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Original Article

Association of Hepcidin with Hepatitis C induced Diabetes Mellitus

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Abstract

Background: To compare serum hepcidin levels of patients with hepatitis C induced diabetes mellitus with healthy controls

Methods: Sixty individuals were included in the study. Thirty were diagnosed cases of chronic hepatitis C who developed diabetes mellitus during the course of HCV infection. Thirty age and gender matched healthy controls were included. Individuals with acute hepatitis C, familial diabetes mellitus, iron deficiency anemia, recent history of blood transfusion, iron or erythropoietin supplementation and inflammatory diseases like rheumatoid arthritis, renal, cardiac, pulmonary diseases and on interferon therapy were excluded from the study. Blood samples were collected from all the individuals and serum hepcidin levels were measured by ELISA.

Results: Significant decrease in serum hepcidin levels was found in patients with the chronic hepatitis C and diabetes mellitus having mean value of 2.7±1.03ng/ml as compared with controls having mean value of 28.5±5.3ng/ml. The difference among the two groups was significant at p-value of <0.001.

Conclusion: Serum hepcidin levels in patients with chronic hepatitis C with diabetes mellitus are significantly less as compared to healthy controls.

Key Words: Hepcidin, hepatitis C induced diabetes mellitus

Introduction

Hepatitis C virus (HCV) is notorious for causing acute and chronic liver disease worldwide.[1] It is an RNA virus that belongs to family Flaviviradae. According to an estimate in 85% of the cases, acute HCV infection becomes chronic and 10% of the population in Pakistan is chronically infected with Hepatitis C virus.[2] HCV infection becomes chronic when its RNA persists in blood for more than 6 months and is frequently associated with extra-hepatic manifestations such as diabetes, arthralgias and thyroiditis. About one third of the chronically infected population develops diabetes.[3] The important feature of pathogenesis of HCV induced diabetes is the development of insulin resistance. [4] Numerous mechanisms have been proposed to explain this insulin resistance including upregulation of inflammatory cytokines, hypophosphorylation of insulin receptor substrate-1 and 2, upregulation of gluconeogenic and lipogenic genes, accumulation of lipids and targeting lipid storage organelles. [2] The infection by hepatitis C virus is characterized by hepatocyte injury and iron overload has been identified as one of the factors behind it. [5]

Hepcidin, a biologically active hepatic peptide, is known as the major regulator of body iron metabolism.[6] It is synthesized primarily by hepatocytes. However, kidney tissue, pancreatic beta cells, macrophages and adipocytes have also been reported as the other sites of hepcidin production.[7] Regulation of iron homeostasis is mainly carried out by binding iron efflux channels called ferroportin [8] which are responsible for iron export from enterocytes and macrophages.[7] Hepcidin binds these iron exporting channels and cause their internalization and degradation thus decreasing iron export into blood.[8] The expression of hepcidin is regulated at transcription level by bone morphogenetic protein (BMP). Other cofactors involved in iron dependent regulation of hepcidin expression are haemochromatosis protein HFE, TfR2 (Tf receptor2), HJV (haemojuvelin), TMPRSS6.[8] Its transport in blood is in free form as well as bound to α-macroglobulin and the excretion is by kidney. [9] Conditions involving oxidative stress, hypoxia, erythropoietin or vitamin D therapy have been reported to decrease serum hepcidin levels whereas conditions involving inflammation, infection or iron supplementation increase serum hepcidin levels.[5, 9-12] Excess iron resulting from altered levels of hepcidin activates inflammatory cells and hepatic stellate cells and catalyzes the production of reactive oxygen species (ROS) and inflammatory cytokines which then evoke fibrosis. [8,13] Chronic HCV infection causes iron accumulation by hepcidin suppression which has been proposed as major mechanism responsible for causing glucose intolerance by influencing insulin signaling. [14] The accumulated iron causes increased glucose production by hepatocytes, increased fatty acid

oxidation and decreased glucose oxidation in skeletal muscles and adipocytes and altered levels of adipokines in adipocytes.[15] Furthermore, it causes oxidative stress, a factor independently responsible for causing insulin resistance. The resultant hyperinsulinemia then causes rapid iron uptake by liver since insulin redistributes transferrin receptors from an intracellular compartment to cell membrane and further exacerbates iron overload.[16]

Patients and Methods

In this descriptive study thirty adults of either gender with Blood Sugar Fasting (BSF) <110mg/dl (for group I), diagnosed patients with HCV infection for >6months and diabetes mellitus (for group II) were included via non-probability purposive sampling. After recording detailed history of every individual followed by general physical examination, individuals with acute Hepatitis C, familial diabetes, interferon therapy, iron deficiency anemia, recent history of blood transfusion, iron or erythropoietin supplementation and inflammatory diseases like rheumatoid arthritis, renal, cardiac, pulmonary and hepatic diseases (other than HCV infection) were dropped . Blood sampling was then done after a 12 hour overnight fast for the measurement of serum glucose, serum insulin and serum hepcidin levels. 5ml of blood was collected by venipuncture under aseptic measures from each individual and was transferred to gel separator tubes. It was allowed to clot and the clotted sample was then centrifuged at 2000-3000rpm for 20 minutes. The separated serum was then pippetted out into the polypropylene tubes for storage at -20^{o}C until analysis .Serum glucose levels were estimated at the time of sampling while serum hepcidin levels were measured by enzyme linked immunosorbent assay (Glory sciences, human hepcidin ELISA kit). . Means and standard deviations were calculated for quantitative variables like age, fasting blood sugar levels (BSF) and serum hepcidin levels whereas percentage and frequency were calculated for categorical variables. Serum hepcidin in the two groups were compared by independent t-test and correlation between the BSF and serum hepcidin levels was found by Pearson's correlation and p-value of <0.05 was considered statistically significant.

Results

In present study, sixty individuals were included. Group 1 (n=30) involved healthy controls of mean age of 63.1± 12.3 years while the group 2 (n=30) included patients with hepatitis as well as diabetes mellitus, having mean age of 67.3±10.6 years. There was no significant difference between the ages of the two groups. In group 1, 21 were male while 9 were female. On the other hand group 2 included 22 male and 8 female. There was no significant difference between the gender representations in the two groups (Table 1).The mean values of BSF in mmol/L in the two groups were compared via independent samples t-test as well and were found to be significantly different in the two groups at p-value of <0.001. The BSF values were noted to be significantly higher in group 2 (12.1± 3.1mmol/l) as compared to group 1 (5±1.3mmol/l) (Table 2). The significantly negative correlation of BSF with serum hepcidin revealed about 77% association between the two parameters and showed that glucose levels in blood are significantly increased with the decreasing hepcidin levels in patients with CHC induced diabetes.Mean values of serum hepcidin were found to be significantly different in the two groups at p-value of 0.000.

Table 1: Comparison of ages and male to female of the healthy controls and cases

Parameters	Group 1 (healthy controls)	Group 2 (Cases with HCV and DM)
Age	63.1±12.3 years	67.3±10.6years
N	30	30
Male: female	21 : 9	22 : 8

Table 2: Comparison of serum hepcidin levels and BSF (mmol/L) between the two groups

Parameters	Group 1 (Healthy controls)	Group 2 (cases with HCV and DM)	p-value
Blood sugar fasting (mmol/L)	5±1.3	12.1± 3.1	0.000*
Serum hepcidin (ng/ml)	28.5± 5.3	2.7± 1.03	0.000*

*p-value is significant (<0.001)

The mean values of group 2 (2.7±1.03ng/ml) were found to be significantly lower as compared to the values observed in group 1 (28.5± 5.3ng/ml) (Table 2). Levels of serum hepcidin showed significant negative correlation with BSF levels at r-value of -0.769 and p-value of 0.000. The above mentioned observations not only confirm the suppressing action of CHC on serum hepcidin levels but also validate the negative association of hepcidin with rising BSF seen in HCV induced diabetes.

Discussion

In present study decreased serum hepcidin levels were found in patients with hepatitis C induced diabetes mellitus as compared with the healthy controls. It shows that suppression of hepcidin is associated with the development of diabetes mellitus

in chronic hepatitis C. The probable mechanism behind the development of this extrahepatic manifestation is the role of hepcidin in the regulation of iron metabolism. Thus, suppression of hepcidin results in decreased internalization and degradation of iron transporting channels, ferroportin. The ensuing increased and uninhibited transport of iron then causes iron overload. This iron accrual causes decreased glucose oxidation and increased lipid breakdown in adipocytes, results in increased fatty acid oxidation and decreased glucose oxidation in muscles and in hepatocytes causing increased glucose production ,thus causing insulin resistance and hyperglycemia and forming a picture identical to diabetes mellitus.[15] Accumulated iron stimulates inflammatory cells and hepatic stellate cells which then play role in mounting reactive oxygen species and progression of hepatic fibrosis, independently infamous as a risk factor for the development of insulin resistance.[16] The results of our study are comparable to Girelli et al. who measured serum hepcidin in 81 untreated chronic hepatitis C patients and 57 healthy controls and found a significant decrease in CHC patients 33.7 versus 90.9 ng/ml respectively (p-value <0.001).[17] Similarly Tsochatzis et al. found a decrease in serum hepcidin levels in CHC patients as compared to healthy individuals. They included 96 chronic cases of hepatitis C and 30 controls and found significantly decreased serum hepcidin levels by ELISA, 14.6±7.3 versus 34.3±17.3 ng/ml at the p-value of <0.001. [18] The values in our study are although generally lower which could be due to difference in the kit used yet, the difference between the serum hepcidin levels of controls and cases is nearer to that reported by Tsochatzis et al. (20.3ng/ml) that is 25.1ng/ml. The difference reported by Girelli et al. is much larger which may be explained by the large difference between the ages of controls and cases (35 versus 42.2 years). The association of hepcidin with standard endocrine type 2 diabetes mellitus has been reported by Wang et al. in Sprague-Dawley rats. He showed a 40% decrease in hepatic hepcidin expression in rats with streptozotocin induced type 2 diabetes mellitus. [19] Gan et al. reported lowering of risk of diabetes mellitus in individuals with hepcidin suppressing mutations of TMPRSS 6 (transmembrane protease serine 6).[20].

Conclusion

1.Aberrations in glucose metabolism have been frequently linked with altered body iron regulation and in present study similar association is found between hepatitis C induced diabetes mellitus and hepcidin, the major iron regulating hormone.

2.Suppressed levels of hepcidin,in hepatitis C patients, explains the iron accrual and resulting alteration in glucose metabolism thus providing a substance that can help in assessment of disease progression and stratification for the risk of development of diabetes mellitus in patients with chronic hepatitis C.

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