ORIGINAL ARTICLE

Serum Hepcidin Levels in Diabetic and Non-diabetic Chronic Hepatitis C Patients and Its Relation with Serum Iron Levels

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ABSTRACT

Background and Objective: Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV) that primarily affects the liver. Research shows that HCV infection leads to a marked reduction of serum hepcidin levels which might be a factor in causing systemic iron overload. The present study was aimed to determine the cause of iron overloadwhich might be due to reduced levels of serum hepcidin in chronic hepatitis C patients.

Methods: This cross-sectional study was conducted in Department of Physiology at University of Health Sciences, Lahorefrom January 2012 to January 2014. A total of 54 male patients of chronic hepatitis C were recruited for this study and divided into two groups, group A (CHC with diabetes) and group B (CHC without diabetes). Both groupswere tested for serum hepcidin, serum iron and serum ferritin levels. ELISA technique was used to measure serum hepcidin. Serum ferritin levels were measured via CODA analyzer. Serum iron levels were measured by colorimetric method. Frequencies and percentages were calculated for serum hepcidin, serum iron and serum ferritin levels in group. The data was analyzed using SPSS (Statistical Package for Social Sciences) version 20.0. Data was considered significant where P-value was ≤ 0.05.

Results: Patients in both groups showed less serum hepcidin levelsalong with less serum iron levels in 93% cases of both groups. The patients in group A showed raisedferritin levels in 26% cases and normal ferritin levels in 63% cases. Whereas in group B 04% cases showed raisedferritin levels and 92% patients hadnormal ferritin levels; whichcould be a marker of ongoing chronic inflammation in CHC patients. Non-significant negative correlation was observed between serum iron and serum hepcidin inCHC population.

Conclusion: Hepatitis C virus and decreased serum iron levels in study population may be the reason of less serum hepcidin levels. Raised/normal ferritin reflects chronic inflammation in patients. Non-significant negative correlation between serum iron and serum hepcidin leads the focus towards increasing the sample size in further studies to see any significant negative correlation between studied parameters.

KEYWORDS: Hepcidin, ferroportin, chronic hepatitis C, LEAP - (Liver antimicrobial peptide).

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INTRODUCTION

Iron, a vital biocatalyst indispensable for life, exists in nature in two forms, i.e. oxidized insoluble iron and a reduced soluble iron.[1] The role of iron in biochemical activities such as sensing oxygen, transfer of electrons in electron transfer chain and formation of complexes with organic ligands etc. makes it necessitous for life.[2]Iron in excess, forms free radicals so its regulation is important to prevent organ dysfunction.[1] Iron from diet exists in two forms i.e. heme and non-heme iron. The vectorial passage of iron from the enterocytes into the blood faces many barriers such as enterocyte membrane, translocation into cytosol and finally through basolateral membrane of enterocyte into blood. Depending upon the iron need in the body, basolateral iron exporter, ferroportin plays an important role in maintaining the iron pool in plasma. Recently discovered 25 amino acid antimicrobial peptide, a novel hormone, hepcidin is linked with the regulation of basolateral ferroportins.[3]

Hepcidin, LEAP-1 (Liver expressed antimicrobial peptide) was discovered from plasma ultra-filtrate and it plays very important role in iron homeostasis.[5] Hepcidin binds to ferroportins, mammalian cellular iron exporters, present on the basolateral surface of enterocytes, reticuloendothelial cells, macrophages, hepatocytes and placental cells.[6] Hepcidin on its interaction with ferroportins phosphorylates tyrosine residues which are present on the cytosolic phase. After phosphorylation, clathrin mediated internalization of hepcidin-ferroportin complexes takes place. Lysosomes degrade the ferroportins after removal of phosphates and ubiquination.[7]On the availability of enough iron stores in blood, signals are sent to the liver for more expression of hepcidin which internalizes the ferroportins preventing the iron efflux from the enterocytes. Contrary to this, when iron blood stores are less, the hepcidin expression is decreased accordingly and more ferroportins are expressed on enterocytes and macrophages facilitating iron release in blood.[8,9] Hepatitis C virus (HCV) by increasing histone deacetylase activity (HDAC) generates reactive oxygen species (ROS) which hypoacetylates transcriptional factors responsible for induction of hepcidin. Less hepcidin expression due to ROS in CHC infection leads to more iron absorption, eventually leading to more iron deposition in liver. HCV also alters the membrane permeability and allows calcium exit from the endoplasmic reticulum (ER). Calcium when enters the mitochondria, inhibits complex I and oxidative phosphorylation. As a result, cell has to depend more on the glycolytic pathways for energy and ROS is generated which decreases hepcidin expression and serves as a causative factor in iron overload.[10]

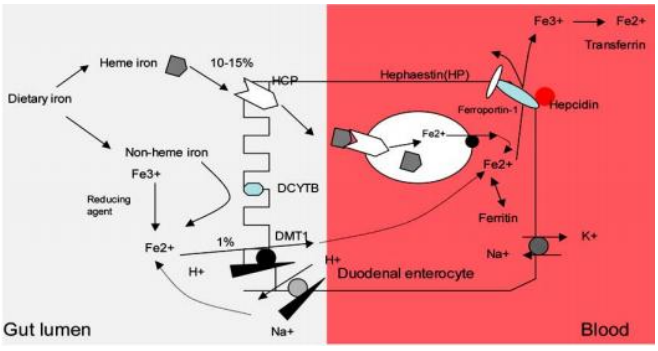

Fig.1: Digestion and absorption of heme and non-heme iron in blood.
DCYTB: duodenal cytochrome b, DMT1: divalent metal transporter, Fe2+: Ferrous iron, Fe3+: Ferric iron, H+: Hydrogen ion, HP: Haphestin, K+: Potassium, Na+: Sodium.[4]

In view of these considerations, this study was designed to evaluate the free serum iron, serum ferritin and serum hepcidin levels in chronic HCV population and correlation of serum hepcidin and serum iron in HCV patients. Rationale of this study was that early detection of decreased hepcidin levels in CHC population can be used as a therapeutic tool against iron overload by providing them with hepcidin agonists or hepcidin hormone preparations.

METHODS

This cross-sectional study was performed in Department of Physiology at University of Health Sciences in Lahore, Pakistan. A total of 54 patients from Jinnah hospital, Lahore were included after taking written informed consent from January

2012 to January 2014. Cases were divided into two groups, group A were chronic hepatitis C (CHC) with diabetes and group B were CHC cases without diabetes. Group A and B were males of 35 – 65 years age and HCV status were diagnosed by PCR. Patients infected with hepatitis B, HIV, terminally ill patients infected with hepatitis C, hepatic carcinomas and decompensated cirrhotic patients or those having BMI more than 30 kg/m^2 were excluded. Complete demographic information was taken along with the detailed history.CHC status was confirmed by PCR reports. Patients were not taking any interferon treatment during the time of study and there was no history of past treatment with interferons. Detailed physical examination was done for the proper selection of patients. For blood sample collection, 5 ml of venous blood was drawn. Blood was collected in non-coated vacutainers (yellow top) for separation of serum. Serum was separated by centrifugation at 3000 rpm for ten minutes within half an hour. The required amount of serum as mentioned in the instructions on the kit was saved in properly labelled eppendorf tubesfor all the parameters. Estimation of serum hepcidin was performed by human hepcidin (Hepc) ELISA kit. Hepcidin was added in the monoclonal antibody-enzyme wells, precoated with human hepcidin monoclonal antibodies. After incubation, biotin-labelled hepcidin antibodies were added in the well making sandwich ELISA. Streptavidin-HRP was added in the wells to form complexes. After incubation, washing was done for the removal of uncombined unused enzyme. The colorof liquid changed into blue after addition of chromogen A and B. Finally, it gave a yellow coloras a result of the effect of acid. The colorwas strongly associated with the concentration of human hepcidin. Serum iron levels were determined by colorimetric method. Cuvettes were labeled separately for the blank, standard and the sample. Samples were thawed and 50 µl of sample (serum) and 250 µl of standard were taken in labelled eppendorf tubes. In the reagent blank, standard and sample cuvettes, 1.00 ml of buffer and 0.05 ml of reductant was added. After mixing, it was incubated for at least 15 minutes at 20 – 25 degree centigrade. Final absorbance was read against the reagent blank. Determination of serum ferritin was quantitatively done and samples were pipetted in the assigned well of coated plates. An enzymatic tracer (100 µl) was added to assigned wells. After covering and protecting it from light, it was incubated for 60 minutes at room temperature. The wells were washed three times with 300 µl of diluted wash solution. TMB substrate, 100 ul was added and then it was incubated again for 10 minutes in dark at room temperature (2 – 28°C). After adding the stop solution, microplate was shaken gently. Absorbance was read against the blank at 450 nm.

STATISTICAL ANALYSIS

The data was entered and analyzed using IBM SPSS (Statistical Package for Social Sciences) version 20.0. Shapiro-Wilk's statistics was used to check the normal distribution of data. Data was considered to be non-normally distributed whereP-valuewas ≤ 0.05. Median with IQR was given for non-normally distributed quantitative variables. Frequencies and percentages were given for categorical variables. Spearman's rho correlation (rho) was used to observe correlation between non-normally distributed quantitative variables. A P-value of ≤ 0.05 was considered statistically significant for all analysis.

RESULTS

Study population included 54 males known CHC patients, which were divided into two groups. Each group included 27 cases. Group A included CHC with diabetes and group B was comprised of CHC patients without diabetes. The frequency distribution and percentages of serum hepcidin, serum iron and serum ferritin levels in groups A and B is shown in Table-1 given below. There was negative, non-significant correlation between hepcidin and iron (-0.078, P = 0.700) in CHC diabetic population and CHC population without diabetes (Fig.2).

DISCUSSION

The present study was designed with a purpose to assess the link between serum hepcidin and serum iron in chronic hepatitis C patients. In group A, 81.5% population showed decreased serum iron levels along with decreased serum hepcidin levels in 93% people. Decreased levels of iron regulatory hormone, serum hepcidin in group A could be due

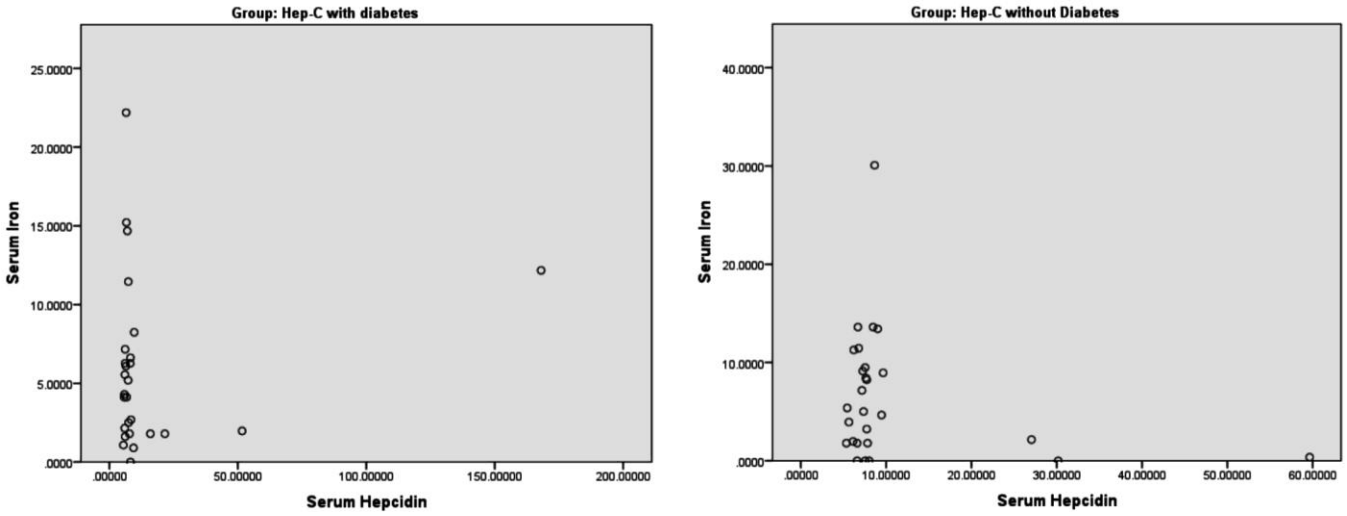

Fig.2: Correlation between hepcidin and iron, (A) Group A- CHC with diabetes (B) Group B-CHC without Diabetes r = Spearman's rho correlation coefficient, *= significant at P-value ≤ 0.05

Table-1: Frequency distribution and percentages of serum hepcidin, serum iron and serum ferritin levels in groups A and B.

Groups	Group A CHC with Diabetes (n = 27)		Group B CHC (n = 27)	
Parameter	Serum Hepcidin (ng/ml)			
	Frequency	Percentage	Frequency	Percentage
< 29 ng/ml	25	93	25	93
29 – 254	02	07	02	07
> 254	Nil	Nil	Nil	Nil
Median (IQR)	7.25 (6.11 – 8.37)		7.54 (6.59 – 8.65)	
Parameter	Serum Iron (µmol/L)			
	Frequency	Percentage	Frequency	Percentage
< 10.6	22	81.5	21	78
10.6-28.3	5	18.5	05	18.5
> 28.3	Nil	Nil	01	04
Median (IQR)	4.29 (1.79 – 7.16)		5.01 (1.79 – 9.49)	
Parameter	Serum Ferritin (ng/ml)			
	Frequency	Percentage	Frequency	Percentage
< 20	03	11	01	04
20 – 400	17	63	25	92
> 400	07	26	01	04
Median (IQR)	161 (37 – 431)		114 (60 – 201)	

CHC: Chronic hepatitis C, IQR: Interquartile Range

to two reasons, i.e. decreased serum iron levels and hepatitis C virus as supported by a study.[11] Hepatitis C virus itself is notorious in decreasing serum hepcidin levels.[12] Majority of the patients of group A showed raised/normal ferritin (26%/ 63%) levels which may be a sign of ongoing chronic inflammation. In group B, decreased serum hepcidin (93%) and serum iron levels (78%) with normal ferritin levels (92%) were observed that were consistent with the parameters of chronic illness.[13] Hepatitis C infection could be a causative factor in decreasing serum hepcidin levels in this particular group.

Correlation analysis of serum hepcidin with serum iron was done in group A and group B by applying Spearman's correlation test.In current study, group A when accessed for correlations between serum hepcidin with serum iron (r = -0.078, P = 0.700), a negative non-significant correlation was observed. Similar results were obtained by a study conducted on 30 CHC patients and 20 healthy volunteers as controls in which CHC patients showed less serum hepcidin levels along with less serum iron and raised serum ferritin levels.[14] Serum ferritin levels in CHC diabetic group (group A) signaled towards inflammatory process and it is also considered a reliable marker of fibrosis.[15] Elevated serum ferritin levels in present study population of CHC diabetic group (group A) may not be related with raised serum iron. Tissue organ grading for iron could give an indication of systemic iron overload along with serum ferritin levels. The effect regarding raised serum ferritin levels seen in CHC diabetic population was in accordance with another study in which they commented on raised ferritin levels as a cause of insulin resistance in spite of less serum iron

levels.[16] Raised serum ferritin levels, along with less serum iron and less hepcidin were also reported by another study, results of which are quite similar with present study.[14] Less serum iron in both groups with significant difference (*P* = 0.007) in serum ferritin levels in current study, especially in group A as compared to group B gave an idea that hepatic iron grading should be done to see the iron status of the patient. In present study, a non-significant negative correlation was observed between serum hepcidin and serum iron (r = -0.012, *P* = 0.953) in group B. Another study was performed on 38 CHC patients and 38 normal people, in which high serum hepcidin levels were observed in CHC population, these results were quite opposite to current study where serum hepcidin level showed non–significant correlation with serum iron (r = 0.216, P = 0.06) but significant positive correlation with hepatic iron (r = 0.378, P = 0.01).[17] A study conductedon 45 CHC patients and 15 healthy controls to access serum preprohepcidin and serum iron parameters observed no significant correlation between serum preprohepcidin and serum iron results of which are quite different from current study.[16]

CONCLUSION

The present study was designed to access the relationship between hepcidin and iron in chronic hepatitis C population. This study could not provide any mounting evidence of significant correlation between serum hepcidin and serum iron. Studies shall be conducted to access hepatic iron grading, for that cases shall be properly informed and consent shall be taken. Future studies shall also focus on assessment of mRNA hepcidin levels.

LIMITATIONS OF THE STUDY

Small sample size was a limitation in this study. Future studies shall be conducted with larger sample size to establish significant correlations between parameters if any.

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CONFLICT OF INTEREST

None to declare

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Author's Contribution

MG: Conception and design of study, drafting and critical revision of manuscript.
JS: Drafting of manuscript and data analysis.
MUB, RNH: Revising it critically for important intellectual content.
KPL: Conception and design of study and revising it critically.
F: Analysis and data interpretation and revising it critically.
ALL AUTHORS: Approval of the final version of the manuscript to be published.