Association of Anti-oxidative Capacity of HDL With Subclinical Atherosclerosis in Non-alcoholic Fatty Liver Disease

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Research

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) patients are at the substantial risk for the for developing cardiovascular disease (CVD). High-density lipoprotein (HDL) is well known to have protective effects against the development of atherosclerotic CVD. One of the major antiatherogenic effect of HDL is its anti-oxidative function.

Objectives: This study investigated the association of anti-oxidative function of HDL with NAFLD and CVD risk.

Methods: A total of 143 subjects including 51 NAFLD and 92 control subjects were included in this cross-sectional study. HDL oxidative index (HOI) was determined spectrophotometrically using a cell-free method in the presence of a fluorescent substrate dichlorofluorescein diacetate (DCFDA). Paraoxonase 1 (PON1) activity, superoxide dismutase (SOD) activity and malondialdehyde (MDA) plasma levels were assessed in both groups.

Results: The subjects with impaired HDL anti-oxidative function ($\text{HOI} \geq 1$) had lower PON1 paraoxonase activity, lower SOD activity, higher MDA levels and higher carotid intima-media thickness (cIMT) values compared to the controls. HDL oxidative index (HOI) was positively correlated with MDA levels and cIMT and negatively correlated with SOD activity. NAFLD patients with $\text{HOI} \geq 1$ had elevated serum levels of liver enzymes and higher LS values.

Conclusions: Alteration in circulating oxidative stress markers may diminish the anti-oxidative function of HDL. Impaired anti-oxidative capacity of HDL might be related to NAFLD severity and subclinical atherosclerosis in NAFLD patients.

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is the most leading cause of chronic liver disease which affects an estimated 20–30% of the general population (1). NAFLD is strongly associated with the other metabolic conditions such as obesity, type 2 diabetes mellitus, and cardiovascular diseases (1). Despite liver-related morbidity and mortality, cardiovascular disease (CVD) is the most important cause of mortality in the NAFLD population (2). Population based studies demonstrated increased CVD risk in NAFLD patients compared with matched healthy controls. It appears that NAFLD is mechanistically involved in the atherosclerosis development (3).

NAFLD patients exhibit an atherogenic dyslipidemia that is characterized by hypertriglyceridemia, low levels of high-density lipoprotein cholesterol (HDL-C), and high levels of low-density lipoprotein cholesterol (LDL-C) (4). Several studies demonstrated an association of higher plasma high-density lipoprotein (HDL) cholesterol levels with a lower risk of atherosclerotic cardiovascular disease (CVD) morbidity and mortality. However, there are multiple clinical observations which failed to support the causal relationship between HDL-C and CVD (5, 6). This has led to the conception that the HDL particle

functionality might be a better predictor of CVD risk than HDL cholesterol mass levels (7). HDL particle has numerous antiatherogenic functions. One of the important anti-atherogenic properties of HDL is its anti-oxidative function, the ability to suppress LDL oxidation which in turn decreases the atherogenicity of LDL particles (8).

Several studies addressed the anti-oxidative function of HDL in CVD and other disorders with high cardiovascular risk (8–11). The anti-oxidative property of HDL was not significantly different in non-MI patients and MI patient with or without ST elevation (8). The subjects with stable coronary artery disease had a similar HDL anti-oxidative function compared to the controls. However, the subjects who had acute coronary syndrome had less anti-oxidative capacity compared with the control subjects (9). Comparison of HDL anti-oxidative function in diabetic patients and healthy volunteers showed an impaired HDL anti-oxidative function in the diabetic patients (10). Additionally, the anti-oxidative function of HDL was impaired in the patients with PCOS (11). Previous studies showed impaired HDL cholesterol efflux capacity in NAFLD (12, 13). However, no study evaluated the anti-oxidative function of HDL in NAFLD patients.

Increasing data have demonstrated that NAFLD patients have abnormal circulating markers of oxidative stress, such as increased malondialdehyde (MDA), superoxide dismutase activity and PON1 activity (14, 15). Having a disturbed anti-oxidative system along with a high risk of CVD in these patients suggest that impaired antioxidant activities of HDL, as an important player in CVD, may be the potential link between these phenomena. In this study, we aimed to investigate the relationship between HDL anti-oxidative capacity, NAFLD markers and carotid intima-media thickness (cIMT) in NAFLD.

Material And Methods

In the current study, 92 Controls and 51 NAFLD patients were selected from from Golestan Cohort Study (16). The was a cross-sectional study and approved by the medical ethics committee of the Semnan University of Medical Sciences (ethics code: IR.SEMUMS.REC.1397. 331) and all participants gave written informed consent. All participants were male, aged 50–81 years old. The diagnosis of NAFLD was based on abdominal ultrasonography. Subjects were excluded if they met one of the following criteria: A history of alcohol abuse (30 g/d), diabetes, viral hepatitis, autoimmune liver disease, hemochromatosis, Wilson's disease. None of the patients were taking medication that has been reported to induce hepatitis.

Ultrasound assessment was performed using an Accuvix XQ ultrasound unit (Medison, South Korea) equipped with a 3–7 MHz curved-array and a 5–12 MHz linear-array transducer for the evaluation of liver, abdominal fat and carotid arteries as previously described (16). Visceral Adipose Tissue thickness (VAT) was measured between the anterior wall of the aorta and the internal face of the rectus abdominis muscle perpendicular to the aorta. (16). Carotid intima-media thickness (cIMT) was assessed as the distance between the lumen–intima interface and the media– adventitia interface, measured at its thickest point on the distal (far) wall of the common carotid arteries, 1.5–2 cm proximal to the carotid bulb. The average of right and left sides was used for cIMT analysis (16). Liver stiffness (LS) was

measured by transient elastography using the FibroScan 502 machine (EchoSense, Paris, France, 5MHz) (16).

Anthropometric parameters were measured in accordance with the standardized protocols. The body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2). WC was measured mid-way between the lowest rib and the iliac crest. Blood pressure was measured after 5 min rest at the left arm in a sitting position using an automatic manometer.

Fasting blood samples were obtained from the participants following an overnight fasting. Serum and plasma were isolated and frozen in aliquots at -80°C for further analysis. Biochemical parameters including Fasting blood glucose (FBG), serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and levels of alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyl transferase (GGT) were measured by automated enzymatic methods using commercial kits (Pars Azmoon, Iran).

Paraoxonase 1 (PON1) enzymatic activity was measured according to a method described before. Paraoxon (Sigma-Aldrich, Germany) was used as the substrate and PON1 activity was assessed by measuring the rate of substrate hydrolysis to p-nitrophenol. Briefly, 5 μL of plasma was added to a mixture of 1.0-mM paraoxon in 195 μL of 50 mM glycine buffer containing 1mM calcium chloride (pH 10.5) and incubated at 37° for 10 minutes in 96 well plates. The formation of p-nitrophenol was recorded at 412 nm and activity was expressed as μmol p-nitrophenol/L/plasma/min (17).

Superoxide dismutase (SOD) activity was measured by a SOD activity assay kit (Teb Pazhouhan Razi, Iran) following the manufacturer's instructions. The kit measures SOD activity by utilizing tetrazolium salt as a substrate which converts to a water-soluble formazan dye upon reduction by superoxide anion. The formazan formation rate is inhibited by the presence of SOD in the samples.

Plasma MDA levels were determined using an MDA assay kit (Teb Pazhouhan Razi, Iran) following the manufacturer's instructions. The method principle was based on the spectrophotometric measurement of the colored compound that is formed during the reaction of MDA and thio- barbituric acid (TBA) at 530 nm.

The high-density oxidative index (HOI) measures the ability of apoB-depleted plasma to inhibit LDL oxidation in the presence of dichlorofluorescein diacetate (DCFDA) (Sigma-Aldrich, Germany). A cell-free assay was performed as previously described with some modifications. Before analysis, apolipoprotein (apo) B depleted plasma was prepared as previously described. Briefly 40 μL 20% polyethylene glycol (Merck, Germany) in Glycine was added to 200 μL plasma, mixed and incubated on ice for 30-minute. Then the mixture was centrifuged at 2200 g for 30 minutes, the HDL-containing supernatant was collected, kept on ice, and used for the further measurements. LDL was obtained from (Man lab Co., Iran) which was isolated from pooled fresh sera of healthy volunteers by sequential ultracentrifugation. LDL was diluted in PBS to a final cholesterol concentration of 100 $\mu\text{g}/\text{mL}$ and oxidized by CuSO_4 (100 $\mu\text{mol}/\text{L}$) for 6 hours at 37°C . Oxidized LDL with a concentration of 1.4 $\mu\text{g}/\text{mL}$, DCFDA with a concentration of

0.725 µg /ml and apo B–depleted plasma from the participants with PBS to a final volume of 175 µl were added to the separate wells of a 96-well flat bottom polystyrene microtiter plates and incubated at 37°C for 60 min. Fluorescence intensity was measured with a at an excitation wavelength of 485 nm and emission wavelength of 530 nm using the synergy h1 hybrid multi-mode microplate reader (BioTek, USA). The HDL oxidative index (HOI) was calculated as the ratio of fluorescence intensity in the presence of apo-B-depleted plasma samples divided by the fluorescence intensity in the absence of apo-B-depleted plasma. An HOI < 1 was considered as anti-oxidative HDL function and an HOI ≥ 1 was considered as pro-oxidative HDL.

The Kolmogorov-Smirnov test was used to test for normal distribution of data. Categorical data are presented as percentages, continuous data as the mean ± SD or median (IQR) as appropriate. Comparison between two groups was performed using independent-sample t test or Mann-Whitney U test for continuous data and the chi-squared test for categorical data. Differences among the four subgroups as classified based on the presence of NAFLD and HOI were determined using the Kruskal-Wallis test. When analysis among the four groups was significant, pairwise tests were performed using the Mann-Whitney test. Pearson correlation was applied to determine the correlations between HOI and the other parameters in the whole study population. Significant differences were defined by P < 0.05 (*) or (#), P < 0.01 (**) or (##), and P < 0.001 (***) or (###).

All of the statistical analyses were performed using the IBM SPSS Statistic 27 and GraphPad prism 9.

Results

Basic clinical and laboratory characteristics of the study groups are presented in Table 1. BMI, waist circumference (WC), waist-to-hip ratio, fasting blood glucose (FBG), TG, visceral fat, AST, ALT, GGT, and LS were significantly higher in the NAFLD group compared with the control group. NAFLD patients had lower HDL-C than the control subjects. There was not any significant difference in age, total cholesterol (TC), LDL-C, and cIMT between the two groups.

Table 1
Baseline characteristics

Parameter	Control (n = 92)	NAFLD (n = 51)	P-value
Age (years)	59.0 (55.0–66.0)	56.0 (54.0–61.0)	0.273
BMI (kg/m ²)	24.83 ± 3.62	29.34 ± 3.47	< 0.001
WC (cm)	93.29 ± 11.12	103.67 ± 9.65	< 0.001
WHR	0.97 (0.92-1.00)	0.98 (0.94–1.03)	0.002
Weight (kg)	68.75 (61.00-79.50)	80.75 (73.50–89.00)	< 0.001
TC (mg/dl)	205.51 ± 39.40	207.34 ± 37.00	0.779
LDL-C (mg/dL)	121.61 ± 33.76	124.61 ± 28.71	0.594
HDL-C (mg/dL)	57.49 ± 12.58	51.94 ± 11.12	0.007
TG (mg/dL)	112.0 (82.0-158.0)	148.5 (112.5–186.0)	0.016
Systolic blood pressure (mmHg)	131.55 ± 21.34	144.96 ± 23.74	0.001
Diastolic blood pressure (mmHg)	79.96 ± 11.03	86.88 ± 12.43	0.001
FBG (mg/dL)	92.71 ± 9.00	99.57 ± 11.21	< 0.001
Visceral fat (%)	46.57 ± 20.76	70.26 ± 18.01	< 0.001
AST (U/L)	18.5 (15.0–22.0)	22.0 (19.5–27.0)	< 0.001
ALT U/L)	15.5 (11.0–22.0)	31.5 (23.0–41.0)	< 0.001
GGT (U/L)	22.37 (17.40–29.10)	28.65 (23.53–38.50)	< 0.001
LS (kPa)	3.80 (3.30–4.30)	5.05 (4.25–6.60)	< 0.001
cIMT (mm)	0.8 (0.75–0.87)	0.81 (0.74–0.91)	0.863
HOI	0.92 ± 0.19	0.95 ± 0.24	0.405
HOI ≥ 1 frequency (%)	32.96	40.38	0.468
SOD (U/mg protein)	10.71 ± 1.51	8.60 ± 2.44	< 0.001
MDA (μM)	33.29 (25.08–42.33)	34.02 (30.40-47.74)	0.771

Baseline characteristics according to disease state. Comparisons between groups were performed using Independent Student's t-test or Mann-Whitney U test as appropriate. Continuous data are expressed as mean ± standard deviation (SD) or median and (interquartile range), categorical data as percentages. BMI: Body mass index; WC: waist circumference; TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; TG: Triglycerides; AST; aspartate amino transferase; ALT: alanine amino transferase; GGT: gamma glutamyl transferase; LS: liver stiffness; cIMT: Carotid intima-media thickness; MDA: malondialdehyde; PON1: Paraoxonase 1; SOD: superoxide dismutase; HOI: high-density oxidative index. p < 0.05 was considered statistically significant.

Parameter	Control (n = 92)	NAFLD (n = 51)	P-value
PON1 (U/L)	24.44 (17.77–31.11)	42.21 (27.77–52.22)	< 0.001
Hypertension (%)	27.90	47.36	0.021
Baseline characteristics according to disease state. Comparisons between groups were performed using Independent Student's t-test or Mann-Whitney U test as appropriate. Continuous data are expressed as mean $\pm$ standard deviation (SD) or median and (interquartile range), categorical data as percentages. BMI: Body mass index; WC: waist circumference; TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; TG: Triglycerides; AST: aspartate amino transferase; ALT: alanine amino transferase; GGT: gamma glutamyl transferase; LS: liver stiffness; cIMT: Carotid intima-media thickness; MDA: malondialdehyde; PON1: Paraoxonase 1; SOD: superoxide dismutase; HOI: high-density oxidative index. $p < 0.05$ was considered statistically significant.			

There was not any significant difference in the mean of HOI between NAFLD and control groups. We found that 61 (66.30%) controls and 30 (58.82%) patients had a preserved anti-oxidative function with an $\text{HOI} < 1$, and 31 (33.69%) controls and 21 (41.17%) patients presented pro-oxidative HDL serum measurements with an $\text{HOI} \geq 1$. We stratified the subjects into two groups, $\text{HOI} < 1$ and $\text{HOI} \geq 1$. The subjects with $\text{HOI} \geq 1$ had higher levels of MDA ($P = 0.043$), lower PON1 activity ($P = 0.028$), lower SOD activity ($P = 0.012$), and higher cIMT values ($P = 0.022$) (Fig. 1).

We further stratified the study population into 4 groups according to combined strata of status and HOI above and below 1. Interestingly, NAFLD patients with $\text{HOI} \geq 1$ had higher LS ($P = 0.034$), AST ($P = 0.039$), ALT ($P = 0.036$) and cIMT ($P = 0.042$) levels than NAFLD patients with $\text{HOI} < 1$ (Fig. 2).

Correlation analysis demonstrated that HDL antioxidant capacity (HOI) correlated positively with plasma MDA levels ($r = 0.298$, $P = 0.001$) and cIMT ($r = 0.197$, $P = 0.025$) in the whole population. There was a significant negative correlation between HOI and the SOD activity ($r = -0.242$, $P = 0.004$) in the whole population (Table 2).

Table 2
Correlation coefficients between HOI and
metabolic and anthropometric
parameters

Parameter	Correlation	P value
MDA	0.298	0.001
PON1	-0.117	0.201
SOD	-0.242	0.004
Age	0.056	0.503
cIMT	0.188	0.031
Visceral Fat	0.01	0.909
WC	0.011	0.9
BMI	0.042	0.622
WHR	0.012	0.891
AST	0.059	0.488
ALT	0.067	0.432
GGT	0.075	0.374
LS	0.062	0.474
FBG	0.077	0.358
HDL-C	-0.044	0.603
LDL-C	0.032	0.705
TG	-0.009	0.919

Discussion

Cardiovascular diseases are the main cause of death in NAFLD patients (18). Impaired anti-oxidative capacity of HDL turned out to be a good predictor of cardiovascular disorders (19). Our findings showed that the subjects with an impaired anti-oxidative capacity of HDL tend to present higher oxidative stress markers. We also found that impaired anti-oxidative capacity of plasma HDL was associated with higher levels of hepatic fibrosis markers and higher cIMT in NAFLD.

A previous study on anti-oxidative properties of HDL in patients with coronary syndrome found higher HOI in patients with acute coronary syndrome or stable coronary artery disease compared with controls (9). Another study reported total HDL antioxidant capacity in systemic lupus erythematosus patients who have increased risk of cardiovascular diseases were significantly reduced compared to the controls (20).

But there was no significant difference in anti-oxidative properties of HDL in MI patients with or without ST elevation and non-MI participants (8). We also did not find a significant difference in HOI values between the NAFLD and the control groups. The frequency of $\text{HOI} \geq 1$ was higher in NAFLD compared to the controls, albeit not significant. Consistently, it has been reported that impaired anti-oxidative capacity of plasma HDL was more frequent in women with polycystic ovary syndrome (PCOS) compared with the control group (11). These conflicting results might be due to the different study populations and methods used in the studies.

In the current study, we found higher level of MDA in the NAFLD group compared to the control group. In parallel, previous studies reported increased levels of MDA in the NAFLD patients compared to controls (14, 15, 21). Numerous pieces of evidence unraveled the important role of oxidative stress in the pathogenesis of NAFLD. Oxidative stress causes by the imbalance between the production of reactive oxygen species (ROS) and the intrinsic antioxidant capacity of a biological system (22). ROS oxidizes various important classes of biological molecules including lipids of all cellular membranes and lipoproteins (23). HDL oxidation generates hydroperoxides in HDL which subsequently produces several reactive intermediates such as MDA (23). A Previous study showed increased levels of MDA in HDL subfractions isolated from the plasma of acute coronary syndrome patients compared to control subjects and it was along with a pro-inflammatory effect of HDL (24). In consistent, we found higher levels of plasma MDA in the subjects with pro-oxidative HDL ($\text{HOI} \geq 1$) compared to the subjects with $\text{HOI} < 1$. Moreover, our findings showed a significant positive correlation between HOI values and MDA levels.

Our results showed a higher level of PON1 paraoxonase activity in the NAFLD than the controls. Conversely, other studies showed lower PON1 serum concentration in the NAFLD group compared to the control group (25, 26). However, in consistent with our findings, a large population study which used fatty liver index for the diagnosis of NAFLD showed that in men PON-1 activity was significantly higher in the NAFLD population compared to the non-NAFLD population (27). PON1 has been shown to be critical for the anti-oxidative function of HDL particle. A previous study demonstrated a lower PON-1 activity in patients with an $\text{HOI} \geq 1$ than the subjects with an $\text{HOI} < 1$ (7). Consistently, we found lower levels of PON1 paraoxonase activity in the $\text{HO} \geq 1$ group compared to the $\text{HOI} < 1$ group.

With respect to SOD activity, we observed decreased activity of SOD in NAFLD patients. Similarly, other studies reported lower activity of SOD in NAFLD patients (14, 28, 29). In our study, SOD activity was inversely correlated with fatty liver and obesity indices such as ALT, LS, BMI, WHR and FBS. A previous report of elevated levels of triglyceride in hepatic cells of SOD knockout mice suggests the contribution of SOD in the development of fatty liver (30). Our findings showed a lower SOD activity in the subjects with $\text{HOI} \geq 1$ compared to the subjects with $\text{HOI} < 1$.

Collectively, higher MDA level, lower PON1 and lower SOD which reflects an impaired intrinsic antioxidant system in the subjects with $\text{HOI} \geq 1$ confirms previous observations showing the relationship between oxidative stress and impaired HDL antioxidant capacity. As it was mentioned earlier, the oxidation of lipid

and protein constituents in HDL is the pivotal cause of impaired HDL functions (31). On the flip side, it has been shown that HDL oxidation causes degradation of PON1 paraoxonase activity (32).

Moreover, we found that NAFLD patients with the impaired antioxidant function of HDL ($\text{HOI} \geq 1$) had higher ALT, AST and LS. Previous studies in NAFLD patients demonstrated the association of circulating oxidative stress biomarkers with disease severity (22). In the light of the important role of oxidative stress in the progression of NAFLD, one can speculate that NAFLD patients with higher liver enzymes and liver fibrosis have higher levels of pro-oxidative and other detrimental factors which cause HDL dysfunctional. However, owing to the multiple anti-oxidative and anti-inflammatory properties of HDL, the possibility that an impaired anti-oxidative capacity of HDL may affect disease severity in NAFLD patients cannot be ruled out.

Finally, we observed higher levels of cIMT in the subjects with $\text{HDL} \geq 1$ compared to the subjects with $\text{HOI} < 1$. We also found a significant positive correlation between HOI and cIMT. However, a previous study on young adults did not find an association between the impaired anti-oxidative capacity of HDL and cIMT (17). cIMT is considered as a surrogate marker of cardiovascular risk (33). Our findings regarding the association of HOI with cIMT supports the notion that impaired anti-oxidative capacity of HDL can be a good predictor of cardiovascular issues. Of note, we found that NAFLD patients with the impaired antioxidant function of HDL ($\text{HOI} \geq 1$) had higher cIMT. This would suggest that the antioxidant function of HDL might be one of the potential mediators of the relationship between NAFLD and CVD.

There are some limitations to this study. The study was cross sectional in nature, which does not provide information about possible causal relationships among NAFLD markers, HDL anti-oxidative capacity and oxidative stress markers. So, further prospective studies are required to specifically address the potential role of HDL anti-oxidative function in the development and progression of NAFLD. We used apolipoprotein B-depleted plasma samples instead of isolated HDL to measure the anti-oxidative capacity of HDL. Polyethylene glycol precipitation is considered as a reproducible and rapid method to extract HDL from plasma samples (9, 19), but there is a possibility that anti-oxidative effects are influenced by other proteins rather than HDL associated proteins. However, a previous study showed comparable HOI values determined with apo B depleted sample and ultracentrifugation isolated HDL.

Conclusions

In summary, our results showed that impaired anti-oxidative capacity of HDL was associated with higher circulating levels of oxidative stress markers and cIMT, suggesting the possible contribution of disturbed anti-oxidative system in causing HDL dysfunctional which in turn can elevate the risk of CVD. The relationship between impaired anti-oxidative capacity of HDL and NAFLD markers suggest either this disease state might attenuate the anti-oxidative effect of HDL or HDL anti-oxidative functionality might contribute to NAFLD pathogenesis. In perspective, further studies are required to elucidate the causal relationship among oxidative stress, the anti-oxidative capacity of HDL, NAFLD pathogenesis and risk of CVD in NAFLD.

Abbreviations

NAFLD: Non-alcoholic fatty liver disease

CVD: Cardiovascular disease

HDL: High-density lipoprotein

DCFDA: Dichlorofluorescein diacetate

PON1: Paraoxonase 1

HOI: HDL oxidative index

SOD: Superoxide dismutase (SOD)

MDA: Malondialdehyde (MDA)

CIMT: Carotid intima-media thickness

LDL-C: low-density lipoprotein cholesterol

VAT: Visceral Adipose Tissue thickness

LS: Liver stiffness

FBG: Fasting blood glucose

TC: Total cholesterol

TG: Triglyceride

HDL-C: high-density lipoprotein cholesterol

ALT: Alanine amino transferase

AST: Aspartate amino transferase

GGT: Gamma glutamyl transferase

Declarations

Ethics approval and consent to participate

All participants gave written informed consent. This study was approved by the medical ethics committee of the Semnan University of Medical Sciences (ethics code: IR.SEMUMS.REC.1397. 331).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PS designed the study, analyzed the data and wrote the manuscript. AP and HP supervised the study and edited the manuscript. ARR did ultrasonography and fibroscan. SK, FAY, and NS performed the experiments and collected the human subjects. All authors approved the manuscript.

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Figures

Figure 1

Comparison of cIMT, plasma levels of MDA, PON1 and SOD in the subjects with preserved anti-oxidative HDL capacity (HOI < 1) and impaired anti-oxidative HDL capacity (HOI ≥ 1). The differences between two groups were analyzed by independent Student's t-test or Mann-Whitney U test and were presented as mean ± standard deviation or median with 95% confidence interval respectively. MDA: malondialdehyde, PON1: Paraoxonase 1, SOD: superoxide dismutase. p < 0.05 was considered statistically significant.

Figure 2

Comparison of AST, ALT, LS and cIMT in the subjects stratified by a HOI below or above 1 and disease state. Kruskal-Wallis test was used to compare among four groups and pairwise tests were performed using the Mann–Whitney test. Data were presented as median with 95% confidence interval. AST; aspartate amino transferase; ALT: alanine amino transferase; LS: liver stiffness, cIMT: Carotid intima-media thickness. $p<0.05$ was considered statistically significant. * indicates significant difference HOI<1 and HOI ≥ 1 . # indicates significant difference between control and NAFLD groups.