Trace Minerals and Antioxidant Enzyme Profile of Chronic Liver Disease Patients in Kano, Northwest, Nigeria

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Abstract

Background: Chronic liver disease is a major cause of death especially in low- and middle-income countries where increasing prevalence has been noted recently. Free radicals-mediated tissue injury plays an important role in hepatic fibrogenesis while antioxidant vitamins and a few trace minerals reduce tissue damage by scavenging free radicals. Aim: To determine the blood levels of some selected antioxidant enzymes and trace minerals in chronic liver disease patients in Kano. Methods: Eighty-nine chronic liver disease patients and eighty-nine age and sex-matched controls were recruited for this study. Atomic absorption spectrophotometry and enzyme-linked immunosorbent assay were used to measure trace minerals and antioxidant enzymes respectively. Data analysis was done using SPSS version 21.0. Results: Results showed that Glutathione Peroxidase (2.54±1.45 ng/mL vs 6.00±2.64 ng/mL), SOD (23.29±15.27 ng/mL vs 52.48±26.72 ng/mL), Zn (0.82±0.22 mg/L vs 1.07±0.26 mg/L) and Se (2.66±0.60 mg/L vs 3.32±0.55 mg/L) levels were significantly lower (p<0.05) compared to their respective controls. Trace minerals (except Cu) and antioxidant enzyme levels decrease significantly with increased severity of chronic liver disease. Conclusion: The findings suggested that trace minerals and antioxidants are reduced in chronic liver disease patients and reduction correlated significantly with advanced disease.

Keywords: Antioxidant Vitamins; Chronic Liver Disease; Trace Minerals.

Introduction

Chronic liver disease has been identified as a major health problem and is a major cause of morbidity and mortality in most developing and developed countries (Allan et al., 2010). The prevalence of chronic liver disease (CLD) is about 18.5%, globally, while the prevalence of liver cirrhosis (LC) is between 4.5% and 9.5%, the annual incidence of hepatocellular carcinoma (HCC) is around 5.6% and has an annual mortality rate of 2 million deaths per year (Marcellin and Kutala, 2018). Worldwide, the common aetiologies of CLD are hepatitis B virus (HBV 43%) infection, hepatitis C virus (HCV 24%), alcoholic liver disease (ALD 19%), nonalcoholic steatohepatitis (NASH 10%) and others (5%) (Cheemerla and Balakrishnan, 2021). The occurrence of the aetiology varies with geographical regions of the world, while HCV, ALD and NASH are common in Europe and North America, HBV is common in Africa, Asia and South America (Blachier, Leleu, Peck-Radosavljevic, Valla and Roudot-Thoraval, 2013). A retrospective study in Southeast, Nigeria, reported that liver disease accounted for 7.9% of medical admissions and HCC and LC accounted for 44.3% and 20.4% respectively (Nwokediuko, Osuala, Uduma, Alaneme, Onwuka, and Mesigo, 2013). A national survey of hepatitis B in Nigeria showed a prevalence of 12.2% among the general population (Olayinka et al., 2016). Moreover, vaccination against hepatitis B in Nigeria is lower than in many sub-Saharan African countries resulting in hepatitis B being the most common cause of liver disease in Nigeria (Owolabi and Ojo, 2008).
Oxidative stress as a result of increased free radicals causes the destruction of different cellular components such as DNA, proteins and lipids/fatty acids leading to cell damage and apoptosis. It also releases some critical proinflammatory cytokines resulting in hepatic inflammation, fibrosis and cirrhosis (Cichoż-Lach, and Michalak, 2014). Both enzymatic and non-enzymatic antioxidant systems are essential for cellular response in order to deal with oxidative stress under physiological conditions. Therefore, antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) and non-enzymatic electron receptors such as glutathione (GSH) are affected and used as indices to evaluate the level of oxidative stress. Essential trace elements/minerals such as zinc (Zn), selenium (Se), copper (Cu), manganese (Mn) and Iron (Fe) not only act as micronutrients but also play vital roles in multiple metabolic processes in the liver by acting as cofactors in the antioxidant systems. Oxidative stress is regarded as one of the pathological mechanisms that result in initiation and progression of various liver diseases such as chronic viral hepatitis, alcoholic liver diseases and non-alcoholic steatohepatitis (Feng, Wang, Ye, Li, Cheung, and Nagamatsu, 2011; Sagnelli, Struffolini, Pirisi, Babudieri, Colloredo, Russello, Coppola, Gaeta, Cacopardo, De Luca, and Almasio, 2018). This study aims to evaluate the trace mineral and antioxidant enzyme activity in patients with chronic liver disease in Kano. Studies on trace minerals and antioxidant status may help in the management of patients with chronic liver disease. The results of this study will add to the existing knowledge and assist in better management of chronic liver disease.

Methods and Materials

Study Design
The study was a cross-sectional study carried out among chronic liver disease patients attending a gastroenterology clinic at Aminu Kano Teaching Hospital, Kano, Northwestern Nigeria.

Sample Size Determination
The sample size was computed (Lwanga and Lemeshow, 1991) to be 89 using a prevalence of 6.2% (Bassi, Builders, Osaronowen, Maduagwuna, Ibrahim, and Dankyau, 2017).

Study Population and Sampling
A total of one hundred and seventy-eight (178) adults (18-60 years) were recruited for the study including eighty-nine (89) chronic liver disease patients as the study group and eighty-nine (89) age and sex-matched apparently healthy staff and students in AKTH. Research participants who satisfied the study inclusion criteria and consented were selected consecutively until the desired sample size was attained.

Exclusion Criteria
Individuals known to have diabetes mellitus, human immunodeficiency virus/acquired immunodeficiency syndrome, chronic kidney disease and cardiovascular diseases were excluded. Additionally, patients and controls that are pregnant, on micronutrient supplementation as well as smokers were also exempted.

Data Collection
Following informed consent, a semi-structured interviewer-administered questionnaire was used to elicit data on subjects' demographic, socioeconomic and medical information including age, gender, alcoholism, smoking and drug addiction/medication among others. The questionnaire was pretested before administration and data was collected with the help of trained research assistants. About ten millilitres (10ml) of blood sample was collected by venipuncture into plain tubes. The samples were properly labelled, allowed to clot and retract, spun and separated to obtain the serum and then frozen at -200C until required for analysis.

Ethical Consideration
The approval to undertake this research was obtained from the Ethical Committee of Aminu Kano Teaching Hospital with reference number NHREC/21/08/2008/AKTH/EC/2759. Study
participants were duly informed about the research work and consent forms signed by the patients. The study objective and procedures were fully explained to participants prior to their recruitment. They were informed about the content of the interview to enable them to understand the procedures and to ensure their approval. Participation was totally voluntary; thus, individuals had the right to or not to partake in the study. Only individuals who consented were interviewed and samples were collected. A special coding system was adopted, and the data obtained were handled by researchers alone to ensure confidentiality.

**Analytical Methods**

The concentrations of serum copper, zinc and selenium were determined using the Agilent 200 series atomic absorption spectrophotometer (AA240FS). Serum samples were digested by the conventional wet acid method by adopting the method of Memon, Tasneem, Hassan, and Nasreen, 2007. This was then aspirated into the AAS flame, where it became atomized. The amount of light intensity absorbed in the flame is proportional to the element in the sample (Yahaya, Shehu, and Dabai, 2013). The serum level of GPx and SOD were measured using Human GPx ELISA kit manufactured by Melsin Medical Company Limited, China. The kit uses enzyme-linked immunosorbent assay-double antibody sandwich principle.

**Statistical Analysis**

Data were presented as mean±SD. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago, IL, U.S.A.). The variables were normally distributed, hence independent t-test was used to compare variables between the patient and control group. Subsequently, one-way analysis of variance (ANOVA) was used to determine significance based on the severity of liver disease. ANOVA was also used to check significant differences based on the duration of the illness. The differences between the various means were compared using the Bonferroni Post Hoc test. Pearson's correlation analysis was conducted to test for association between trace minerals, antioxidants and liver enzymes. The level of significance was set at p<0.05.

**Results**

Table 1: Socio-demographic Characteristics of Chronic Liver Disease Patients and Controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients Frequency (n=89)</th>
<th>Controls Frequency (n=89)</th>
<th>Patients Percentage</th>
<th>Controls Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60</td>
<td>65</td>
<td>67.4</td>
<td>73.0</td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
<td>24</td>
<td>32.6</td>
<td>27.0</td>
</tr>
<tr>
<td>Age Groups (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26-35</td>
<td>32</td>
<td>37</td>
<td>36.0</td>
<td>41.6</td>
</tr>
<tr>
<td>36-45</td>
<td>25</td>
<td>23</td>
<td>28.1</td>
<td>25.8</td>
</tr>
<tr>
<td>46-55</td>
<td>22</td>
<td>21</td>
<td>24.7</td>
<td>23.6</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>10</td>
<td>44</td>
<td>11.2</td>
<td>49.4</td>
</tr>
<tr>
<td>Married</td>
<td>72</td>
<td>45</td>
<td>80.9</td>
<td>50.6</td>
</tr>
<tr>
<td>Divorced</td>
<td>7</td>
<td>0</td>
<td>7.9</td>
<td>0.0</td>
</tr>
<tr>
<td>Level of Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-formal</td>
<td>11</td>
<td>0</td>
<td>12.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Primary</td>
<td>16</td>
<td>0</td>
<td>18.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Secondary</td>
<td>37</td>
<td>0</td>
<td>41.6</td>
<td>0.0</td>
</tr>
</tbody>
</table>
The socio-demographic characteristics of the study groups are presented in Table 1. The majority of the participants in the patient and control groups were males, 67.4% and 73.0% respectively. Age group 26-35 yrs showed the highest frequency (36.0%) and most were from the married population. Most of the patients have a secondary level of education (41.6%) and most are traders (39.3%).

**Figure 1:** Pattern of Chronic Liver Disease among Study Subjects

CHB= chronic hepatitis B, CHC= chronic hepatitis C, PLCC= primary liver cell carcinoma

Chronic hepatitis B (CHB) was the most frequently seen liver disease (75.3%). The distribution of liver cirrhosis was 11.2% while that of chronic hepatitis C (CHC) was 6.7%. Of the total patients recruited, 4.5% presented with both CHB and CHC co-infection. Only 2.2% of cases of primary liver cell carcinoma (PLCC) were reported as presented in Figure 1.

**Table 2:** Levels of Liver Enzymes and Bilirubin (Mean±SD) in Patients with CLD and Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients</th>
<th>Control</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=89</td>
<td>n=89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>59.40±29.63</td>
<td>26.81±8.58</td>
<td>9.968</td>
<td>0.000</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>62.83±32.88</td>
<td>26.36±9.41</td>
<td>10.061</td>
<td>0.000</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>54.13±28.06</td>
<td>17.74±8.17</td>
<td>11.748</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Table 2 shows the mean±SD levels of liver enzymes and bilirubin in the study groups. The activities of liver enzymes and bilirubin were significantly higher in patients with CLD than in control groups (p=0.000).

Table 3: Antioxidants and Trace Minerals (mean±SD) in Patients with CLD and Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients</th>
<th>Control</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (ng/mL)</td>
<td>2.54±1.45</td>
<td>6.00±2.64</td>
<td>10.860</td>
<td>0.000</td>
</tr>
<tr>
<td>SOD (ng/mL)</td>
<td>23.29±15.27</td>
<td>52.48±26.72</td>
<td>8.949</td>
<td>0.000</td>
</tr>
<tr>
<td>Cu (mg/L)</td>
<td>3.51±0.68</td>
<td>2.48±0.39</td>
<td>12.381</td>
<td>0.000</td>
</tr>
<tr>
<td>Zn (mg/L)</td>
<td>0.82±0.22</td>
<td>1.07±0.26</td>
<td>6.969</td>
<td>0.000</td>
</tr>
<tr>
<td>Se (mg/L)</td>
<td>2.66±0.60</td>
<td>3.32±0.55</td>
<td>7.649</td>
<td>0.000</td>
</tr>
</tbody>
</table>

GPx= glutathione peroxidase, SOD= superoxide dismutase, Cu= Copper, Zn= Zinc, Se= Selenium, CLD= chronic liver disease, n= number of subjects

The level of antioxidants in patients was seen to be significantly lower than that in the control group (p=0.000). The mean copper level was significantly higher in patients with CLD (3.51±0.68mg/L) when compared to controls (2.48±0.39mg/L). Conversely, the zinc and selenium levels were seen to be significantly lower when compared to the control (p=0.000) as shown in Table 3.

Table 4: Trace Minerals and Antioxidant Enzyme Level Based on Severity of CLD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grade 0 (n=41)</th>
<th>Grade 1 (n=39)</th>
<th>Grade 2 (n=9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (ng/mL)</td>
<td>2.63±1.48\textsuperscript{a}</td>
<td>2.75±1.39\textsuperscript{a}</td>
<td>1.19±0.81\textsuperscript{b}</td>
<td>0.011*</td>
</tr>
</tbody>
</table>

Mohammed, I.Y et al (2023)
SOD (ng/mL) 24.74±14.47a 24.56±16.55a 11.06±5.50b 0.038*
Cu (mg/L) 3.35±0.56a 3.50±0.72a 4.26±0.59b 0.001**
Zn (mg/L) 0.84±0.20a 0.84±0.23a 0.65±0.19b 0.049*
Se (mg/L) 2.72±0.63a 2.67±0.56a 2.37±0.52a 0.272

GPx= glutathione peroxidase, SOD= superoxide dismutase, Cu= Copper, Zn= Zinc, Se= Selenium, CLD= chronic liver disease, n= number of subjects

The trace minerals (except copper) and antioxidants reduce significantly with increased severity of CLD (p< 0.05). Copper increased significantly across the severity groups (p=0.001). This is presented in Table 4.

Discussion
The majority of subjects in our study were Hausas, this may be because the most predominant inhabitants in this locality are Hausa/Fulani according to the population census (National Population Commission, 2006). The demographic profile of the respondents revealed that most of the cases were aged between 26-35 years (Table 1). This is comparable with a study in Ilorin, Kwara State where they found most CLD patients to be in their third and fourth decades of life (Olokoba, Aderibigbe, and Kayode, 2010). This may suggest a high risk of exposure to causative factors because of the sexually active lifestyle of this age group.

Approximately two-thirds of the CLD patients were males (67.4%) as shown in Table 1. This pattern of male preponderance has been well-documented by many researchers (Okonkwo, 2009; Nwokediuko, Osuala, Uduma, Alaneme, Onwuka, and Mesigo, 2013); Sage, Waiya, Ali, Garba, and Muhammad, 2018. A remarkable increase in the pattern of male preponderance in the EPANCRON study in Italy was also reported (Sagnelli, Stroffolini, Sagnelli, Pirisi, Babudieri, Colloredo, Russello, Coppola, Gaeta, Cacopardo, De Luca, and Almasio 2018). Numerous variables contribute to the greater risk of chronic HBV infection in men, which has been extensively researched (Durazzo, Belci, Collo, Prandi, Pistone, Martorana, Gambino, and Bo, 2014). However, it is unclear, though, whether males are exposed to more infections or whether their immune systems function less effectively. Our study also revealed that most of the patients possess a secondary level of education (41.6%). Despite the high level of education among the cases in this study, there may be poor perception as regards the risk factors for liver disease, though the levels of awareness of patients were not sought at the time of the study.

Carbohydrates were found to be the major dietary sources in the studied population most of which are grains as established by responses of patients obtained by the questionnaire. The possible role of environmental factors such as contamination of food with aflatoxin cannot be excluded. One of the staple diets of inhabitants of this locality is grains. Studies have shown that food items are most likely to be contaminated by aflatoxin (Igetei, Otegbayo, Ndububa, Lesi, Anumudu, and Hainaut, 2008). This may be a likely problem in this part, due to the poor processing of grains (either by drying or storage) which invariably encourage the growth of Aspergillus flavus, the fungus which produces aflatoxin. Although our study revealed a low distribution of primary liver cell carcinoma (PLCC), it has been reported that exposure to aflatoxin increases the susceptibility to p53 mutation which is implicated in the pathogenesis of PLCC.
Most of the patients were distributed across several occupations with traders having the highest percentage (Table I). It is a known fact that the most predominant occupation of Kano citizens is business owing to the fact that Kano State is the hub of commerce (Ikenwa, 2019).

Based on the pattern of CLD among study subjects, CHB had the highest frequency of 75.3% with PLCC being the lowest (2.2%) as illustrated in Figure 1. This parallels the report of (Ali, Ikani, Onyekwelu, Obi, and Ogbonna, 2020) in Southwest, Nigeria that reported the leading cause of CLD cases was HBV infection with a high prevalence of 83.1%. This implies that HBV is the leading cause of CLD in this environment and this is compatible with previous reports in Kano (Zage, Waiya, Ali, Garba, Muhammad, 2018) and other parts of Nigeria and the African continent (Lesi, Kehinde, and Anomneze, 2004; Blankson, Wiredu, Gyasi, Adjei, and Tetty, 2005; Okonkwo, 2009; Ayele and Gebre-Selassie, 2013). This high HBV prevalence may be due to the low vaccination rate against hepatitis B which has a wider risk exposure. In the 2016 National seroprevalence survey (Olayinka, Oyemakinde, Balogun, Ajudua, Ngu, and Aderinola, 2016) on HBV infection factors associated with testing positive for HBV infection were dental procedures outside the health facility (odds ratios [OR] = 3.4, 95% CI = 1.52–7.70), local circumcision (OR = 1.73, 95% CI = 1.17–2.57), and uvulectomy (OR = 1.65, 95% = 1.06–2.57). Hepatitis C viral infection also contributed to the aetiology of CLD among the cases but with a lower prevalence which is similar to previous findings Nwokediuko, Osuala, Uduma, Alaneme, Onwuka, and Mesigo, 2013). The high HBV versus lower HCV occurrence in the same study group could be attributed to the fact that HCV transmission is basically through contact with infected blood, while, in addition to exposure to infected blood, HBV can also be transmitted through other body fluids including saliva, giving it a wider risk exposure. This study also recorded HBV/HCV co-infection which may be due to the similar routes of transmission (Fig. 1).

In the present study, the liver enzymes and bilirubin levels were significantly higher in patients with CLD (Table 2) than in the control group (p<0.05) similar to other findings (Ibrahim, 2013; Zage, Waiya, Ali, Garba, and Muhammad, 2018). These elevations are mostly due to transaminases (ALT and AST) which form part of the sensitive indicators for liver injury and are produced by hepatocyte changes in cell permeability, hepatocellular degeneration, necrosis and inflammation can cause the release of ALT and AST from hepatocytes and subsequent increase of serum values. Bilirubin is derived from haemoglobin degradation. Impaired liver function leads to the accumulation of bilirubin in the blood and causes yellowing of the skin and eyes, called jaundice (Thapa and Walia, 2007).

The result from this study showed a marked decrease in the antioxidant status of patients with CLD compared to healthy controls (p<0.05) as in Table 3. This is in tandem with reports which showed a decrease in SOD but increased GPx in patients with viral hepatitis C in Turkey (Salem, El-Refaei, and Badra, 2003; Yalcin, Gulesci, Bilgin, and Koltas, 2020). The body cells produce free radicals during normal metabolic processes which are being mopped up or neutralized by antioxidants. In general, the body is able to maintain a balance between antioxidants and free radicals. However, during liver damage, there is oxidative stress that occurs because of increased free radical species which causes depletion of antioxidants. Decreased activity of both SOD and GPx in these patients may also be due to a deficiency of certain trace minerals which forms an integral part of these enzymes (El-Hassan, Abdelrazik, Abdelaziz, and El-Iraqi, 2004). This low antioxidant status gives way to oxidative stress which cause deleterious effect on body organs such as the liver. This may suggest a loss of antioxidant capability in patients with CLD.
Disturbances in the concentration of essential trace minerals (Cu, Zn and Se) compared to control subjects were also found in these patients (Table 3). The patients had low Zn and Se concentrations and high Cu concentrations compared to controls. The low Zn and Se with high Cu is consistent with some findings in patients with viral hepatitis (Guo, Chen, and Ko, 2013) and in patients with hepatocellular carcinoma (Mekky, Hasanain, Adel-Naiem, Orabi, and Osman, 2018). Upregulation of Zn-importing proteins by pro-inflammatory cytokines and oxidative stress reduces plasma Zn concentration (Luizzi, Lichten, Rivera, Blanchard, Aydemir, Knutson, Ganz, and Cousins, 2005). Se is another potent antioxidant that acts as an anti-inflammatory agent (Walston, Xue, Semba, Ferruc, Cappola, Ricks, Guralnik, and Fried, 2006). The liver synthesizes and secrete selenium-rich selenoprotein P into the plasma to supply extrahepatic tissues with selenium. During liver damage, this synthetic function may be impaired leading to deterioration of the hepatic reserve hence the low level observed in the presence of inadequate dietary intake. The release of Cu during tissue damage mediated by inflammatory responses may account for increased circulating Cu concentrations (Guo, Wang, Chen, and Yang, 2011). Biliary excretion serves as the major route of copper excretion/elimination. It is known that CLD may lead to cholestasis because of either a functional defect in bile formation (at the level of the hepatocytes) or impairment in bile secretion or flow (at the bile duct level), causing impaired biliary excretion of copper, hence the elevated level. Alteration in some trace minerals may lead to their interaction, such as zinc and copper; the excesses of one trace mineral may lead to the deficiency of another (Festa, Anderon, Dowdy and Ellersieck, 1985) as observed in our study. A couple of studies in Egypt reported high concentrations of copper among hepatitis B, C patients and in children with CLD respectively (Sayed, El-Ayyat, El-Dusoki, Zohairy, M. and Mohammed, 2005; Ibrahim, 2013).

Conclusion
Our study demonstrated an increased oxidative stress level in chronic liver disease patients. Both antioxidant enzymes and their corresponding trace minerals are depleted consequently.

Recommendation
Further studies are required to prove if micronutrient supplementation will boost their defence against free radical injury, limit tissue damage and improve overall outcomes.

Conflict of interest
There are no conflicts of interest to declare.

References


Prevalence of Hepatitis B virus among patients diagnosed with liver cirrhosis disease in Kano, Northern Nigeria.

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