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Study of Association between Serum Hepsin Level and Lymphocyte-to-C-reactive Protein Ratio in Patients with Diabetes

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Abstract

Background: Hepsin is known as a cell-surface serine protease expressed predominantly in the liver. Hepsin-deficient mice show resistance to high-fat diet-induced obesity, hyperlipidemia, and hyperglycemia. Up to the present, the physiological function of hepsin has not been fully determined. Hepsin may play significant and specific roles in diabetes.

Objectives: This study aimed to evaluate the relationship between hepsin protein concentrations in serum and type 2 diabetes mellitus (T2DM) and elucidate possible associations with disease activity andinflammatory and metabolic parameters. To the best of our knowledge, this is the first study evaluating the relationship between hepsin, lymphocyte-to-C-reactive protein ratio (LCR), and type 2 diabetes in humans in the existingliterature.

Methods: This case-control study included 60 patients (30 males and 30 females) diagnosed with type 2 diabetes, according to American Diabetes Association's criteria, and 30 healthy controls (14 males and 16 females) with similar demographic characteristics. Several laboratory parameters were assessed including fasting glucose, total cholesterol, insulin, hemoglobin A1c, gamma-glutamyl transferase, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, C-reactive protein, atherogenic index of plasma, LCR, monocyte-to-neutrophil ratio,neutrophil-to-lymphocyte ratio, and serum hepsin levels.

Results: The type 2 diabetes group had significantly higher LCR than controls (P<0.016). Correlation analysis in the patient group showed a statistically significant relationship between hepsin and LCR (rho=0.296,P=0.02). Hepsin was negatively correlated with CRP in the patient group (rho=-0.333, P=0.01). Correlation analysis in the patient group showed a statistically significant relationship between hepsin and cholesterol (rho= 0.29,P= 0.02). Age was positively correlated with hepsin in the patient group (*rho*= 0.267, P=0.04). There was no statistically significant difference in serum hepsin levels between the diabetes group and the control group (P=0.157).

Conclusion: To the best of our knowledge, this is the first study assessing the hepsin levels in patients with T2DM. Our results indicated that increased levels of hepsin could be associated with the inflammatory processes. Similar results were not found for diabetes. However, it is recommended that similar studies should be conducted in larger patient populations.

Keywords: Diabetes, Hepatocyte growth factor, Hepsin, Lymphocyte-to-C-reactive proteinratio, Obesity, Serine protease, Transmembrane protease serine 1, Type II transmembrane serine proteases

1. Background

Hepsin (HPN) is a type II transmembrane serine protease (TTSP) encoded by the transmembrane protease serine 1 (TMPRSS1) gene on chromosome 19 and is highly expressed in the liver (1, 2). It is a member of the TTSP family of 17 proteolytic enzymes, seven of which are found in humans (3).Structurally, it consists of an N-terminal cytoplasmic domain, a transmembrane domain, and a C-terminal extracellular compartment containing the scavenger receptor cysteine-rich(SRCR) domain and a catalytic amino acid triad (Ser-His-Asp) (2, 4, 5).

Hepsin can modify the plasma membrane and intercellular and extracellular matrix, the same as other TTSPs (5, 6). The location and structure of hepsin areideal for its protease activity and modification ofthe extracellular matrix which play a role in enzyme networks (3). This well-characterized structure ofhepsin is similar to the structure of other serine proteases responsible for physiological homeostases, such as digestion, respiration, immune reactions, reproduction, coagulation cascade, and growth (2, 6).Although some TTSPs are secreted in different tissues and cell types, they have a tissue-specific expression pattern. Due to this similarity, hepsin, which is highly expressed on hepatocyte surfaces, is expected to function in liver tissue (2-4).Despite the tissue-specific expression pattern of the hepsin and its TTSP-specific structure, its function is not fully understood (2, 7).Previous studies have shown that hepsin plays a role in cell growth and preservation of cell morphology, blood coagulation, and numerous pathologies, including cancer growth, especially prostate cancer, tumor invasion, and metastasis(4, 6).Furthermore, hepsin is the activator of pro-HGF, the precursor of hepatocyte growth factor (HGF), whose high plasma levels are closely associated with obesity, diabetes, and metabolic syndrome (8, 9, 10). The HGF/c-Met

signaling pathway is primarily affected by overexpression and deficiency of hepsin (2, 11).More specifically, high pro-HGF concentration was found in hepsin-deficient mice, while low levels of HGF were detected (2). This supports the idea that increased hepsin expression may be associated with diabetes and obesity as well as oncogenesis (1, 4, 8). Moreover, hepsin, whose transmembrane and cytoplasmic domains were found to be identical in mice, rats, and humans, is also associated with embryonic development, post-natal survival, and hearing in mice (2, 12).

Recent mice studies have shown that this enzyme is an important regulator of liver metabolism and energy homeostasis (2). In mice experiments, it was found that lipid and glucose levels were low in knock-out mice that did not produce hepsin, and resistance to obesity developed even in high-fat feeding (2).This suggests that hepsin can play important and specific roles in diabetes and may be used to reduce the prevalence of diabetes. Furthermore, a new study published in 2020 showed that hepsin was found in the extracellular environment and reported the presence of this enzyme in serum (1).

Lymphocyte-to-C-reactive protein ratio (LCR) is a recently identified marker of systemic inflammation, which is a combination of inflammatory markers. It is a marker that could be used prognostically in some diseases, including oncological and obstructive sleep syndrome. (13).

2. Objectives

In the light of all this information, this study aimed to evaluate the relationship between hepsin protein concentrations in serum and type 2 diabetes mellitus (T2DM) and elucidate possible associations with disease activity and inflammatory or metabolic parameters.No previous study has examined the role of hepsin in diabetes in humans up to the present.

3. Methods

This study included 60 patients (30 males and 30 females) and 30 healthy volunteers with similar demographic characteristics including14 (45%) males and 16 (55%) females, as the group of control. The study protocol was approvedby the Ethics Committee of the Faculty of Medicine in Nigde Omer Halisdemir University (Niğde/Turkey, ethics code: 2021/49), andthe study was conducted following the provisions of the Declaration of Helsinki. The informed written consentwas taken from the patients.

3.1. Inclusion and Exclusion Criteria

The inclusion criteria included 1) patients diagnosed with type 2 diabetes, 2) patients with hemoglobin A1c (HbA1c) values above 6.5% which is an ADA criterion for diabetes, 3) the age range of 18-65 years, 4) the absence of any infective disease, and 5) the absence of any oncological or systemic disease. However, those diagnosed with prostate, kidney, or breast cancer andviral hepatitis, those who received chemotherapy or cancer treatment, and patients with liver or other organ failure were excluded from the study.

The control group consisted of healthy volunteers with illness who met no exclusion criteria and had demographic characteristics similar to the patient group.

Blood samples from the study participants were collected between 8:00 and 10:30 in the morning, after 12 hours of fasting and 15 minutes of rest. Venous blood was collected into vacuumed Becton Dickinson tubes with gel and clot activator (BD, United States). The tubes were centrifuged at 3000 g for 5 minutes after at least 15 minutes to make sure that the blood was coagulated.

Glucose, gamma-glutamyl transferase (GGT), high-density lipoprotein (HDL), total cholesterol, triglycerides, and uric acid levels were measured using the enzymatic and spectrophotometric methods on an Advia 1800 analyzer (Siemens Diagnostics, Erlangen, Germany). Insulin levels were measured on an XPT analyzer (Siemens Diagnostics, Erlangen, Germany) using the chemiluminescent immunoassay method. HbA1c levels were determined by high-pressure liquid chromatography (HPLC) using an ADAMS A1c HA-8180V analyzer (Arkray, Japan). Complete blood counts were performed using a Sysmex XN 1100 analyzer (Sysmex Diagnostic,Japan).

Hepsin serum levels were measured using a SunRed enzyme-linked immunosorbent assay (ELISA) kit on a Biotek ELx 808 analyzer (BioTek Diagnostics, Maryland, United States).

Hepsin kit was supplied by SunRed (China, Catalog no. DZE-201-12-3942) with an assay rangeof 0.25-70 ng/mL and a sensitivity of 0.208 ng/mL. Samples were assessed with an intra-assay coefficient of variation <10% and an inter-assay coefficient of variation<12%.

Serum samples for hepsin measurements were stored in aliquots at -20 ºC. Samples were thawed at 2 ºC-8 ºC one day before the analysis and were taken out of the refrigerator early the next day and heatedto analysis temperature.

3.2. Statistical analysis

Statistical analyses were conducted using Jamovi (Version1.2.27.0, Sydney, Australia). Shapiro-Wilk test was used to determine the stability of data to the normal distribution, and an independent t-test was used to evaluate the normal distribution of variables. Mann-Whitney U test was used to compare groups without normal distribution. With in-group

associations of variables (the patient group or the control group) were determined using Pearson's correlation test for normally distributed variables. For non-normally-distributed variables,within-group associations of variables were identified using Spearman's correlation test. An alpha level of 0.05 was adopted for all statistical tests.

4. Results

Table 1 presents the main demographic findings of patients (n=60) and controls (n=30). The patients and the control group were similar in terms of age, gender, height, weight, and body mass index (BMI). Although BMI values were similar (P=0.289), the T2DM group had a significantly higher waist circumference (WC) ratio than healthy controls (P<0.035). High level of atherogenic index was observed in 46.6% of the patients, while a low-level atherogenic index was observed in 45% of the patients. In total, 28(46.6%),5 (8.4%), and 27 (45%) of T2DM patients were classified as atherogenic index of plasma (AIP) stage I (low risk), stage II (intermediate risk), and stage III (high risk), respectively.

A statistically significant difference was observed between the diabetic patient group and the control group in terms of serum LCR levels (P=0.016). Moreover, a statistically significant difference was determined in serum AIP levels between the diabetic patient group and the control group (P=0.002). However, there was no statistically significant difference in serum hepsin levels between the diabetes group and the control group (P=0.157) (Table 2).

Correlation analysis in the patient group showed a statistically significant low correlation between hepsin and cholesterol (rho=0.29, P=0.02).

A statistically significant difference was determined between hepsin and L/CRP in the patient group (rho=0.296, P=0.02). In addition, hepsin was negatively correlated with CRP (rho=-0.333, P=0.01).

In the patient group, AIP was positively correlated with both LCR and GGT (rho=0.273, P=0.04, and rho=0.373, P=0.006, respectively).

Age was positively correlated with hepsin in the patient group (rho=0.267, P=0.04). Moreover, the correlation between GGT and glucose was also positive (rho=0.406, P=0.03) in this group. A negative correlation was observed between GGT and HDL-C

Table 1. Demographic characteristics of the patients with type 2 diabetes and healthy controls

	Patients (n=60)		Controls (n=30)		*P**
	Mean ± SD	**Min-max (median)**	**Mean ± SD**	**Min-max (median)**	
Age (years)	52.8 ± 7.29	161 - 225	46.7 ± 13.4	17-62 (34)	0.369
Gengern (%) Female	30(50)		16(55)		0.608
Male	30(50)		14(45)		
Height(cm)	169±9.5	148-186(167)	167±8.7	154-186(167)	0.518
Weight(kg)	69.6±9.51	49-89(70)	65.6±8.6	52-82(64.5)	0.077
BMI (kg/m2)	24.4 ± 2.48	20.8-29.8 (24.2)	23.5 ± 2.23	18.8-27 (23.8)	0.289
Waist circumference (cm)	84.6 ± 15.1	60-110 (86)	73.9 ± 13.2	59-102 (72.5)	0.035
AIP stage n(%) Low	27 (45)				
Inter	5 (8.4)				
High	28 (46.6)				

BMI: body mass index; AIP: Atherogenic index of plasma; SD: standard deviation; min-max: minimum-maximum; Significance level P<0.05*

Table 2. Comparison of laboratory parameters in patients with type 2 diabetes and healthy controls

	Patients		Controls		*P**
	Median	**IQR**	**Median**	**IQR**	
Hepsin(ng/mL)	9.98	161 - 225	9.76	90 - 96.8	0.157
Triglycerid (mg/dL)	174	124 - 251	128	90.5 - 159	**0.003**
HDL (mg/dL)	45.5	39 - 57	53.5	43 - 61.3	0.079
LDL (mg/dL)	112	93.5 - 139	109	101 - 126	0.605
Cholesterol (mg/dL)	201	173 - 229	194	176 - 214	0.696
Fasting plasma Glucose (mg/dL)	161	130 - 225	92	90 - 96.8	<0.001
GGT (U/L)	25	14 - 31	18.5	14 - 20	0.025
Fasting plasma Insulin (mU/L)	11	7.4 - 17	8.2	8.2 - 12.6	0.108
HbA1c (%)	8	6.95 - 9.95	5.3	5.38 - 5.6	<0.001
Uric Acid (mg/dL)	4	4 - 5.95	5	4.47 - 6	0.087
AIP	0.2	0.01-0.44	0.001	0.001-0.217	0.002
CRP (mg/dL)	1.92	0.79-4.75	0.92	0.35-2.43	0.049
MPV	10.6	10-11	10.4	10-11	0.482
MNR	0.14	0.11-0.17	0.135	0.12-0.18	0.724
NLR	0.6	0.4-0.78	0.63	0.63-0.77	0.624
LCR	1.25	0.53-3.04	2.03	1.12-6.77	0.016

Note. Independent Student's t, Mann-Whitney U test, Significance level P<0.05*

AIP: Atherogenic index of plasma; CRP: Serum reactive protein; MPV: Mean platelet volume; MNR:Monocyte neutrophil ratio; LNR: Lenfocyte neutrophil ratio; LCR: Lymphocyte-to-C-reactive protein ratio; HbA1c: Hemoglobin A1c; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; GGT:Gamma-glutamyltransferase; IQR: Inter quantile range

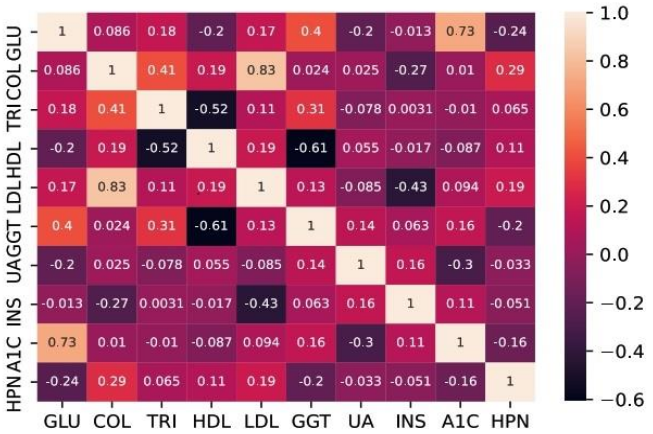

Figure 1. Heatmap of the Spearman correlation coefficients between hepsin, glucose, insulin, uric acid, GGT, triglyceride, cholesterol, HDL, and LDL levels of the patient group

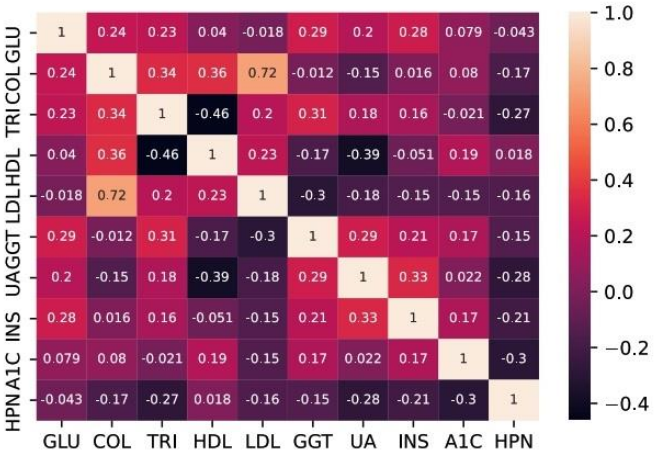

Figure 2. Heatmap of Spearman correlation coefficients between hepsin, glucose, insulin, uric acid, GGT, triglyceride, cholesterol, HDL, and LDL levels of the control group

(rho=-0.608, P=0.02). Furthermore, a significant relationship was determined between mean platelet volume and HbA1C and between glucose and uric acid (P=0.008 and P=0.009, respectively).

No significant differences were observed in LCR ratio values, according to AIP stages in patients with T2DM (Table 3).

The comparisons showed no significant differences in hepsin values, according to AIP stages in patients with T2DM (Table 3).

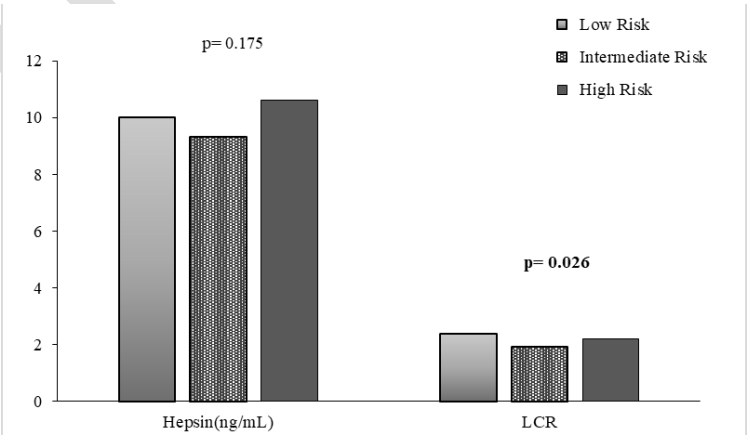

Figure 3. Comparison of mean values of hepsin and L/CRP level in patients with type 2 diabetes regarding Atherogenic index of plasma stage

5. Discussion

The first striking finding of this study was that T2DM patients had significantly higher levels of LCR compared to controls considering age, gender, and BMI; however, there was no difference in hepsin levels. The presence of correlations between hepsin and cholesterol, between hepsin and LCR, between hepsin and CRP, and between LCR and AIP in T2DM patients was another remarkable finding of this study.

In the study conducted by Shuo Li et al. (2020) using knock-out mice models (HPN -/-) and wild type(WT) mice, tissues of mice that did not produce hepsin were examined histologically. Low blood glucose and lipids, high proportion of brown adipose tissue, high liver glycogen, and impaired HGF/c-Met signal were detected in HPN -/- mice. It was found that resistance to obesity and diabetes developed even in high-fat feeding. Based on this supportive and regulatory role of hepsin in energy and lipid metabolism in the liver, the authors suggest that hepsin canbe a therapeutic target in the treatment of obesity and diabetes (2).However, no other studies have examined the role of hepsin in diabetes.

In a mice study conducted by Aljakna et al. using N-Ethyl-N-Nitrosourea mutagenesis, a decrease in hepsin levels led to a decrease in HDL, triglyceride, and cholesterol (14). In the present study, a statistically significant difference was observed only in plasma cholesterol levelsofpatients with T2DM. Another study on the functional significance of Hepsin compared HPN -/- mice (generated by homologous recombination) with WT mice. Tissues were examined histologically. Hepatic proteins and enzyme levels were similar inthe study groups. No difference was found between tissue weights and serum concentrations of all proteins except bone-derived alkaline phosphatase (9,15).Apart fromthe results of some studies in the literature ontheimportant and specific roles of hepsin in the coagulation cascade, maturation of hepatocytes, and embryonic development, some studieshave shown that hepsin isnot necessary for embryonic development and normal hemostasis (16-19).

In this study, we examined serum hepsin levels and theirrelationships with other diagnostic markers in selected patients diagnosed with T2DM. Our results showed that there wasno difference between the diabetic patients and the control group in terms of hepsin serum levels. However, correlation analysis performed in the patient group showed a statistically significant relationship between hepsin and cholesterol. The presence of a correlation between cholesterol and hepsin in the patient group suggests that hepsin may be effective in lipid metabolism in humans. In the study of Li et al., histological examination showed that hepsin acts cellularly in adipose tissue and disrupts adipocyte browning by disrupting the c-Met signal (2). Therefore, available information supports the idea that hepsin may be effective in lipid and energy metabolism. However, it is unclear whether hepsin levels in serum reflect the concentration in liver hepatocytes. Hepsin is an enzyme produced in many tissues, especially the liver, kidney, and pancreas.

Levels of hepsin, LCR (which is a new systemic inflammatory index), several other markers and indices ininflammation and metabolism were measured in T2DM patients (13).LCR value was found to be significantly lower in patients with T2DM. In addition, LCR correlated with hepsin and AIP. Okugawa et al. have explained the relation of low LCR rate with the impairment of the immune reaction resulting from adecrease in the lymphocyte count and subsequent systemic inflammation (13). A decrease in lymphocyte numbers led to an impairment in immune response, while an increase in CRP levels indicatedsystemic inflammation. In line with the current results, higher levels of LCR rate determined in patients with T2DM can provide significant information for predicting diabetes (20).However,large-scale prospective studies are needed to determine whether LCR, a recently defined indicator of systemic inflammation, may have a role in the diagnosis and follow-up of patients with diabetes.Due to high correlational associations between hepsin and LCR and between hepsin and CRP, there may be an important link between hepsin and systemic inflammation (20).

Another possible explanation for the relationship betweenhepsin, LCR, and diabetes may be the low-grade inflammation which is common in T2DM patients, and the associated increased inflammatory cytokines levels (20). Furthermore, since all of them areactivators of pro-HGF - the HGF precursor closely related to obesity - a high level of correlation between LCR and CRP and higher levels of LCR in diabetic patients may indicate a possible pathogenetic link between hepsin and T2DM (8-10).

5.1. Limitations

Regarding the strength of this study, one can refer to the fact that this is the first study evaluating hepsin and LCR and their relationships with other metabolic and inflammatory parameters in T2DM patients. Nevertheless, there are some limitations.Small sample size can be considered thefirst limitation of this study. Second, the patients included in this study were taking antidiabetic medications.Eventually, we did not know how much of serum hepsin was of liver origin.

6. Conclusion

To the best of our knowledge, this is the first study assessing the hepsin levels in patients with T2DM. Our results indicated that increased levels of hepsin could be associated with the inflammatory

processes. Similar results were not found for diabetes. However, we believe that similar studies withlarger patient populations are needed. Eventually, it is recommended tomeasure tissue hepsin levels in T2DM patients.

The first striking finding of this study wasthat T2DM patients hadsignificantly higher levels of LCR, compared to controls considering age, gender, and BMI; however, there was no difference in hepsin levels.As the second remarkable finding of this study,we found a relationship between hepsin and cholesterol, hepsin and LCR, hepsin and CRP, and LCR andAIP in T2DM patients.

Footnotes

Conflicts of Interest: The authors declare no conflict of interest regarding the publication of the present study.

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