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Full Length Research Paper

Iron overload in chronic Hepatitis C patients with *Candida albicans* infection

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Patients with chronic hepatitis C (CHC) often have increased liver iron. The distribution of pathogenic microorganisms including *Candida albicans* in human body mainly depend up on the concentration of iron which changes during hepatitis C virus (HCV) infection. This study aimed to measure iron overload and its correlation to *C. albicans* infection in patients with CHC. According to the presence of *C. albicans* antibodies measured using, patients were classified into 40 CHC patients with *C. albicans* positive IgG antibodies and 40 CHC patients negative for *C. albicans* IgG antibodies. Serum iron, transferren saturation and ferritin were estimated using spectrophotometric analysis. *C. albicans* IgG antibodies were estimated in 50% (40/80) of CHC patients. Statistical analysis showed that age but not gender was associated with HCV infection and duration (*P*<0.001). Comparisons with demographic and laboratory parameters showed that age (*P*<0.001); BMI (*P*<0.001); HCV-RNA (*P*<0.002); Hb (*P*<0.001); ALT (*P*<0.001); AST (*P*<0.001) were all associated with *C. albicans* infection in HCV patients compared with both CHC and control groups. A significant increase in serum iron levels, Ferritin, Transferrin and Transferrin saturation among CHC patients with *C. albicans* compared to both CHC patients and control group. The increase in iron status showed a significant correlation between aspartate aminotransferase (AST), alanine aminotransferase (ALT), body mass index (BMI), Age, Hb and HCV-RNA. This study finding suggests that iron overload disorder plays a significant role in the pathogenesis of *C. albicans* in CHC patients.

Key words: Iron overload, ferritin, transferrin saturation, hepatitis C virus- RNA (HCV-RNA), *Candida albicans*.

INTRODUCTION

Persistent infection with hepatitis C virus (HCV) is a major cause of chronic liver disease, with an estimated 170 million infected people worldwide (Shepard et al., 2005; Bialek and Terrault, 2006). It is well established that about 20% of patients with chronic hepatitis C (CHC) will progress to cirrhosis within 20 years from infection (Fattovich et al., 2004). CHC patients frequently develop mild to moderate iron overload (Bonkovsky et al., 2002). Many experimental and clinical studies (Beinker et al., 1996; Chapoutot et al., 2000; Angelucci et al., 2002), though not all (Guyader et al., 2007; Nahon et al., 2008), suggest that excessive iron in CHC is a cofactor promoting the progression of liver damage and increasing the risk of fibrosis, cirrhosis, and Hepatocellular carcinoma (HCC). Hepatic iron concentration has been inversely associated with the response to antiviral therapy (Olynyk et al., 1995; Lebray et al., 2004). Freely available iron can severely damage or destroy the whole mechanism of natural resistance, leading to rapid bacterial or fungal growth in tissue fluids. In addition, there are ample data to show that the availability of iron can and does play a critical role in many different clinicalinfections (Bullen et al., 2005).

Iron overload has been implicated as a risk factor for infections due to a variety of fungi, including *Candida (*Altes et al., 2004). Many fungi have developed sophisticated mechanisms for acquiring iron (Howard, 1999). When excess iron is available, fungal virulence is enhanced, and immune response is impaired (Bullen et al., 2005).

Candida albicans is a common fungal saprophyte for humans, growing on mucosal surfaces, including mouth

Table 1. Clinical and demographic characteristics of the study population. Values were expressed as Mean ± SD.

Parameter	Controls (20)	CHC patients (40)	CHC with candidasis (40)	P-value
Age (Year) (mean ± SD)	29±6.5	44.6±4.3	46.3±4.9	0.001
Gender (Male/Female)	12/8	36/4	38/2	-
BMI (mean ± SD)	16.5±4.8	21.5±4.2	22.2±4.6	0.001
HCV genotype		4; 2 (39/1)	4;2 (38/2)	-
HCV-RNA (IU/ml)		5.94×10^5	6.7×10^5	0.002
C. albicans IgG ELISA		---	48.1±10.95	_
Blood culture test of *C. albicans*				
100 cells/ml of blood			+++	
10 cells/ml of blood			+++	
1 cell/ml of blood			++	
Duration of CHC (years)		4.72 ± 1.7	6.5 ± 2.7	0.001
Duration of Candidasis (years)		---------	3.5 ± 1.7	_

P = positive results: +++ / ++ = confluent growth; + = uncountable colonies. Values are mean ± SD.

and gut, of up to 60% of normal individuals (Glick and Siegel, 1999). Under special circumstances, this pleiomorphic fungus becomes invasive and disseminates via the blood stream. Disseminated *Candida* infections, occurring most frequently in the setting of neutropenia, cause tremendous morbidity and mortality (Rex et al., 2000). Iron is an essential nutrient for *C. albicans*, and iron uptake may play a special role in promoting virulent infections. However, the iron sources that support intravascular infection and the fungal iron uptake systems that mediate iron acquisition in the intravascular space have not been defined (Ramanan and Wang, 2000).

The majority of iron in healthy human exists as heme in hemoglobin and a smaller amount is bound to transferring. It was reported that *C. albicans* has a hemolytic activity, and plasma membrane proteins capable of binding hemin and hemoglobin (Luo et al., 2001; Pendrak et al., 2004; Santos et al., 2003; Weissman and Kornitzer, 2004). The uptake pathway for hemin iron depends on the intracellular enzyme heme oxygenase (Hmx1p) to release iron from the porphyrin chelate (Santos et al., 2003; Pendrak et al., 2004). The role of this system during intravascular infection has not been tested. Finally, a reductive system exists, located in the plasma membrane of *C. albicans* and consisting of three activities, each encoded by multiple genes. This represents a challenge for clinical medicine to investigate the role of iron metabolism availability in chronic CHC with *C. albicans* infections.

MATERIALS AND METHODS

Patients

A total of 100 individuals selected from patients admitted to different Hospitals in Riyadh, Saudi Arabia. Out of these twenty age and sex matched healthy individuals (12 men and 8 women, between 14 and 66 years of age) with a mean age of 29±6.5 were selected as controls. Eighty chronic Hepatitis C patients (35 men and 20 women, aged from11 to 64 years of age) with a mean age of 44.6±4.3 were included in this study. Patients were classified according to the presence of *C. albicans* antibodies by ELISA into 40 CHC positive with *C. albicans* antibodies and confirmed by blood culture test and 40 CHC patients with negative *C. albicans* antibodies.

Patients and control subjects who were smokers and had past or concurrent diseases like anemia, abnormal lipid profile, diabetes mellitus, cardiovascular diseases, hepatitis B virus (HBV) were excluded from the study. Also subjects with iron supplementation or overweight and obesity (BMI: ≥ 25 and ≥ 30 kg/m^2) were excluded from the study. All subjects completed a structured questionnaire with questions regarding demographic data, tobacco usage and daily medication use. The demographics and baseline characteristics of patients and controls are presented in Table 1. The study protocol was approved by ethical committee of King Khalid University Hospital.

Polymerase chain reaction (PCR)

All chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). The HCV RNA was extracted by the silica method. PCR was performed as described by Boom et al. (1990).

HCV quantitative test

HCV RNA quantification was done by using Smart Cycler II Real-time PCR (Cepheid, Sunnyvale, Calif. USA) with HCV RNA quantification kits (Sacace Biotechnologies, Italy). HCV- RNA was isolated according to the manufacturer's instructions. Briefly, serum samples were treated with cell lysis solution containing 5 µl internal control (IC) (Sacace Biotechnologies Caserta, Italy). The presence of quantitative HCV IC allows not only to monitor the extraction procedure and to check possible PCR inhibition but also to verify possible losses of the RNA during extraction procedure thus enabling to calculate precisely the HCV viral load. Also, the presence of two positive controls of the extraction: Pos1 HCV low viral load and Pos2 HCV medium viral load which carried through

all steps and allow quality control of the conducted analysis. HCV-RNA pallet was solubilized in 1% DEPC (Diethyl pyrocarbonate treated water).

HCV RNA quantification was performed in which amplification and diagnosis were detected at the same time using fluorescent probes to investigate amplification after each replicating cycle according to the manufacturer's instructions of HCV quantitative kit (Sacace Biotechnologies Caserta, Italy). The lower and upper detection limits of the used assay were 250 and 5.0×10^8 IU/mL, respectively. Specimens yielding values above the upper limit were diluted 100-fold, retested and the obtained values were multiplied by this dilution factor to get the actual HCV RNA concentration in international units (IU) per ml.

HCV genotyping

HCV genotyping technique was done for sera of HCV positive patients using PCR technique. The method was done as described Okamoto et al. (1992).

C. albicans IgG ELISA

The *C. albicans* IgG antibodies were identified in the serum of HCV patients using ELISA test kit (Cat. No.: RE56171, IBL Immuno Biological Laboratories).

C. albicans blood culture

A total of 80 samples taken from BacT/Alert blood culture bottles (Organon Teknika Corporation, Durham, N.C.), including samples from bottles inoculated with blood from 40 CHC patients with positive *C. albicans* IgG antibodies using ELISA, were tested. *C. albicans* strain ATCC 24433 and blood samples of CHC with negative *C. albicans* IgG antibodies were used as positive and negative control samples. After the addition of yeast cells, inoculated bottles were immediately placed in the automated BACTEC 9240 incubator system.

This system incubates specimens at 35°C for 5 days with continuous agitation and uses a fluorescent technology to detect the quantity and rate of CO_2 production (indicative of microbial growth). Blood culture bottles were removed from the incubator after the automated system determined that they were positive. Also, two milliliter aliquots from bottles shown to contain yeasts by Gram staining were removed and stored at 30°C. Routine phenotypic culture identification consisted of isolation from positive blood culture bottles on chocolate and Sabouraud dextrose agar plates (BBL, Division of Becton-Dickinson, Cockeysville, Md.), assessment for germ tube and chlamydospore formation, and API 20C (BioMerieux, Hazelwood, Mo.). Five to ten colonies were suspended in sterile saline and centrifuged for 10 min at 1,500 rpm. The pellet was washed twice and a suspension of turbidity equivalent to 1000 cells/ml was prepared. The number of cells in this suspension was confirmed by counting the cells in Neubauer's chamber (Carvalho, 1978).

Direct species identification

Subcultures of positive blood cultures were carried out on solid medium CHROM agar (KIMA, Padova, Italy). The original suspension of 1000 cells of *C. albicans*/ml was serially diluted to obtain inoculums tenfold more concentrated than the final tests, that is, 100, 10 and 5 cells/ml.

One milliliter of these suspensions was inoculated into each of the bottles or tubes, which had been previously inoculated with 9 ml of blood. Accordingly, to perform tests with 100 cells of Candida/ml of blood, 1 ml of the suspension containing 1000 cells/ml was used; to obtain tests containing 10 cells of Candida/ ml of blood the inoculum with 100 cells/ml was used, and so on up to 1 cell/ml of blood. After the inoculation the samples were incubated at 35°C for 24 h.

All yeast isolates observed on CHROM agar were identified by colony morphology and pigmentation (Horvath et al., 2003). Each plate was read on day 1 to 4 with emphasis placed on recording the colony color (green), size, texture, presence of color diffusion into the surrounding agar, and gram stain method.

Laboratory data

Laboratory test results used, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed using standard kits (Max Discovery™ Color Endpoint Assay kits Cat. No. BO_ 5605-01 and BO_3460-08, Bioo Scientific Co., USA.). Availability of the results of routine laboratory studies such as complete blood count (CBC), serum samples were also used to measure contemporaneously iron, transferrin, and ferritin.

Estimation of serum iron

Simple, direct and automation-ready procedures for measuring iron concentrations were designed to measure total iron directly in serum without any pretreatment using BioAssay Systems' iron assay kit (QuantiChrom™ Iron Assay Kit, DIFE-250). The improved method utilizes a chromogen that forms a blue colored complex specifically with Fe^{2+}. Fe^{3+} in the sample is reduced to Fe^{2+}, thus allowing the assay for total iron concentration. The intensity of the color, measured at 590 nm, is directly proportional to the iron concentration in the serum. The optimized formulation substantially reduces interference by substances in the raw samples.

Estimation of serum ferritin using enzyme linked immunosorbent assay (ELISA)

Serum ferritin was analyzed by an immunometric assay. The ferritin quantitative test is based on a solid phase ELISA (human ferritin enzyme immunoassay kit, Catalog Number: 40-052-115015). The assay system utilizes one rabbit anti-ferritin antibody for solid phase (micro titer wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution.

The test sample allowed reacting simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45 min incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated at room temperature for 20 min, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of ferritin is directly proportional to the color intensity of the test sample.

Estimation of serum transferrin and transferrin saturation

Serum transferrin was analyzed by an immune enzymatic ELISA assay. Human Transferrin ELISA kit (Alpha Diagnostic Int., ELISA Kit Cat.# 1210) is based on binding of human transferrin from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish

Table 2. Biochemical characteristics of the study population. Values were expressed as Mean ± SD.

Biomarkers	Controls (20)	CHC patients 40	CHC with candidasis(40)	P-value
Hb (g/dl)	14.3±1.0	15.0±0.062	14.94±2.48	< 0.001
ALT (IU/l)	20.9±2.1	79.5±13.1	104.6± 15.1	< 0.001
AST (IU/l)	24.4±3.9	67.8± 12.0	78.03±15.9	< 0.001
Serum total Iron (µg/dL)	79.0±22.1	125.84±4.41	147. 6 ± 6.12	< 0.001
Serum Transferren (g/l)	2.3±0.23	4.58±0.68	5.74±1.43	< 0.001
Serum Transferren (%)	24.5±4.1	31.9±4.4	41.53±9.3	< 0.001
Serum Ferritin (µg/1)	70.5±14.6	139.7±4.85	149.3± 7.5	< 0.001

peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of transferrin present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm, and the concentration of transferrin in samples and control is read off the standard curve. Transferrin saturation was calculated by dividing the serum iron level by total iron-binding capacity (TIBC) and multiplied by100 as shown in the following equation:

$$\text{Transferrin saturation (\%) (T sat)} = \frac{\text{Serum iron}}{\text{TIBC}} \times 100$$

Statistical analysis

Variables were summarized by standard descriptive statistics and expressed as mean ±SD. The results obtained were analyzed statistically using the unpaired student's 'z' test, to evaluate the significance of differences between the mean values. Spearman's correlation was used to calculate the association of variables between groups. *P* values > 0.05 were considered statistically significant. All statistical analysis was performed using the SPSS 11.5 package program.

RESULTS

The 80 subjects were predominantly (90-95%) male with a mean age of (45-46) years; 96% of patients were infected with HCV genotype 4. *C. albicans* IgG antibodies were estimated in 50% (40/80) of CHC patients, the results confirmed with blood culture test which indicates the presence of *C. albicans* (Table 1). Statistical analysis showed that age but not gender was associated with HCV infection and duration (*P*<0.001). Comparisons with demographic and laboratory parameters showed that age (*P*<0.001); BMI (*P*<0.001); HCV-RNA (*P*<0.002); Hb (*P*<0.001); ALT (*P*<0.001); AST (*P*<0.001) were all associated with *C. albicans* infection in HCV patients compared with both CHC and control groups (Table 2).

In subjects with HCV infection the mean serum concentrations of iron were significantly (*P*<0.001) higher compared to healthy subjects (Table 2). Serum levels of iron among HCV patients with *C. albicans* infection were also significantly higher (*P*<0.001) than such levels among CHC patients (*P*<0.001) (Table 2).

Mean serum levels of ferritin were markedly higher among subjects with HCV infection compared to healthy subjects (*P*<0.001). Interestingly, serum levels of ferritin were much higher among CHC patients with *C. albicans* infection when compared with CHC patients (*P*<0.001) (Table 2). In HCV patients with *C. albicans* infection, the mean serum of transferrin and transferrin saturation (95% CI, *P*<0.001) were higher compared to both CHC patients and healthy controls (Table 2).

In CHC patients, Serum levels of ferritin were significantly (*P*<0.02 and *P*<0.01) and directly correlated with serum levels of ALT and AST. Similarly, serum levels of iron and transferrin saturation were significantly positively correlated with serum levels of ALT and AST, whereas Hb % were inversely correlated (*P*<0.03) with these measurements of iron status. Only, Ferritin and Tranferren saturation were significantly correlated with Age and BMI (*P*<0.006; *P*<0.005) respectively. However, HCV-RNA levels showed negative correlations with serum levels of iron, Ferritin, Transferren and Transferren saturation (Table 3).

In CHC with *C. albicans* infection, Serum levels of ferritin were significantly (*P*<0.01) and directly correlated with serum levels of ALT and AST. Similarly, serum levels of iron and transferrin saturation were significantly positively correlated with serum levels of ALT and AST, whereas Hb % were inversely correlated (*P*<0.01) with these measurements of iron status. Only, serum level of iron and Transferren saturation were significantly correlated with Age and BMI (*P*<0.006; *P*<0.0003) respectively. However, HCV-RNA levels showed a negative correlation with serum levels of iron, Ferritin, Transferren and Transferren saturation (Table 4).

DISCUSSION

Most of the HCV-infected patients develop a chronic slowly progressive liver disease that may result in cirrhosis and hepatocarcinoma. Several factors have been proposed to explain this unfavorable evolution such as male gender, age at infection and alcohol abuse (Poynard et al., 2001). Recently, the role of iron has been pointed out as an important element in the natural

Table 3. Correlations of serum levels of Ferritin, iron and transferrin saturation with liver enzyme levels, viral overload (Serum HCV-RNA levels), Hb and demographic parameters among CHC patients.

Level or count	Serum levels of iron		Serum levels of Ferritin		Serum levels of Tranferren		Serum levels of Tranferren %	
	r	P	r	P	r	P	r	P
Age	- 0.194	0.22	0.426	0.006	- 0.21	0.2	0.10	0.57
BMI	0.016	0.92	0.04	0.79	- 0.102	0.52	0.435	0.005
Hb	- 0.076	0.03	- 0.013	0.03	- 0.313	0.03	-0.156	0.03
ALT	0.143	0.02	0.049	0.02	0.194	0.02	0.218	0.02
AST	0.386	0.01	0.175	0.01	0.285	0.01	0.278	0.01
Viral Load	0.159	0.32	0.105	0.51	0.010	0.93	0.063	0.69

Table 4. Correlations of serum levels of Ferritin, iron and transferrin saturation with liver enzyme levels, Serum HCV-RNA levels), Hb and demographic parameters among CHC with positive *C. albicans* antibodies.

Level or count	Serum levels of iron		Serum levels of Ferritin		Serum levels of Tranferren		Serum levels of Tranferren %	
	r	P	r	P	r	P	r	P
Age	0.238	0.14	0.075	0.64	0.048	0.76	-0.427	0.006
BMI	-0.539	0.0003	0.086	0.59	0.019	0.91	0.101	0.53
Hb	-0.035	0.01	-0.015	0.01	-0.109	0.01	-0.214	0.01
ALT	0.012	0.01	0.235	0.01	0.178	0.01	0.182	0.01
AST	0.418	0.01	0.034	0.01	0.240	0.01	0.375	0.01
Viral Load	0.003	0.98	0.092	0.57	0.136	0.4	0.21	0.19

history of hepatitis C. In fact, serum iron stores are frequently increased in chronic HCV-infected carriers but little is known about the significance of these abnormalities (Fujita et al., 2007; Desai et al., 2012).

The present study shows that serum levels of Ferritin in CHC patients were significantly (P<0.02 and P<0.01) and directly correlated with the increase in serum levels of ALT and AST. This matched with Rigamonti et al. (2002) who reported that serum levels of ferritin were directly correlated with the concentration of iron in the liver and with grading and staging scores for chronic hepatitis. In the same manner, Hofer et al. (2004) reported that higher serum levels of ferritin at baseline, but not a higher serum transferrin saturation value or a higher concentration of iron in the liver, were predictors of a poor response to antiviral therapy in patients with chronic CHC. Most studies reported that, higher levels of iron have been associated with the failure of IFN treatment for CHC and with the development of more-severe fibrosis in individuals with CHC (Bonkovsky et al., 2002; Pietrangelo et al., 2002; Van Thiel et al.,1994).

Similarly, serum levels of iron and transferrin saturation in CHC patients were significantly correlated with the increase in serum levels of ALT and AST. The finding of abnormal results of liver function tests, particularly for ALT, AST, and GGTP levels, often triggers evaluations that lead to the diagnosis of liver disease (Prati et al., 2002).

Higher serum levels of iron were strongly associated with liver disease, especially HCV infection. Ruhl and Everhart (2003) recently analyzed the associations of serum concentrations of iron and antioxidants with abnormal ALT activity in patients with HCV and HBV infection, and they found that the risk for apparent liver injury was associated with higher transferrin saturation and serum iron values. In fact, higher values of transferrin saturation and serum levels of iron were consistently associated with elevated ALT, AST, or GGTP activity. This result extends earlier findings that suggested a role for iron in the development of nonhemochromatotic liver diseases (Yano et al., 2002; George et al., 1998; Fargion et al., 1997; Iwasa et al., 2002).

In the present study, Hb % of CHC patients was inversely correlated (P<0.03) with these measurements of iron status (serum iron, Ferritin, transferren and transferren saturation). The iron excess in hepatitis C may be due to hereditary hemochromatosis, hematologic diseases, multiple transfusions, porphyria cutanea tarda and chronic alcohol abuse. However, if these factors are absent the mechanisms involved in iron overload remain unclear (Ganne-Carrie et al., 2000; Martinelli et al., 2000).

However, Ferritin and Transferrin saturation of the studied CHC patients were significantly correlated with

age and BMI (*P*<0.006; *P*<0.005) respectively. This matched with Ortiz et al. (2002) who concluded that obesity, advanced age at infection, and elevated ALT levels predict rapid disease progression, suggesting that measures aimed at weight reduction may play a significant role in hepatitis C management. Nevertheless, when we analyzed data stratified for iron burden, we found a positive correlation between HCV RNA and serum iron status in CHC patients, which speculate that increasing iron load enhance the production of viral proteins.

This agrees with Theurl et al. (2004) who found a positive association between excess liver iron levels, eIF3 expression, and HCV expression when liver-biopsy samples from HCV-infected patients were analyzed. Iron and its binding proteins have immunoregulatory properties, and shifting of immunoregulatory balances by excess or deficiency in iron may produce severe deleterious physiological effects (Walker and Walker, 2000).

Our data suggested that increased serum iron status in HCV patients may affect the clinical course of HCV infection through impairment or modulation of host immune response or trigger HCV viral expression. Some studies on selected populations confirmed a significant association between a high prevalence of *C. albicans* together with Oral *Lichen planus* (OLP) in HCV patients with excess iron status (Weiss et al., 1990; Chuang et al., 1999; Palekar and Harrison, 2005). Two potential mechanisms by which excess of iron increases the risk of fungal infections has been described. As an important cofactor for enzymes involved in many basic cellular functions and metabolic pathways, iron is as essential a nutrient for the fungi as it is for humans (Schaible and Kaufmann, 2004). So, in the present study we also discussed the iron status and its correlation to the infection of *C. albicans* in CHC patients. Serum levels of ferritin were significantly (*P*<0.01) correlated to the increase in serum levels of ALT and AST. Similarly, serum levels of iron and transferrin saturation were significantly correlated with the elevated serum levels of ALT and AST.

Our data matched with others, who reported that in chronic hepatitis C patients with end stage liver disease, associated with iron overload and did not have genetic markers for hereditary hemo-chromatosis, but still had a worse outcome compared with those without iron overload (Kowdley et al., 2005), and that iron overload has been implicated as a risk factor for infections due to a variety of fungi, including Candida (Altes et al., 2004; Kowdley et al., 2005). The growth of a wide range of fungi in body fluids, cells, tissues, and intact vertebrate hosts has been shown to be stimulated by excess iron. Second, a virtually iron-free environment is required for the proper function of innate and acquired immune responses (Bullen et al., 2005). Thus, iron excess leads to direct impairment of the natural defense systems of body which ultimately, leads to enhancement of fungal virulence, and immune response is impaired (George et al., 1997; Howard, 1999).

In the present study, hemoglobin (Hb %) of CHC patients with *C. albicans* were inversely correlated (*P*<0.01) with these measurements of iron status. The data demonstrate that Hb % is greatly and stably enhances the severity of *C. albicans* infection. This matched with Yan et al. (1996) who reported that hemoglobin may be an important regulator of Fibronectin binding activity in *C. albicans* that may play an important role in the initiation and dissemination of *C. albicans* infections. Thus Hb % may play a role in the pathogenesis of *C. albicans* whereas, it was found that hemoglobin binds to a specific cell surface receptor of *C. albicans* and induces the expression of a *C. albicans* hemeoxygenase (CaHmx1p). This enzyme confers a nutritional advantage for growth and pathogenesis in mammalian hosts by limiting the immune response (Pendrak et al., 2004).

This study shows that, serum level of both iron and transferrin saturation were significantly correlated with age and BMI (*P* < 0.006; *P*< 0.0003) respectively. This matched with Zafon et al. (2010) who reported that obesity might increase the risk of iron disorder and exhibit high serum ferritin levels due to chronic inflammation.

Similarly, HCV-RNA levels showed a positive correlation with serum levels of iron, ferritin, transferrin and transferrin saturation. This is in accordance with Liu et al. (2012) who reported that, the cellular mechanisms in patients with iron overload are utilized for translating viral mRNA into protein through enhancement of the levels of eukaryotic initiation factor 3 (eIF3), which is essential for HCV translation.

Similarly, using a semi quantitative reverse transcription-PCR assay, Kakizaki et al. (2000) found that supplementation of an HCV infected non-neoplastic human hepatocytes cell line with different concentrations of iron salts enhanced HCV replication and viral protein expression.

Conclusion

This study suggests that iron overload disorder plays a significant role in the pathogenesis of *C. albicans* along with HCV in chronic hepatitis patients. A better understanding of the interplay between HCV, iron status and fungal infection may help to create novel effective strategies of the treatment of CHC patients with concomitant *C. albicans* infection.

REFERENCES

Altes A, Remacha AF, Sarda P, Sancho FJ, Sureda A, Martino R Briones J, Brunet S, Canals C, Sierra J (2004). Frequent severe liver iron overload after stem cell transplantation and its possible association with invasive aspergillosis. Bone Marrow Transplant

34:505-509.

Angelucci E, Muretto P, Nicolucci A, Baronciani D, Erer B, Gaziev J Ripalti M, Sodani P, Tomassoni S, Visani G, Lucarelli G (2002). Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. Blood 100:17-21.

Beinker NK, Voigt MD, Arendse M, Smit J, Stander IA, Kirsch RE (1996). Threshold effect of liver iron content on hepatic inflammation and fibrosis in hepatitis B and C. J. Hepatol. 25:633-638.

Bialek SR, Terrault NA (2006). The changing epidemiology and natural history of hepatitis C virus infection. Clin. Liver Dis. 10:697-715

Bonkovsky HL, Troy N, McNeal K, Banner BF, Sharma A, Obando J, Mehta S, Koff RS, Liu Q, Hsieh CC (2002). Iron and *HFE* or *Tfr1*mutations as comorbid factors and progression of chronic hepatitis C. J. Hepatol. 37:848-858.

Bonkovsky HL, Troy N, McNeal K, Banner BF, Sharma A, Obando J, Mehta S, Koff RS, Liu Q, Hsieh CC (2002). Iron and HFE or TfR1 mutations as comorbid factors for development and progression of chronic hepatitis C. J Hepatol. 37:848-854.

Boom R, Sol CJ, Salimans M, Jansen CL, Wertheim-van DPM, Noordaa J (1990). Rapid and simple method for purification of nucleic acids. J Clin. Microbiol. 28(3):495-503.

Bullen JJ, Rogers HJ, Spalding PB, Ward CG (2005). Iron and infection: The heart of the matter. FEMS Immunol. Med. Microbiol. 43:325-330.

Carvalho WF (1978). Contagem das células sangüیneas. In: Técnicas médicas de hematologia e imuno-hematologia. 2.ed. Belo Horizonte, Coop. Ed. Cult. Médica. p. 54-65.

Chapoutot C, Esslimani M, Joomaye Z, Ramos J, Perney P, Laurent C, Fabbro-Peray P, Larrey D, Domergue J, Blanc F (2000). Liver iron excess in patients with hepatocellular carcinoma developed on viral C cirrhosis. Gut 46:711-714.

Chuang TY, Stitle L, Brashear R, Lewis C (1999). Hepatitis C virus and lichen planus: A case-control study of 340 patients. J. Am. Acad. Dermatol. 41(5 Pt 1):787-789.

Desai T K, Bortman J, Al-Sibae R, Bonkovsky H (2012). The Role of Iron in Hepatitis C Infection. Curr. Hepatitis Rep. 11:41–47. DOI 10.1007/s11901-011-0120-4

Fargion S, Fracanzani AL, Sampietro M, Molteni V, Boldorini R (1997). Liver iron influences the response to interferon alpha therapy in chronic hepatitis C. Eur. J. Gastroenterol. Hepatol. 9:497-503.

Fattovich G, Stroffolini T, Zagni I, Donato F (2004). Hepatocellular carcinoma in cirrhosis: incidence andrisk factors. Gastroenterology 127:S35-S50.

Fujita N, Horiike S, Sugimoto R, Tanaka H, Iwasa M, Kobayashi Y , Hasegawa K, Ma N, Kawanishi S, Adachi Y, Kaito M (2007). Hepatic oxidative DNA damage correlates with iron overload in chronic hepatitis C patients. Free Radical Biol. Med. 42:353-362.

Ganne-Carrie N, Christidis C, Chastang C (2000). Liver iron is predictive of death in alcoholic cirrhosis: a multivariate study of 229 consecutive patients with alcoholic and/or hepatitis C virus cirrhosis: a prospective follow up study. Gut 46:277-282.

George DK, Goldwurm S, MacDonald GA, Ducot B, Pelletier1 G, Beaugrand M, Buffet C (1998). Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. Gastroenterology 114:311-318.

George MJ, Snydman DR, Werner BG, Griffith J, Falagas ME, Dougherty NN, Rubin RH (1997). The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Cytogam, MedImmune Inc. Gaithersburg, Maryland. Am. J. Med. 103:106-113.

Glick M, Siegel MA (1999). Viral and fungal infections of the oral cavity in immunocompetent patients. Infect. Dis. Clin. N. Am. 13:817-831.

Guyader D, Thirouard AS, Erdtmann L, Rakba N, Jacquelinet S, Danielou H, Perrin M, Jouanolle AM, Brissot P, Deugnier Y (2007). Liver iron is asurrogate marker of severe fibrosis in chronic hepatitis C. J. Hepatol. 46:587-595.

Hofer H, Osterreicher C, Jessner W, Penz M, Steindl-Munda P, Wrba F (2004). Hepatic iron concentration does not predict response to standard and pegylated-IFN/ribavirin therapy in patients with chronic hepatitis C. J Hepatol. 40:1018-1022.

Horvath LL, Hospenthal DR, Murray CK, Dooley DP (2003). Direct isolation of *Candida* spp. From blood cultures on the chromogenic medium CHROM agar Candida. J. Clin. Microbiol. 41:2629- 2632.

Howard DH (1999). Acquisition, transport, and storage of iron by pathogenic fungi. Clin. Microbiol. Rev. 12:394-404.

Iwasa M, Kaito M, Ikoma J (2002). Dietary iron restriction improves aminotransferase levels in chronic hepatitis C patients. Hepatogastroenterology. 49:529–31.

Kakizaki S, Takagim H, Horiguchi N, Toyoda M, Takayama H, Nagamine T, Mori M, Kato N (2000). Iron enhances hepatitis C virus replication in cultured human hepatocytes. Liver 20:125-128.

Kowdley KV, Brandhagen DJ, Gish RG, Bass NM, Weinstein J, Schilsky ML, Fontana RJ, McCashlan (2005). Survival after liver transplantation in patients with hepatic iron overload: The national hemochromatosis transplant registry. Gastroenterology 129:494-503.

Lebray P, Zylberberg H, Hue S, Poulet B, Carnot F, Martin S, Chretien Y, Pol S, Caillat-Zuckman S, Bréchot C, Nalpas B (2004). Influence of HFE gene polymorphisms on the progression and treatment of chronic hepatitis C. J. Viral. Hepat. 11:175-182.

Liu Y, An D, Sun R, Jin L, Wang Q (2012). Inhibition of translation initiation factors might be the potential therapeutic targets for HCV patients with hepatic iron overload. Med. Hypotheses 78(1):142-143.

Luo G, Samaranayake LP, Yau JY (2001). *Candida* species exhibit differential *in vitro* hemolytic activities. J. Clin. Microbiol. 39:2971-2974.

Martinelli AL, Franco RF, Villanova MG, Figueiredo JF, Secaf M (2000). Are haemochromatosis mutations related to the severity of liver disease in hepatitis C virus infection? Acta Haematol. 102:152-156.

Nahon P, Sutton A, Rufat P, Ziol M, Thabut G, Schischmanoff PO, Vidaud D (2008). Liver iron, HFE genemutations, and hepatocellular carcinoma occurrence in patients with cirrhosis. Gastroenterology 134:102-110.

Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugia A, Tanaka T, Sato K, Tsuda F, Miyakawa Y, Mayumi M (1992). Typing hepatitis C virus by PCR with type-specific primers. Application to clinical surveys and tracing infectious sources. J. Gen. Virol. 73:673-679.

Olynyk JK, Reddy KR, Dibisceglie AM, Jeffers LJ, Parker TI, Radick JL, Schiff ER, Bacon BR (1995). Hepatic ironconcentration as a predictor of response to interferon-alfa therapy in chronic hepatitis-C. Gastroenterology 108:1104-1109.

Ortiz V, Berenguer M, Rayón JM, Carrasco D, Berenguer J (2002). Contribution of obesity to hepatitis C-related fibrosis progression. Am. J. Gastroenterol. 97(9):2408-2414.

Palekar NA, Harrison SA (2005). Extra manifestation of hepatitis C. South Med J. 98(10):1019-1023.

Pendrak ML, Yan SS, Roberts DD (2004). Sensing the host environment: recognition of hemoglobin by the pathogenic yeast *Candida albicans.* Arch. Biochem. Biophys. 426:148-156.

Pendrak ML, Chao MP, Yan SS, Roberts DD (2004). Heme oxygenase in *Candida albicans* is regulated by hemoglobin and is necessary for metabolism of exogenous heme and hemoglobin to alpha-biliverdin. J Biol. Chem. 279(5):3426-3433

Pietrangelo A, Montosi G, Garuti C, Contri M, Giovannini F, Ceccarelli D (2002). Iron-induced oxidant stress in nonparenchymal liver cells: mitochondrial derangement and fibrosis in acutely iron-dosed gerbils and its prevention by silybin. J. Bioenerg. Biomembr. 34:67-79.

Poynard T, Ratziu V, Charlote F, Goodman Z, McHutchison J, Albrecht J (2001). Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. J. Hepatol. 34:730-739.

Prati D, Taioli E, Zanella A, Ella Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F (2002). Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann. Int. Med. 137:1-10.

Ramanan N, Wang Y (2000). A high-affinity iron permease essential for *Candida albicans* virulence. Science 288:1062-1064.

Rex JH, Walsh TJ, Sobel JD, Filler SG, Pappas P G, Dismukes WE, Edwards JE (2000). Practice guidelines for the treatment of candidiasis. Infect. Dis. Soc. Am. Clin. Infect. Dis. 30:662-678.

Rigamonti C, Andorno S, Maduli E, Morelli S, Pittau S, Nicosia G, Boldorini R, Sartori M (2002). Iron, hepatic stellate cells and fibrosis in chronic hepatitis C. Eur. J. Clin. Invest. 32(1):28-35.

Ruhl CE, Everhart JE (2003). Relation of elevated serum alanine aminotransferase activity with iron and antioxidant levels in the

United States. Gastroenterology 124:1821-1829.

Santos R, Buisson N, Knight S A, Dancis A, Camadro JM, Lesuisse E (2003). Haemin uptake and use as an iron source by *Candida albicans*: role of *CaHMX1*-encoded haem oxygenase. Microbiology 149:579-588.

Schaible UE, Kaufmann SH (2004). Iron and microbial infection. Nat Rev Microbiol. 2:946-953.

Shepard CW, Finelli L, Alter MJ (2005). Global epidemiology of hepatitis C virus infection. Lancet Infect. Dis. 558-567

Theurl I, Zoller H, Obrist P, Datz C, Bachmann F, Elliott RM, Weiss G (2004). Iron regulates hepatitis C virus translation via stimulation of expression of translation initiation factor 3. J. Infect. Dis. 190(4): 819-825.

Van Thiel DH, Friedlander L, Fagiuoli S, Wright HI, Irish W, Gavaler JS (1994). Response to interferon alpha therapy is influenced by the iron content of the liver. J. Hepatol. 20:410-415.

Walker EM Jr, Walker SM (2000). Effects of iron overload on the immune system. Ann. Clin. Lab. Sci. 30(4):354-365.

Weiss G, Umlauft F, Urbanek M, Herold M, Loyevsky M, Offner F, Gordeuk VR (1990). Associations between cellular immune effector function, iron metabolism, and disease activity in patients with chronic hepatitis C virus infection. J. Infect. Dis. 180(5):1452-1458.

Weissman Z, Kornitzer D (2004). A family of Candida cell surface haem-binding proteins involved in haemin and haemoglobin-iron utilization. Mol. Microbiol. 53:1209-1220.

Yan S, Nègre E, Cashel JA, Guo N, Lyman CA, Walsh TJ, Roberts DD (1996). Specific induction of fibronectin binding activity by hemoglobin in Candida albicans grown in defined media. Infect. Immun. 64(8):293029-5.

Yano M, Hayashi H, Wakusawa S, Sanae F, Takikawa T, Shiono Y (2002). Long term effects of phlebotomy on biochemical and histological parameters of chronic hepatitis C. Am. J. Gastroenterol. 97:133-137.

Zafon C, Lecube A, Simó R (2010). Iron in obesity. An ancient micronutrient for a modern disease. Obes. Rev. 11(4):322-328. Epub. 2009 Jul 10.