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Research Article
Micronutrients in the Clinical Management of Hepatitis C Virus Infected Patients

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Abstract

Background and Objective: Hepatitis C virus (HCV) is a blood borne virus. Various nutrients have been identified to be associated with suppression or promotion of HCV proliferation. This research aimed to assess the dietary quality and the micronutrient status of patients infected with hepatitis C virus. **Materials and Methods:** A total of 130 patients diagnosed with hepatitis C who attended outpatient clinic at University of Nigeria Teaching Hospital and 120 healthy control subjects were recruited for this study. A 24 h dietary recall was obtained using a well-structured questionnaire; serum levels of zinc and iron were measured in the HCV patients and control subjects. **Results:** The result showed that less than 50% of the study sample consumed fruits, vegetables, meat/fish/egg and milk/dairy products. The plasma zinc level was below the optimal range in 94 (72.3%), within the optimal range in 31 (23.8%) and above the optimal range in 5 (3.8%) patients. A significant decrease in total iron and transferrin saturation levels and a significant increase in total iron binding capacity (TIBC), ferritin and transferrin levels was observed in the infected male and female patients when compared with the control subjects. **Conclusion:** Reduction in the dietary iron intake and consuming high amount of foods rich in zinc along with conventional therapies should be encouraged as diet alone could not supply all the required amount of zinc to HCV patients.

Key words: Hepatitis C Virus, micronutrient, nutrients, zinc, dietary iron

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hepatitis C virus (HCV) is a positive-sense enveloped RNA flavivirus that infects the hepatocytes through exposure to infected body fluids[1,2]. Like many viruses, HCV has the ability to impede antiviral and apoptotic responses to favour its own persistence[3]. Nigeria has a high burden of viral hepatitis C at a prevalence rate of 2.2%[4] and approximately 70% of acute HCV infection progresses to chronic liver disease. The current standard of care for the management of chronic HCV infection is based on the combination of interferon and ribavirin. The suppression or promotion of HCV proliferation have been identified to be associated with various nutrients[5] and balanced micro- and macro-nutrient consumption is an important issue in the clinical management of HCV patients[6]. It was observed that a high intake of macronutrient is associated with more advanced fibrosis in HCV patients[7]. Some micronutrients are not only utilized by viruses such as the hepatitis C virus to spread but are also necessary to produce an effective immune response to viral infections[8].

In Hepatitis C virus infection, the induction of an acute inflammatory response driven by pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α can lead to hepatocyte oxidative stress and mitochondrial dysfunction[9]. Hepatocyte oxidative stress could lead to chronic hepatocyte damage which stimulates inflammation that can lead to the development of liver fibrosis and, ultimately, cirrhosis and cancer[10]. HCV infection is associated with oxidative stress which plays an important role in the onset and progression of liver disease[11]. Numerous micronutrients serve as key components of the hepatic antioxidant response and these micronutrients can become significantly impaired upon micronutrient deficiency. A major example is zinc which is tightly bound to metallothionein (MT), a chaperone protein which is found in the liver[12] and functions as intracellular sensors of oxidative stress which acts to scavenge free radicals[13]. Zinc is found in a variety of foods including meats, cereals, grains, beans and dairy products[14,15] and plays an important role in cell growth, immune system, wound healing, membrane integrity differentiation, apoptosis, metabolism and is involved in cytoprotection of hepatocytes against oxidative stress[16,17]. Zinc is required for optimal innate immune defences including phagocytosis, natural killer cell activity, generation of oxidative bursts, cytokine production and complement activity[18,19]. In hepatitis C virus infection, inflammatory cytokines such as interleukin (IL)-6 stimulate hepatic zinc uptake via the Zip14 zinc transporter, resulting in transient hypozincemia[20]. The elevated cytosolic zinc up-regulates metallothionein expression, which exerts numerous antioxidant and anti-viral effects[12]. Hypozincemia, a deficiency of zinc is associated with the progression of chronic liver diseases[21,22]. Another example is iron which plays a key role in DNA and protein synthesis, electron transport, erythrocyte production, cell proliferation, regulation of gene expression and cellular respiration[23]. Being an oxidant with free radical activity, excess of iron leads to the breakdown of cellular membranes, ultimately leading to damage the organs such as kidneys, heart, lungs and liver[24].

Liver is the major organ responsible for the storage and metabolism of many micronutrients such as iron and zinc[25], chronic HCV replication has the potential to negatively influence the state of these micronutrients. As such, there could be an imbalance between the dietary micronutrient content and micronutrient availability during infection. This study was initiated aimed to assess the dietary quality and the micronutrient status of hepatitis C patients.

MATERIALS AND METHODS

Subjects: This study recruited 130 Hepatitis C patients (60 males and 70 females) attending clinic at University of Nigeria Teaching Hospital (UNTH) Ituku- ozalla, Enugu state. The subjects were considered for recruitment if they were: confirmed hepatitis C patients, expressed willingness to participate and gave their informed consent, able to complete all questionnaires, within the age range of 18-40 years and not on vitamins and mineral supplements. The subjects were excluded if they were not hepatitis C patients, unwilling to participate or unable to give informed consent, on drugs and suffered from any other illness. One hundred and twenty (120) subjects that were not infected with hepatitis C virus were also recruited to serve as control. The control subjects were considered for recruitment if they were not hepatitis C patients, expressed willingness to participate and gave their informed consent, within the age range of 18-40 years, not on vitamins and mineral supplements. Control subjects that were unwilling to participate or unable to give informed consent, on drugs and suffered from any other illness like malaria were excluded from the study.

Data collection: Data on different foods consumed within the last 24 h was collected using a 24 h food consumption recall questionnaire. A standard protocol was used to take 24 h food recall. The questionnaire included a detailed description of the foods eaten, the type and size of food taken as breakfast, lunch, dinner and in-between meals. The amounts of each

food consumed were estimated in reference to common household measures such as bowls, cups, glasses and spoons. The respondents were shown visual aids to assist them in accurate reporting of food intake.

Dietary diversity score (DDS): Dietary diversity score which quantifies the number of food groups in a diet consumed over a reference period was calculated by summing the number of unique food groups consumed during last 24 h as described by Krebs-Smith *et al.*[26]. Food groups considered were cereals/roots, vegetables, fruits, legumes, meat/fish/egg and milk/dairy products.

Collection of blood samples and assays: Only 3 mL of blood sample was obtained from the cubital fossa under aseptic conditions and transferred into a plain tube for the determination of iron and zinc levels. The plasma zinc levels were determined using atomic absorption spectrophotometer as described by Lee *et al.*,[27]. Serum iron markers included total iron (μmol L^{-1}), transferrin saturation (%), transferrin (g L^{-1}), total iron-binding capacity (μmol L^{-1}) and ferritin (μg L^{-1}) concentrations were measured in serums obtained from fasting patients using standard techniques used in the laboratories of clinical chemistry.

Ethics: The procedures followed in this study were in accordance with the ethical standards of University of Nigeria ethics committee on human experimentation. The study protocol was approved by the College of Medicine Research Ethics Committee, under the Directorate of Research and Publication, College of Medicine, University of Nigeria, Enugu Campus (Protocol No: 097/12/2019). Informed consent was obtained from each of the participant.

Statistical analysis: Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS, version 24, Chicago, Illinois, USA). Frequencies and percentages were used for each variable; the Chi-squared test was used to study the relationship between variables and the t-test was used to determine the difference between means.

RESULTS

24 h dietary recall: Food consumption record obtained through 24 h dietary recall method showed that 86% of the patients had consumed legumes during the previous day of the survey. Food groups like cereals/roots and meat/fish/egg were also consumed by 82 and 43% of the patients respectively. Vegetables and fruits were consumed by 37% and 31% of the patients respectively while 24% had consumed at least one milk/dairy product during the previous day of the survey. Less than 50% of the subjects had consumed fruits, vegetables, meat/fish/egg and milk/dairy products (Fig. 1). Food consumption record of the control subjects obtained

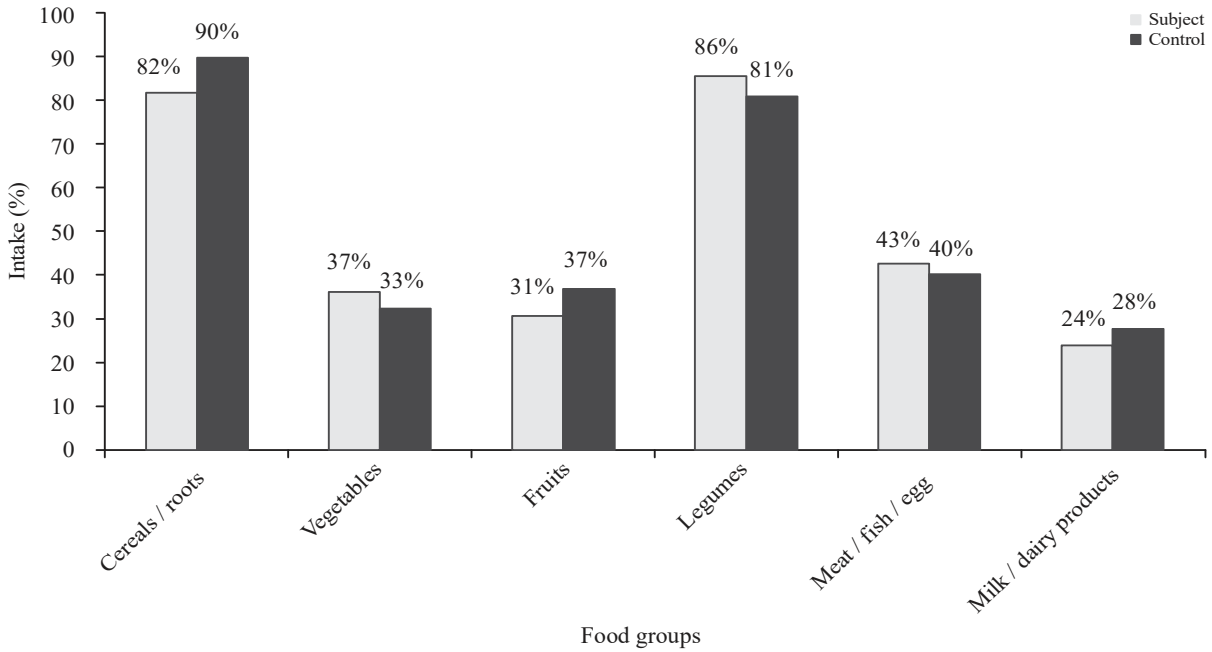

Fig. 1: Food consumption record of HCV patients

Table 1: Plasma zinc levels of HCV infected subjects and the control subjects

Range	Subjects	Control
Below the optimal range (<90 μg dL^{-1})	94 (72.3%)	2 (1.7%)
Optimal range (90-1590 μg dL^{-1})	31 (23.8%)	105 (87.5%)
Above the optimal range (>150 μg dL^{-1})	5 (3.8%)	13 (10.8%)

Table 2: Serum iron markers of male HCV infected subjects and controls

Variables	HCV infected males	Controls
Total iron (μmol L^{-1})	15.81±8.94*	28.33±1.03
Total iron binding capacity (μmol L^{-1})	88.53±4.68*	67.00±4.10
Ferritin (μg L^{-1})	644.35±98.42*	227.67±43.95
Transferrin saturation (%)	17.82±10.21*	42.67±1.37
Transferrin (g L^{-1})	3.54±0.91*	2.68±0.16

*p<0.05 (significant)

through dietary recall showed that 81% had consumed legumes during the previous day of the survey. Food groups like cereals/roots and meat/fish egg were also consumed by 90 and 40% of the patients respectively. Vegetables and fruits were consumed by about 33 and 37% respectively while 28% had consumed at least one milk/dairy product during the previous day of the survey (Fig. 1).

Plasma zinc level: The plasma zinc level varied markedly and was below the optimal range in 94 (72.3%) subjects, within the optimal range in 31 (23.8%) and above the optimal range in 5 (3.8%) subjects. In the control subject, 2 (1.7%) were below the optimal range while 105 (87.5%) and 13 (10.8%) were within the optimal range and above the optimal range respectively (Table 1).

Serum Iron markers in HCV infected subjects and control subjects: Males revealed significant increase ($p<0.05$) in ferritin (644.35±98.42 μg L^{-1}), total iron binding capacity (TIBC) (88.51±4.68 μmol L^{-1}), transferrin (3.54±0.91 g L^{-1}) and significant decrease ($p<0.05$) in iron (15.81±8.94 μmol L^{-1}) and transferrin saturation (17.82±10.21%) compared to control subjects the results showed the values for ferritin (227.67±43.95 μg L^{-1}), TIBC (67.00±4.10 μmol L^{-1}), transferrin (2.68±0.16 g L^{-1}), iron (28.33±1.03 μmol L^{-1}) and transferrin saturation (42.67±1.37%), (Table 2).

Females revealed significant increase in ferritin (502.78±350.14 μg L^{-1}), TIBC (89.30±11.07 μmol L^{-1}), transferrin (3.57±0.44%) and significant decrease in iron (13.43±7.66 μmol L^{-1}), transferrin saturation (14.78±7.79%) compared to control the results showed ferritin (82.67±19.13 μg L^{-1}), TIBC (53.33±2.73 μmol L^{-1}), transferrin (2.13±0.11 g L^{-1}), iron (21.33±1.37 μmol L^{-1}), transferrin saturation (40.00±0.89%), (Table 3).

Table 3: Serum iron markers of female HCV infected subjects and controls

Variables	HCV infected females	Controls
Total iron (μmol L^{-1})	13.43±7.66*	21.33±1.37
Total Iron binding capacity (μmol L^{-1})	89.30±11.07*	53.33±2.73
Ferritin (μg L^{-1})	502.78±350.14*	82.67±19.13
Transferrin saturation (%)	14.78±7.79*	40.00±0.89
Transferrin (g L^{-1})	3.57±0.44*	2.13±0.11

*p<0.05 (significant)

DISCUSSION

Micronutrient deficiency or excess is caused by several factors. It could be due to disease pathologies such as chronic viral infection, geographic location, socioeconomic or nutritional status. Excess or deficiency of micronutrients can significantly affect both the innate and the adaptive arms of antiviral immunity since they are essential for a strong immune response. In most disease conditions, innate immune response is crucial for the protection against hepatitis C. Some of the micronutrients influence antioxidant and inflammatory responses and are necessary to elicit an effective immune response to viral infections but are also utilized by viruses such as the hepatitis C virus to propagate.

Zinc serves important functions in the body and is found in foods such as meats, cereals, grains, beans and dairy products. Fig. 1 shows that the control subjects consumed high amounts of foods which are the main sources of zinc. Looking at the plasma zinc levels of the infected subjects and the control subject, zinc level was below the optimal range in 72.3% of the subjects while zinc level was within the optimal range in 23.8% of the subjects despite consuming high amounts of foods rich in zinc. This could be attributed to many factors one of them is improper utilisation of zinc. Intestinal absorption and utilisation of dietary zinc depends on the overall composition of the diet and a lot of dietary factors have been shown to antagonise zinc absorption from the diet. Apart from the competitive interactions between zinc and other ions that share similar physicochemical properties, the ability of organic compounds to form poorly soluble and stable complexes with zinc can negatively affect the uptake and intestinal absorption of zinc from the diet[28]. The hepatitis C virus itself exacerbates zinc deficiency. Studies have shown that in acute hepatitis C virus infection, in ammatory cytokines such as interleukin-6 stimulate hepatic zinc uptake via the Zip14 zinc transporter, which results in transient hypozincemia[29]. The in ammatory cytokines could also lead to mitochondrial dysfunction and oxidative stress which disrupts zinc homeostasis resulting in zinc deficiency. Optimal level of zinc is beneficial in the management and treatment of

hepatitis C. The antioxidant function, regulation of the imbalance between type 1 T helper (TH1) and type 2 T helper (TH2) cells, enhancement of antiviral effects of interferon, inhibitory effects of zinc in the HCV replicon system and hepatoprotective effects of metallothionein are those therapeutic reasons which indicate that optimal zinc level is beneficial in the treatment of chronic hepatitis C[21,30].

Iron is an oxidant with free radical activity and excess of iron can damage many organs including liver, kidneys, heart and lungs. Meat, poultry, sh, vegetables, legumes, fruits and dairy products are good sources of iron. The dietary recall showed that the subjects consumed low amounts of foods that serve as the main sources of iron in the body and this could cause the decrease in some serum iron markers observed in this study. A comparison of the serum iron markers of the HCV infected males and females with their control subjects showed a significant decrease in total iron and transferrin saturation levels; and a significant increase in total iron binding capacity (TIBC), ferritin and transferrin level. Studies have shown that individuals with HCV have elevated serum ferritin levels and increase in serum ferritin strongly correlated with the degree of hepatic injury which resulted in release of tissue ferritin stored in the liver cells[31]. HCV has also been shown to in uence the absorption of iron through oxidative stress-mediated down-regulation of hepcidin expression[32]. Consequently, elevated enterocyte ferroportin levels and increased intestinal absorption of iron leading to excess levels of some serum iron markers[33]. Elevation of serum ferritin levels and decrease in total iron observed in this study could result from acute or chronic inflammation as part of an acute-phase reaction unrelated to hepatocellular necrosis[34]. Studies have shown that serum or hepatic zinc content is reduced in various types of liver disease such as chronic hepatitis[35-37]. Studies have also shown that mild-to-moderate iron overload is common among patients with chronic HCV infection; up to 30-40% of them showing increased serum iron, transferrin saturation (which is contrary to the findings of this study) and serum ferritin level as observed in this study[38-40].

CONCLUSION

Micronutrient deficiencies is common particularly in developing countries. Serum zinc levels are often reduced in patients with chronic liver disease. For HCV patients zinc supplementation along with conventional therapies should be considered; as diet alone could not supply all the required micronutrients. Reduction in the intake of dietary iron, therapeutic iron reduction, including phlebotomy is therefore recommended for HCV-patients since they have been shown to decrease serum iron level. Iron reduction therapy in HCV patients significantly reduced lipid peroxidation and oxidative stress, which mediate the deleterious effect of iron overload on the liver. The addition of soy protein to food should be encouraged, as it reduces the iron absorption possibly due to its high phytate content thereby reducing serum ferritin.

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