ORIGINAL ARTICLE

Serum Hepcidin Levels in Patients with Chronic Hepatitis C

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ABSTRACT

Background and Objective: *Chronic hepatitis C infection and its associated mortality and morbidity is a big health challenge in Pakistan. Chronic hepatitis C infection leads to marked reduction of serum hepcidin levels leading to systemic iron overload. Present study was aimed to determine the levels of serum hepcidin in chronic hepatitis C patients and controls.*

Methods: *This cross-sectional study was conducted in Department of Physiology, University of Health Sciences, Lahore from January 2012 to January 2014. A total of 81 individualsin each of the two groups were recruited after taking written informed consent. Group A was a control group (with no history of hepatitis) while Group B comprised of chronic hepatitis (CHC) patients. Both groups were tested for serum hepcidin levels measured by ELISA technique. Frequencies and percentages were calculated for serum hepcidin levels in each group. Comparative analysis of serum hepcidin value was done between two groups by applying Kruskal Wallis test.*

Results: *In Group A, 89% subjects had reduced serum hepcidin levels while in Group B, 93% CHCpatients showed decreased serum hepcidin levels. No significant difference in serum hepcidin was seen among both groups.*

Conclusion: *Reduced hepcidin levels are seen in hepatitis C patients. Insignificant difference observed in both groups open the horizon towards other etiological factorsthat may result in decreased serum hepcidin levels in these patients.*

KEYWORDS: *Hepcidin, Liver expressed antimicrobial peptide-LEAP-1, Chronic hepatitis C, ELISA.*

INTRODUCTION

Quest for regulation of iron leads to discovery of a regulatory hormone, LEAP-1 (Liver expressed antimicrobial peptide) from plasma ultra filtrate.[1] LEAP, the novel hormone was isolated from human urine and named it as hepcidin in 2001. Hep revealed its origin from liver and cidin because of antimicrobial activity it carries.[2] Besides the liver, many other organs such as heart, kidney, brain and adipose tissue also secrete hepcidin in minor amounts.[3] Normal serum concentration of hepcidin is 20 – 200 ng/ml[4] and urinary concentration is 10 – 100 ng/ml.[5]

At the molecular level, hepcidin, the iron regulatory hormone binds with one of two tyrosine residues present on the cytosolic phase of ferroportin, iron exporters on enterocytes, macrophages and hepatocytes. After contact with hepcidin, ferroportin undergoes rapid phosphorylation, mediated by Janus Kinase (JAK 2) pathway. Clathrin-mediated internalization of hepcidin-ferroportin complexes takes place after phosphorylation, hence reduction of iron entry in plasma. Removal of phosphates and ubiquitination of ferroportin leads to degradation by lysosomes.[6]

Upregulators for hepcidin expression are increased iron stores, ER (endoplasmic reticulum) stress and inflammation. Anemia, hypoxia, oxidative stress and erythropoiesis are the down regulators for hepcidin.[7]

Hepatitis C virus (HCV) infection, by stimulating the polymorphs and Kupffer cells, serves as a major source of oxidative stress as shown in (Fig-1). HCV by increasing histone deacetylase activity (HDAC) generates reactive oxygen species (ROS) which hyperacetylates transcriptional factors responsible for induction of hepcidin. Reduced hepcidin expression due to ROS in CHC infection leads to more iron absorption eventually leading to more iron deposition in liver.[8]

In view of these considerations, this study was designed to evaluate serum hepcidin levels in patients of chronic hepatitis C as compared to control subjects without hepatitis. Rationale of the study was that early detection of decreased serum hepcidin levels in hepatitis C patients can be used as a preventive tool in CHC patients against iron overload by providing them with hepcidin agonists or hepcidin hormone preparations.

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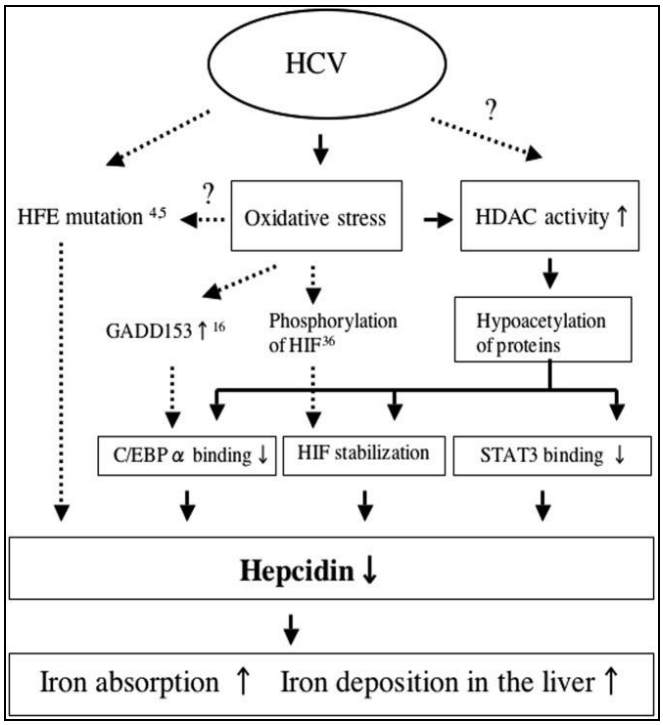

Fig. 1: *Proposed mechanisms responsible for hepcidin and iron regulation by HCV.*[8]
C/EBP α: CCAAT/enhancer-binding protein, HCV: hepatitis c virus, HDAC: Histone deacetylase, HFE: human haemochromatosis gene, HIF: hypoxia-inducible factor, STAT3: Signal transducer and activator of transcription 3.

METHODS

This cross-sectional study was performed at University of Health Sciences, Lahore from January 2012 to January 2014. Ethical approval was taken from the Board of Advance Study of University of Health Sciences, Lahore. Tests for desired parameters were performed in Physiology Laboratory of University of Health Sciences, Lahore. Total 81 subjects (35 to 65 years males) were selected according to the selection criteria by convenient non-probability sampling. Healthy subjects were selected from University of Health Sciences, Lahore. CHC patients were recruited from Jinnah hospital, Lahore. Out of two groups, group A comprised of healthy subjects without any history of hepatitis or significant illness. This group served as control group. Group B comprised of CHC patients confirmed by PCR reports who were not taking any interferon treatments during the time of study.

After written informed consent, relevant demographic information was taken along with detailed history. Detailed physical examination was done for the proper selection of patients according. Patients infected with hepatitis B, HIV, terminally ill patients infected with hepatitis C, hepatic carcinomas and decompensated cirrhotic patients, people having BMI more than 30 kg/m^2 were excluded. No history of past treatment with Interferon in patients of Group B was also an inclusion criterion.

For blood sample collection,8-12 hours of overnight fast was observed in subjects. To determine hepcidin levels, 5 ml of venous blood was drawn from subjects. Blood was collected in non-coated vacutainers (yellow top) for separation of serum. Vacutainers were kept in ice to keep them safe while transportation. Serum was separated by centrifugation at 3000 rpm for ten minutes within half an hour. Required amount of serum was saved in properly labelled Eppendorfat -80°C for future use.

Estimation of serum hepcidin was performed by human hepcidin (Hepc) ELISA kit (Glory Science Co., Ltd, 2400 Veterans Blvd. Suite 16 – 101, Del Rio, TX 78840. USA). 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. Colour of liquid changed into blue after addition of chromogen A and B. Finally, it gave yellow colour at the effect of acid. Colour was strongly associated with the concentration of human hepcidin.

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 if *P-value* was ≤ *0.05*. Median with IQR was given for non-normally distributed quantitative variables. Frequencies and percentages were given for categorical variables.

Kruskal Wallis test was used to compare variables between two and more than two groups respectively. A *P-value* ≤ *0.05* was considered statistically significant for all analysis.

RESULTS

Serum hepcidin levels were reduced i.e. < 29 ng/ml in 89% of population of group A while 11% population showed normal hepcidin levels (29-254ng/ml). In group B, 93 %patients had serum hepcidin levels less than normalwhile 7% patients had normal hepcidin levels i.e. 29-254 ng/ml (Table -1).

Median of serum hepcidin of healthy and CHC population was 7.72 (6.40 – 9.464) ng/ml and 7.25 (6.11 – 8.37) ng/ml. *P-value* after Kruskal Walis test was *0.821* which was considerably higher than significant indicating that no difference between the hepcidin values in two groups.

Table- 1: *Frequency distribution and comparison of serum hepcidin levels in study groups.*

Serum Hepcidin Levels (ng/ml)	Group A: (n = 27) Controls		Group B: (n = 27) CHC		Kruskal Wallis Test p-value*
	Frequency	%	Frequency	%	
Low (< 29)	24	89	25	93	
Normal (29- 254)	3	11	2	7	
Total	27	100	27	100	
Median (IQR)	7.72 (6.40-9.464)		7.25 (6.11-8.37)		0.821

*$P \leq 0.05$ is statistically significant

DISCUSSION

Present study was designed with a purpose to determine the serum hepcidin levels in chronic hepatitis C patients and control subjects. In group A, majority of population (89%) had reduced serum hepcidin levels that led the authors to focus on the probability of iron deficiency in Group A, as described by Wian.[9] Group A having less serum hepcidin levels can be an indicator of negative feedback to overcome iron deficiency as explained by Abosakran.[10] A total of 93% people in group B showed decreased serum hepcidin levels which could be due to two reasons i.e. decreased serum iron levels or hepatitis C virus.[11] Hepatitis C virus itself is notorious in decreasing serum hepcidin levels.[12] Similar results of decreased serum hepcidin levels are reported by Mohamed (2019) who found out a strong positive correlation between hepatitis C virus titers and hepcidin expression.[14]

In the present study, Kruskal Wallis H test was applied to determine the statistical difference of serum hepcidin between groups. No statistically significant difference was observed *(P = 0.821)* between two groups which is in accordance with the study by Ali and colleagues[13] reporting serum hepcidin levels in chronic hepatits C patients in Egypt. They did not find any significant difference in serum hepcidin levels between HCV negative and HCV positivepopulation.

CONCLUSION

HCV might be a factor in reduction of hepcidin levels in CHC population however significant reductions in serum hepcidin levels in healthy population points out towards Fe deficiency. No significant difference in comparison of serum hepcidin values between groups revealed that there might be some other factors other than hepatitis C in decreasing hepcidin levels in CHC population.

LIMITATIONS OF STUDY

Limitations of the study are that this is a single centered study with small sample size. Future studies shall be conducted with larger sample size to establish significant correlations between parameters if any. Future studies shall also focus on assessment of mRNA hepcidin levels.

AUTHOR'S CONTRIBUTION

MG: Conception and design of study, data interpretation, drafting and final approval of the version to be published.
MOS: Data analysis and interpretation.
AK: Data acquisition, study design construction.
SN: Critical revision of the manuscript for intellectual content.
MFJ: Data interpretation.
MM: Drafting and final approval of manuscript.

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CONFLICT OF INTEREST

None to declare.

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