# The Detection of HDL receptor on platelet surface in patients with Coronary artery disease (CAD)

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## ABSTRACT

The human scavenger receptor B1 (hSR-B1/CLA) is a HDL receptor with various anti-atherogenic functions which is expressed on the platelet surface. The anti thrombotic function of HDL includes the modulation of platelet reactivity, coagulation, and endothelial function. The goal of this study is to detect the level of HDL receptor on platelets of CAD patients with atherosclerotic disease. Expressions of the hSRB1 receptor on platelets of 31 CAD patients with atherosclerotic plaque and 20 healthy controls was detected using flow cytometry. Moreover, the lipid panel tests were assayed by Chemistry auto analyzer and compared with healthy controls. Our findings show that abundance of hSR-B1/CLA-1 was significantly reduced on the surface of platelets from CAD patients with atherosclerotic disease compared with healthy control (6/8 % vs. 13/6 %), (P <0,001).
The HDL receptor (hSR-B1/CLA-1) expression on platelets inversely correlates with the risk of coronary heart disease. Our findings suggest that determining the level of hSR-B1/CLA-1 on the platelets may be a useful laboratory marker for CAD investigation.

**Key Words:** CAD; Platelet; HDL Receptor

## INTRODUCTION

Atherosclerosis is the major pathological factor which caused CAD and its thrombotic consequences are a progressive disease predominantly occurring in the large arteries. There is a strong correlation between hypercholesterolemia and developing atherosclerosis plaque [1-3]. The High density Lipoprotein (HDL) plays an important role as an anti atherosclerotic agent. This is largely due to HDL function as a cholesterol vehicle, where HDL promotes cholesterol efflux from lipid-laden macrophages and takes this cholesterol back to the liver for processing [3-7].

Scavenger receptor B1 can induce cholesterol efflux by enabling HDL to bind cells and reorganize lipids within cholesterol-rich domains in the plasma membrane [8]. Studies in both humans and animal models indicated that HDL inhibits platelet activation via actions of HDL on the endothelium which induced them to produce nitric oxide (NO) and prostacyclin and tissue factor. On the other hand, down regulate the platelet-activating factor, such as: thromboxane A2, results in inhibits platelet aggregation [9-11].

Further understanding of signaling by SR-BI will optimize the capacity to harness the mechanisms of action of HDL–SR-BI for cardiovascular benefit [12-14]. The goal of this study is to detect the level of HDL receptor expression on platelets of CAD patients who have atherosclerotic plaque.

## MATERIALS AND METHODS

### *Patients*

31 CAD patients including unstable angina,

myocardial infarction and acute coronary syndrome who visited the cardiovascular clinic of Tajrish hospital (Tehran) during 2015-2016 were under study. The participants gave informed consent in accordance with the Deceleration of Helsinki. Before preparation of any sample, the variations of plaque severity score (PSS) were measured using CT angiography which has been described by Pagali SR, et al [17]. It was ensured that all patients had atherosclerotic plaque for control group; samples from 20 healthy volunteers were collected.

***Platelet preparation***

Platelets were isolated from 10 ml anticoagulant (acid-citrate-dextrose) blood of patients and controls. The platelet-rich plasma was fractionated by centrifugation at 200 *g* for 10 minutes at room temperature.

***Flow Cytometric Analysis***

Platelets were washed three times with wash buffer (PBS); they were next resuspended in FACS buffer (0.1% BSA in PBS) at a concentration of $10^7$ platelets/mL in the same buffer. The cells were then incubated with 20 µL diluted (1:100) Rabbit anti-scavenging Receptor SRB1 antibody (abcam company) and put in ice for 3 hours followed by exposure to 5µL fluoresce in (FITC)-conjugated Goat anti Rabbit IgG H & L antibody ( abcam ) (1: 50 dilution) in ice for 1 hour. For controls, platelets were incubated merely with FITC-conjugated Goat anti Rabbit IgG H & L (FITC) antibody (abcam). For Platelet gating, the Platelets were incubated with phycoerythrin (PE) conjugated anti-CD61 antibody (Dako). For platelets' maintenance, 100µL fixative buffer (0.1% paraformaldehyde in PBS) added to PRP before flow ctometry analysis. Finally, the platelets were analyzed using flow cytometry (model PARTEC) with forward scatter 160 and side scatter 215.

***Biochemistry Assays***

Lipid panel (cholesterol, triglyceride, HDL, LDL, HDL/LDL ratio) , liver enzymes(AST, ALT, LDH), heart enzymes(CPK, CK-MB) and troponin were also measured using auto-analyzer ( model CA-180 FURUNO).

***Statistical analysis***

All experiments were repeated three times and statistical analyses were performed with SPSS software (version 21), using Pearson product-moment correlation coefficient test for correlation between tests. A $P<0.05$ was considered statistically significant**.**

## RESULTS

Out of 31 patients, 21 were male and 10 were femal**e** with the the average age 61.5 SEM 9.5; 13 of controls were male and 7 female, with the average age of 56.8 _SEM 4.2.Table 1 summarizes the clinical and laboratory features of CAD patients with atherosclerotic. The amount of plaque was quantified as mild (score of 1+), moderate (score of 2+), or severe (score of 3+)

**Table 1.** Summary of the Clinical Features of the CAD Patients

| No | Age/Y | Sex | Atherosclerosis plaque/ Scoring | T –Cho mg/dL | LDL-C mg/dL | HDL-C mg/dL |
|---|---|---|---|---|---|---|
| **1** | 56 | M | 2 + | 122 | 44 | 59 |
| **2** | 52 | M | 2 + | 177 | 125 | 33 |
| **3** | 58 | F | 1 + | 168 | 104 | 44 |
| **4** | 50 | M | 1 + | 90 | 112 | 39 |
| **5** | 71 | M | 1 + | 136 | 85 | 28 |
| **6** | 56 | M | 2 + | 232 | 146 | 54 |
| **7** | 79 | M | 1 + | 128 | 72 | 40 |
| **8** | 58 | M | 2 + | 135 | 77 | 34 |
| **9** | 51 | F | 1 + | 226 | 41 | 40 |
| **10** | 65 | F | 1 + | 155 | 63 | 63 |
| **11** | 61 | M | 1 + | 143 | 50 | 52 |
| **12** | 65 | M | 1 + | 141 | 74 | 54 |
| **13** | 49 | F | 1 + | 177 | 122 | 38 |
| **14** | 59 | F | 1 + | 137 | 50 | 55 |
| **15** | 68 | M | 1 + | 132 | 68 | 50 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **16** | 66 | F | 1 + | 222 | 140 | 50 |
| **17** | 77 | M | 1 + | 171 | 107 | 35 |
| **18** | 51 | M | 1 + | 140 | 64 | 48 |
| **19** | 74 | M | 1 + | 129 | 65 | 45 |
| **20** | 61 | M | 1 + | 130 | 55 | 53 |
| **21** | 79 | M | 1 + | 210 | 140 | 63 |
| **22** | 51 | F | 1 + | 129 | 40 | 46 |
| **23** | 63 | M | 1 + | 131 | 63 | 41 |
| **24** | 67 | M | 1 + | 110 | 45 | 39 |
| **25** | 67 | F | 1 + | 218 | 132 | 51 |
| **26** | 46 | M | 1 + | 135 | 70 | 43 |
| **27** | 58 | F | 1 + | 224 | 143 | 51 |
| **28** | 64 | M | 1 + | 173 | 97 | 41 |
| **29** | 57 | M | 1 + | 164 | 65 | 58 |
| **30** | 62 | M | 1 + | 189 | 67 | 39 |
| **31** | 41 | F | 1 + | 163 | 59 | 43 |

**M** indicates male; **F**, female; **T-Chol**, total cholesterol; **LDL-C**, LDL cholestrol; HDL-C, HDL cholesterol, Atherosclerotic plaque scoring as: Mild 1+, Moderate 2+, Sever 3+

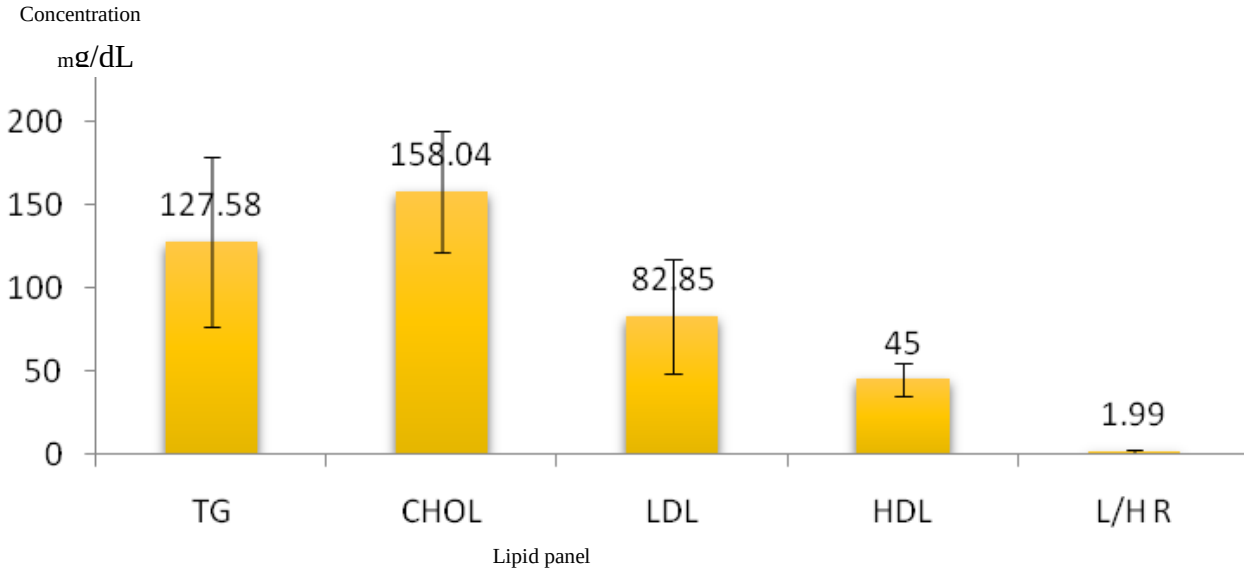


**Figure 1.** The mean and SD of serum lipid panel: triglyceride (TG), Total cholesterol (CHOL), LDL-cholesterol (LDL) , HDL-cholesterol (HDL) and LDL/HDL ratio (L/H R) in patients

The Flow cytometry analysis of hSR-B1/CLA-1 receptors on the Surface of patients and controls were detected on CD61-positive platelets (figure 2).

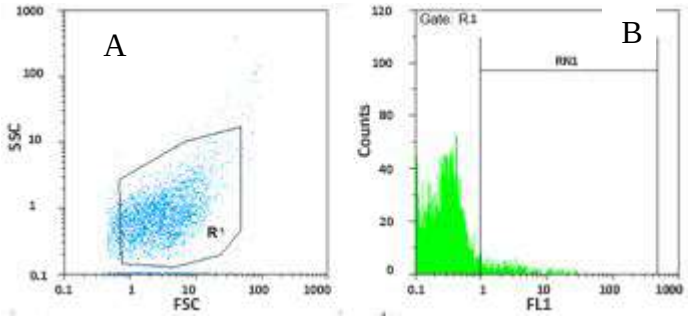


**Figure2.** Flow cytometric analysis for surface expression of hSR-B1 receptor in human platelets with CD $61^{+}$ (A); the mean fluorescence intensity (MFI) of a patient as 6.2 % (Figure B

Differential levels of hSR-B1on platelets from patients with atherosclerotic disease were compared with controls.

A representative profile of hSR-B1surface expression on the platelets from a healthy individual was compared with that of a patient with atherosclerotic disease. The mean of hSR-B1 levels of platelets from the patient was clearly decreased in comparison with healthy controls. Figure 3 shows the mean fluorescence intensity (MFI) of hSR-B1 expression, which are 6.84 % and 13.6 % in patients and controls respectively. Our study shows that hSR-B1 expression in platelets is decreased in patients with atherosclerotic disease compared with the control group (p<0,001).

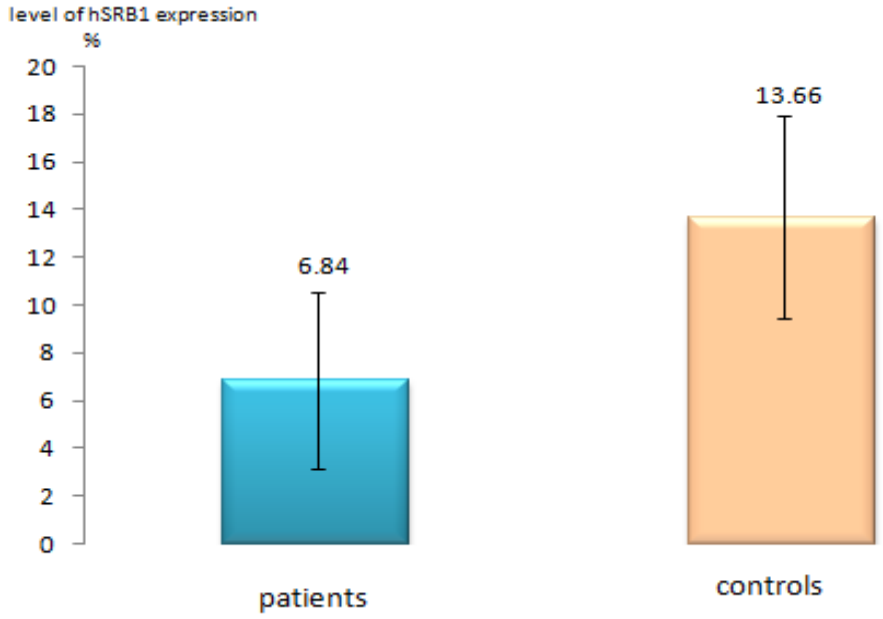


**Figure 3.** The percentage of the mean of hSRB1 expression platelets' patients (left): 6.84% and controls (right) : 13.66% by flow cytometry, ( p <0,001).

The results of the comparison between the level of platelet HDL receptors and serum lipid panel and serum enzymes show that platelet HDL receptor expression did not significantly correlate with plasma cholesterol, HDL, LDL, or triglycerides and heart enzyme, but do have a significant correlation with troponin ( P < 0.049). The comparison between serum biochemical and platelet HDL receptor is included in table 2.

**Table 2.** The relation between the expression of platelet HDL receptors and serum biochemical factors in patients with CAD

| **The relation between HDL receptor and Serum Biochemical factors** | **P value** |
|---|---|
| Troponin | p=0.049* |
| CPK | p=0.913 |
| CK-MB | p=0.387 |
| LDH | p=0.634 |
| AST | p=0.725 |
| Total-Cholesterol | p=0.543 |
| HDL-c | p=0.732 |
| LDL-c | p=0.894 |
| TG | p=0.309 |

## DISCUSSION

The scavenger receptor class B type 1 (SR-B1) is a multi-ligand receptor that has high affinity for HDL and can mediate the bi-directional exchange of lipids between bound HDL and cells like platelets and macrophages [8,15]. In this study, the expression of hSR-B1/CLA-1 in platelets of CAD patients was detected. The results show that the expression of hSR-B1 receptor was significantly diminished in patients (p<0,001) in flow cytometry. SR-B1 is believed to play an important role as a docking receptor for HDL in connection with selective uptake of cholesterol esters. It is tempting to speculate that decreased levels of hSR-B1/CLA-1 in platelets, isolated from patients with atherosclerotic disease, reflect a decrease in RCT and thus enhance their risk for developing the disease [14, 16]. It could be said that the SR-BI as a HDL receptor has considerable role as an anti atherogenic capacity [17].

## CONCLUSION

These findings raise the possibility that a measurement of the hSR-B1/CLA-1 expression on human platelets may provide a valuable insight that reflects the status of RCT in patients with atherosclerosis and can show the risk of cardiovascular disease in future.

## ACKNOWLEDGMENTS

Authors would like to thank the deputy research of Shahid Beheshti University of medical Science and IBTO for supporting our research.

*"The authors declare no conflict of interest"*

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