the test group) were included to serve as control.

Inclusion and Exclusion criteria:
Sudanese patients with end stage renal failure and apparently healthy volunteers were included, while patients with renal failure who tested hepatitis positive were excluded.

Ethical consideration:
Written consent was taken regarding acceptance to participate in the study and reassurance of confidentiality. Before the specimen was collected, the donor knew that this specimen was collected for research purpose.

Data collection:
The Clinical data were obtained from history, clinical examination and hospital follow up records and were recorded on a questionnaire sheet.

Sample collection and processing:
After informed consent and use of a local antiseptic for the skin (70%), 3 ml of venous blood was collected from the forearm of each patient and control by syringe (3ml) using venipuncturing
directly into centrifuge tube which contained anticoagulant for serum preparation. Serum was separated from blood cells after centrifugation for 5 minutes at 5000 r.p.m, at room temperature and the sera were used immediately for estimation of liver enzymes.

**Estimation of serum levels of Alanine transaminase:**

**Principles:**

\[
\begin{align*}
\text{a-oxoglutarate} + l - \text{alanine} & \overset{ALT}{\leftrightarrow} l - \text{glutamate} + \text{pyruvate} \\
\text{pyruvate} + \text{NADH} + H^+ & \overset{\text{LDH}}{\leftrightarrow} l - \text{lactate} + \text{NAD}^+
\end{align*}
\]

Alanine aminotransferase catalyzes the reversible transamination of l-alanine and a-oxoglutarate to pyruvate and l-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of reduced B-nicotinamide adenine dinucleotide (NADH) to B-nicotinamide adenine dinucleotide (NAD). This change in absorbance is directly proportional to the activity of ALT in the sample.

**Assay procedure:**

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>1000 μL</td>
<td>1000 μL</td>
</tr>
<tr>
<td>Dist water</td>
<td>100 μL</td>
<td>–</td>
</tr>
<tr>
<td>Sample</td>
<td>–</td>
<td>100 μL</td>
</tr>
</tbody>
</table>

Mix, incubate for 5 min, then add:

<table>
<thead>
<tr>
<th></th>
<th>250 μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 2</td>
<td>250 μL</td>
</tr>
</tbody>
</table>

Mix thoroughly, read the absorbance after 1 min and monitor time. Read the absorbance again for additional 3 min.

\[
\Delta A/\text{min} = [\Delta A/\text{min sample}] - [\Delta A/\text{min blank}]
\]
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**Calculation:**
The analyzer calculates the activity of each sample automatically with a specified valid calibration factor from calibration process.

\[
1 \text{U/L} = 16.67 \times 10^{-3} \mu \text{kat/L} \quad 1 \mu \text{KAT/L} = 60 \text{U/L}
\]

**Reference Intervals**

<table>
<thead>
<tr>
<th></th>
<th>Conventional Units</th>
<th>S.I. Units</th>
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</thead>
<tbody>
<tr>
<td>Male</td>
<td>≤45 U/L</td>
<td>≤0.75 μkat/L</td>
</tr>
<tr>
<td>Female</td>
<td>≤34 U/L</td>
<td>≤0.57 μkat/L</td>
</tr>
</tbody>
</table>

**Estimation of serum levels of Aspartate transaminase:**

**Reaction Principle:**

\[
L\text{-aspartate + } \alpha\text{-oxoglutarate} \rightleftharpoons \text{oxaloacetate + L-glutamate} \quad ^{AST}\n\]

\[
\text{oxaloacetate + NADH + } H\rightleftharpoons \text{L-malate + NAD++H}_2O \quad ^{MDH}\n\]

(MDH = Malate dehydrogenase, EC1.1.1.37)

In the assay reaction, the AST catalyzes the reversible transamination of L-aspartate and α-oxoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with NADH being oxidized to NAD+. The rate of the photometrically determined NADH decrease is directly proportional to the rate of formation of oxaloacetate and thus the AST activity.

**Assay procedure**

<table>
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<tr>
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<tr>
<td>Reagent 1</td>
<td>1000 μL</td>
<td>1000 μL</td>
</tr>
<tr>
<td>Dist water 100 μL</td>
<td>100 μL</td>
<td>–</td>
</tr>
<tr>
<td>Sample</td>
<td>–</td>
<td>100 μL</td>
</tr>
</tbody>
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Mix thoroughly, read the absorbance after 1 min and monitor time. Read the absorbance again for additional 3 min.
The effect of hemodialysis on the liver enzymes (AST & ALT) in patients with renal failure

\[ \Delta A/\text{min} = [\Delta A/\text{min sample}] - [\Delta A/\text{min blank}] \]

**Calculation:**
The analyzer calculates the activity of each sample automatically with a specified valid calibration factor from calibration process.

\[ 1 \text{U/L} = 16.67 \times 10^{-3} \mu\text{kat/L} \quad 1 \mu\text{KAT/L} = 60 \text{U/L} \]

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<tr>
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<th>S.I. Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>≤35 U/L</td>
<td>≤0.58 μkat/L</td>
</tr>
<tr>
<td>Female</td>
<td>≤31 U/L</td>
<td>≤0.52 μkat/L</td>
</tr>
</tbody>
</table>

**Quality control**
The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before its application for the measurement of test and control samples.

**Data analysis**
data was analyzed using SPSS computer program, the mean and standard deviation of Alanine transaminase and Aspartate transaminase (AST&ALT) activity were obtained and the independent 't.test' used for comparison (p value of ≤ 0.05) was considered significant.

**Results**

![Figure (1): distribution of patients according to age groups.](image-url)
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Figure (2) Comparison of frequency of males and females in the patients group.

Table (1) Mean of body mass index (BMI), of patients with renal failure group and control group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients N=80</th>
<th>Control N=40</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>29±4.1kg/m²</td>
<td>25±3kg/m²</td>
<td>≤ 0.02</td>
</tr>
</tbody>
</table>

Results given in mean ±SD range between brackets. p-value ≤0.05 consider significant.

Table (2) Distribution of patients according to family history disease and other associated disease:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>47</td>
<td>59 %</td>
</tr>
<tr>
<td>Diabetes</td>
<td>37</td>
<td>46%</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>20</td>
<td>20%</td>
</tr>
</tbody>
</table>

Table (3) Means of Alanine transaminase (ALT) and Aspartate transaminase (AST), (u/l) activity in patients with renal failure and control group:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients N=80</th>
<th>Control N=40</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (u/l)</td>
<td>10±4 (4-22)</td>
<td>24±7 (14-37)</td>
<td>0.000*</td>
</tr>
<tr>
<td>AST (u/l)</td>
<td>15±5 (5-27)</td>
<td>34±7 (20-50)</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Results given in mean ±SD. Range between brackets.
The effect of hemodialysis on the liver enzymes (AST & ALT) in patients with renal failure

Table (4) Means of Alanine transaminase (ALT) and Aspartate transaminase (AST), (u/l) activity in patients with renal failure, (pre and post dialysis):

<table>
<thead>
<tr>
<th>Variables</th>
<th>Predialysis N=20</th>
<th>postdialysis N=20</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (u/l)</td>
<td>8±4</td>
<td>17±3</td>
<td>0.000*</td>
</tr>
<tr>
<td>AST (u/l)</td>
<td>13±4</td>
<td>22±4</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Results given in mean± SD.
* P-value ≤0.05 consider significant

Table (5) Mean of alanine transaminase(ALT) activity according to gender

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Number</th>
<th>Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (ALT). (u/l)</td>
<td>43</td>
<td>10±4</td>
<td></td>
</tr>
<tr>
<td>Females (ALT)(u/l)</td>
<td>37</td>
<td>9±4</td>
<td>0.428*</td>
</tr>
</tbody>
</table>

Results given in mean ±SD.
* P-value ≤0.05 consider significant.

Table (6) Mean of aspartate transaminase(AST) activity according to gender

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Number</th>
<th>Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (AST). (u/l)</td>
<td>43</td>
<td>15±6</td>
<td></td>
</tr>
<tr>
<td>Females (AST)(u/l)</td>
<td>37</td>
<td>15±5</td>
<td>0.963*</td>
</tr>
</tbody>
</table>

Results given in mean ±SD
* P-value ≤0.05 consider significant.

Figure (3): A scatter plot shows the correlation between ALT(u/l) level and body mass index (BMI) (r=0.139, p-value≤0.218).
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Figure (4): A scatter plot shows the correlation between AST level and body mass index (BMI); (r=0.048, p-value ≤0.669).

Figure (5): A scatter plot shows the correlation between ALT level and duration of dialysis (r=0.024, p-value ≤0.836).

Figure (6): A scatter plot shows the correlation between AST level and duration of dialysis; (r=-0.083, p-value ≤0.466).
Discussion

Kidney failure is a condition in which the kidneys fail to remove metabolic end product from the blood, so when kidney failure is reached the end stage it must need dialysis. (1)

Hemodialysis affects many substances in the blood by increasing, decreasing or removing them. This study conducted to study the effect of hemodialysis on levels of liver enzymes activity. Two liver enzymes, alanine transaminase and aspartate transaminase (ALT&AST) were chosen for the assessment the effect of dialysis on levels of liver enzymes.

Preliminary investigation and findings obtained from specially designed questionnaire revealed that the majority of patients under dialysis participated in this study were in the average ages of about 55 years. This agreed with previous published results of many authors,(5), whose finding confirmed that, after the age of 30 years, glomerular filtration rate (GFR) progressively declines at an average rate of 8 mL/min/173 m² per decade , and the risk of renal failure increased with age. This result also was agreed with result carried by:(6) (Christian) showed that the average age of a British person with the renal failure is 77 years.

Sex distribution in patients under hemodialysis of this study revealed that 54% were males. This agree with the previous study which documented in the field of nephrology, showed that women seem to be somewhat protected from developing end stage renal failure; the cumulative incidence of the disease remains low during the reproductive ages and begins to rise 10 years later.(7)

Social clinical history index of patients under the study indicated that appositive family history of renal failure of first degree relatives found to be in 34% of cases. These findings may indicate that hereditary play a role in the pathogenesis of renal failure patients. This result agreed with previous study showed
that there is a high prevalence of family history –end stage renal disease among US population, about 23%. (8) Other study showed the same result also family history of renal disease is one of the most important risk factors associated with development of nephropathy. (9)

The findings of this study showed that there was a significant difference in the body mass index (BMI; determined by dividing the weight in kilograms by the height in meter square.) between patients and control, the patients with renal failure susceptible to be more obese than control group. This made the BMI is independent factor of renal failure. This agreed with previous study which found positive correlation between increased (BMI) and risk of renal failure disease. (10)

Another study examined the relationship between increased weight (BMI) and renal function evaluated by the estimated glomerular filtration rate, Increased BMI was consistently associated with reduced glomerular filtration rate. (11)

In this study some of diseases presented in patients with renal failure as appeared in table (2), more than half (59%) of patients in these study were present with hypertension. It is well documented that the persistence of hypertension is one of leading cause of chronic renal failure.

Also (46%) of patients under dialysis participated in this study were present with diabetes.

This agreed with previous study showed that high risk groups that should be screened for chronic kidney disease include patients who have a family history of the disease and patients who have diabetes, hypertension. (12)

Also this result was in agreement with findings done by (Oyetunde) (13), revealed that both hypertension and diabetes were significantly related to chronic renal failure with incidence of (43%). The results showed that diabetes, hypertension and
chronic renal failure were significantly correlated (p-value<0.05).

Also the result agreed with study done by( Janice) (14) which showed that, The key risk factors for kidney disease are hypertension and diabetes, which are both becoming more prevalent in the United States, (40% among patients with renal failure.

In this study the comparison of levels of liver enzyme, alanine transaminase (ALT) activity between cases and control participated in this study showed that decreasing of levels of Alanine transaminase in patients with renal failure under hemodialysis when compared with control. (10 ± 4versus 24±7, p-value = 0.00).

As well as aspartate transaminase (AST) which showed that decreasing of levels of Aspartate transaminase in patients with renal failure under hemodialysis when compared with control.

(15 ± 5 versus 34±7, p-value =0.00).

This result agreed with result carried by (Luis et al) (3) which found that serum levels of the enzymes aspartate transaminase& alanine transaminase in patients with renal failure on hemodialysis; were decreased. It was hypothesized that this reduction could be caused by factors such as the withdrawal of aminotransferases during the hemodialysis session; the high lactate serum levels, which, during biochemical dosages, would rapidly consume Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and result in low levels of aminotransferases; the presence of uremic factors that would inhibit the activity of these enzymes; and, finally, the deficiency of pyridoxine, a cofactor for the synthesis of the aminotransferases.

This agreed with study done by( Ono et al) (15), showed that there were positive correlation between(ALT&AST) and
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pyridoxine, but disagreed with (Jung et al) (16) and (Yasuda et al) (17) whose showed there was no correlation between them.

Also this results are in agreement with the findings in (Mujeeb et al) (18), showed that both AST & ALT were significantly low (p-value <0.05) in both sexes when compared with their counterparts in the control group the decrease in levels of aminotransferases in patients with renal failure who have not undergone dialysis therapy has observed in this study suggests that renal impairment has an impact on the liver enzymes.

As appear in table (4) compare the (AST & ALT) in 20 cases of renal failure, predialysis and post dialysis showed that the levels of (AST & ALT) concentration were significant decreased in pre dialysis when compared to post dialysis, ALT (8±4 versus 17±3 ; p = 0.00) AST (13±4 versus 22±4 ; p =0.00)

This agreed with previous study which collected serum aminotransferases prior to and after hemodialysis sessions and observed a 15-35% increase after dialysis, which supports the hypothesis of hemoconcentration for the rise in aminotransferases observed after the dialysis procedure. (17)

Also this result agreed with other study carried by (Sombolos et al) (19) evaluated 53 patients on hemodialysis and divided them into three groups: hemodialysis, isolated ultrafiltration, and euclidean hemodialysis (without the removal of fluids) and verified the effects of hemodilution in the serum levels of the aminotransferases. In the patients who underwent euclidean hemodialysis, there were no differences between the ALT and AST levels prior to and after the procedure. However, when an isolated ultrafiltration or hemodialysis was performed, there was an increase in the aminotransferase levels when compared with the values prior to and after the procedure the authors concluded that the rise in the aminotransferase serum levels after hemodialysis should
primarily occur due to the hemoconcentration induced by the ultrafiltration.

Also the result agreed with (Isabella et al) (4) which showed the aminotransferase levels were lower in the samples collected before hemodialysis compared with the samples collected after the hemodialysis.

In this study results showed there is no significant difference between (AST & ALT) according to gender (males and females) in cases and control. This result agreed with previous study done by (Mujeebet al) (18) showed that there was no significant difference of transaminases (AST&ALT) according to gender.

In this study findings showed that, there was insignificant correlation between body mass index (BMI) and ALT, (p-value≤0.218), and AST (p-valuep-value≤0.669). This result agreed with study done by (Ajay Kumar) (20), whose showed that No significant relation was found between liver transaminase ( ALT p-value ≤0.21 & AST p-value≤0.28) innormal, overweight and obese individuals.

The findings of this study are disagreed with the previous study done by (Salvaggio;) (21). which showed that the percentage increase in the geometric mean of liver enzymes(AST&ALT) activity of the obese subjects (BMI greater than 30 kg/m2) compared with that of the normal subjects (BMI less than or equal to 25 kg/m2), p-value ≤0.01.

Also In this study as appeared in figures (5&6), which showed no correlation between duration of dialysis and liver enzymes (AST&ALT) activity. It was suggested that; the liver transaminases start to decrease from first time of hemodialysis and continue to decrease after many sessions of hemodialysis. In chronic renal failure, the liver enzymes reach the steady state and are not influenced with hemodialysis sessions. This result agreed with previous study done by (Luis et al), (3) whose recorded the levels of transaminases in patients under
hemodialysis and found that in first times of dialysis (AST&ALT) started to decrease and after many sessions of dialysis reached steady state.

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