Oxidative Low Density Lipoprotein Prohibited Plasmodium Falciparum Clearance in type 2 Diabetes Mellitus Via Cluster Differentiation 36

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Abstract

Background: Cluster of differentiation 36 (CD36) is reported to function as a receptor of erythrocytes infected with Plasmodium falciparum (PF) and as an oxidized low-density lipoprotein (oxLDL). Aim: The aim of this study was to investigate the impact of CD36 in PF parasitized red blood cells in high concentration of oxLDL of T2 diabetes mellitus patients. Material and Methods: This cross-sectional study was conducted among diabetic patients. A total of 45 samples were collected from diabetic patients with more than 8% of HbA1c and more than 170 mg/dL of oxLDL. Results: The mean difference between CD36 negative and positive controls was found to be statistically significant (P ≤ 0.001). The mean difference between CD36 positive control and CD36 in diabetic patients with oxLDL ≥ 170 mg/dL also was statistically significant. Conclusion: High concentration of oxidative low density of lipoprotein more than 170 mg/dL leads to block CD36 receptor on infected red blood. This process believed to contribute in parasite survival by avoiding phagocytic clearance in the spleen.

Keywords: CD36, diabetes mellitus, diabetes mellitus, Oxidative low density lipoprotein, plasmodium falciparum

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Introduction

Cluster of differentiation 36 (CD36) is a multifunctional molecule. It has independent binding sites for different classes of ligands such as modified phospholipids, thrombospondins, and free fatty acids. This enables CD36 responsible for several different cellular processes depending on the nature of the ligand and the type and location of the cell on which it is expressed. On phagocytes, CD36 functions as a scavenger receptor helping in recognition and internalization of apoptotic cells, falciparum malaria-infected erythrocytes.

CD36 also functions as an adhesion molecule, it has been identified CD36 as the receptor that helps in cytoadherence of Plasmodium falciparum (PF) parasitized erythrocytes. It has been reported that CD36 on platelet mediates clumping of PF-infected erythrocytes.

is strongly associated with severe malaria in contrast, CD36 on monocytes or macrophages can help phagocytosis of falciparum-infected erythrocytes. Thus, the location of CD36 receptor can regulate the severity of malarial disease. Several studies have suggested an important role of CD36 in phagocytic clearance of apoptotic and senescent cells.

Low-density lipoprotein (LDL) is the major cholesterol-bearing lipoprotein in human serum. LDL transports cholesterol to various tissues and cells where it will be used. LDL particles contain, on average, 1600 molecules of cholesterol ester, 600 molecules of free cholesterol, 700 molecules of phospholipid (64% phosphatidylcholine (PC), 1.5%...
phosphatidylethanolamine (PE), 26% sphingomyelin (SM), and 11% lysol phosphatidylcholine (LPC), 180 molecules of triglyceride (TG), and 1 molecule of apolipoprotein B-100 (ApoB-100). The phospholipids, free cholesterol and ApoB constitute the outer layer of LDL. The inner core of LDL contains mostly cholesterol ester and TG. Oxidized low-density lipoprotein (oxLDL) changes macrophages to be more an adherent. oxLDL or oxidized linoleic acids, components of oxLDL induce differentiation of monocytes to macrophages with lower expression of chemokine (C-C motif) receptor (CCR) 2 and higher expression of C x 3CR1, which are more adherent to arterial smooth muscle cells; a previous study also showed that cholesterol addition to plasma membrane inhibits macrophage migration.

Materials and Methods

Ethical approval

Ethical clearance will be obtaining from the Ethical Committee Board of the Tropical Medicine Research Institute. The verbal of the consent was taken from patients and take the permission from medical management of Jaber Abu Ezz Diabetes Center and selected individual after being informed with all objectives of the study and its health impact in the future.

This cross-sectional study was conducted in Khartoum state among diabetic patients attending in Jabir Abo Eleiz diabetic center.

Sampling

Venous blood samples were collected in heparin containers for culture, oxLDL, and random blood sugar. For HbA1c in 0.04 mg ethylenediaminetetraacetic acid anticoagulant, 2-5 mL of blood was collected. The samples were mixed well and tested within 6 h. Samples were classified into three groups:

First group contains 15 samples were collected from apparently health people free from any disease as negative control, and in the second group 15 samples were cultured with plasmodium falciparum as CD36 positive control. While in third group 15 samples from diabetic patients with PF (culture) with high concentration oxLDL more than 170 mg/dL were tested for CD36.

Malaria culture is the method to grow malaria parasite outside the body, that is, in an in vitro environment. PF is currently the only human malaria parasite that has been successfully cultured continuously in vitro.

Quality control

All reagents and test equipment were controlled according to the instructions in the procedures manual, manufacturing control, and control sample were used in each test.

Statistical study

Data were analyzed by using Statistical Package for Social Science (SPSS) version 21 and Microsoft Excel 2013. Results were obtained by using student’s t test.

Results

A total of 45 individuals participated in the present study. All these groups were cultured with PF except CD36 negative control group. In the Table: The mean difference of CD36 amount in negative control and positive control was found to be statistically significant at P = 0.001 (P ≤ 0.001). The mean difference of CD36 amount in negative control and diabetic patients with oxLDL ≥ 170 mg/dL was found to be statistically significant at P = 0.001(P ≤ 0.001). The mean difference of CD36 amount in positive control and diabetic patients with oxLDL ≥ 170 mg/dL was found to be statistically significant at P = 0.001. The mean difference percentage of red blood cells (RBCs) containing (CD36); between CD36 negative control and CD36 positive control was found to be statistically significant at P = 0.003. The mean difference of percentage of RBCs containing (CD36) between CD36 positive control and CD36 diabetic patient with high oxLDL ≥ 170 mg/dL was found to be statistically insignificant at P = 0.013.

Discussion

CD36 is a broadly expressed membrane glycoprotein that acts as a facilitator of fatty acid uptake, a receptor for LDL, and malaria-infected erythrocytes. Despite an impressive increase in knowledge of CD36 functions, in depth understanding of the mechanistic aspects of this protein remains elusive. This study focuses on the role of CD36 in PF infection in diabetic patients. CD36 was measured by Flow cytometry uses the principles of light scattering, light excitation, and emission of fluorochrome molecules to generate specific multiparameter data from particles and cells in the size range of 0.5 - 40 μm diameter. Cells are hydrodynamically focused in a sheath of PBS (Phosphate buffer saline) before intercepting an optimally focused light source. Lasers are most often used as a light source in flow cytometry properties of single particles (e.g. cells, nuclei, and chromosomes) during their passage within a narrow, precisely defined liquid stream.

In present study when data of RBCs infected with PF was compared with normal healthy RBCs there was significant (P < 0.001) expression of CD36 in the RBC [Table 1] in addition, comparison between the same
groups also found significant ($P < 0.003$) increasing of RBCs percentage containing CD36. This agree with Ho and White$^{[13]}$ were proposed CD36 a major receptor for PF-infected RBCs.

LDL cholesterol esters in serum are hydrolyzed by cholesterol esterase. LDL cholesterol is then oxidized by cholesterol oxidase to the corresponding ketone liberating hydrogen peroxide, which is then converted to water and oxygen by the enzyme peroxidase. Para aminphenazone (4 aminphenazone) takes up the oxygen and together with phenol forms a pink colored quinoneimine dye, which can be measured at 515 nm/yellow green filter. When the group that contains oxLDL concentration more than 170 ml/dL was compared with the group which contains CD36 in patient with PF malaria only there was significant decrease CD36 expression by oxLDL as appeared in Table 1 ($P < 0.001$), also comparison of same above groups there was significant reduction of the percentage of infected RBCs that contain CD36 ($P < 0.013$), this agreed with the previous study represented that oxLDL plays an important role of CD36 reduction in parasitized RBCs by falciparum malaria, few have investigated its impact on erythrocytes, has been demonstrated that oxLDL has a significant effect on viscoelastic properties of erythrocytes, suggesting that under pathological conditions oxLDL may indeed injure erythrocytes and reduce their deformability in circulation, also found that oxLDL decreased free thiol radicals in erythrocyte membranes, which coincided with the increase of the cross-linking among membrane skeletal proteins.

For the membrane to deform normally, the skeletal network with a “spoked” hexagonal topology must undergo equibiaxial and/or anisotropic extension, where spectrin and other skeletal proteins may also unfold. Inter- or intermolecular cross-linking of membrane proteins would limit topological rearrangement of the network and hinder erythrocyte deformation.$^{[14]}$

The reduction of CD36 expression may have remarkable in the development of severity PF malaria in diabetic patient (T2 DM). oxLDL blocks the CD36 receptor on infected RBCs, oxLDL competitively inhibits the adherence of pRBCs to CD36 and therefore; CD36 may play a role in cardiovascular disease, since excessive uptake of oxLDL by macrophages contributes to atherogenesis. Lipid-loaded macrophages constitute the foam cells seen in the “fatty streak” that characterizes atherosclerotic plaques, and CD36 is upregulated in cells that take up and degrade oxLDL. The innate immunity such as macrophage playing important role to phagocyte parasite, but in the presence of oxLDL that bind with this receptor in parasitized red blood cell the macrophage cannot identify (infected RBCs) and some complication coming, while the adaptive immunity like Fc receptor for immunoglobulin G (IgG), Fcγ RII-B2 will not identify the parasitized RBCs that mean the oxLDL block receptor and IgG does not identify parasite and some complication can be coming. Ectopic expression of FcyRII-B2 conferred upon cells the ability to efficiently take up and degrade oxLDL. As expected for an oxLDL receptor, FcyRII-B2 binds oxLDL with high affinity, and the binding is blocked by oxLDL. Although a monoclonal antibody directed against FcγRII-B2 blocked the uptake of oxLDL in transfected cells, the same antibody was unable to significantly block oxLDL uptake by macrophages the physiological relevance of FcγRII-mediated uptake of oxLDL thus remains uncertain, these play role in adaptive immunity Ab IgG and to become feebleness of this immunity as previously mentioned and increased the severity of malaria PF and lead severe complication.$^{[15]}$

### Conclusion

We conclude that PF might increase the density and amount of CD36 in parasitized red blood cells. High concentration of oxidative low density of lipoprotein more than 170 mg/dL leads to block CD36 receptor on

| Group                                      | Mean     | SD       | DF | $T$   | Sign   |
|--------------------------------------------|----------|----------|----|-------|--------|
| CD36 negative control                      | 0.3600   | 0.12923  | 28 | 10.513| 0.001**|
| CD36 positive control                      | 26.1000  | 9.48194  |    |       |        |
| CD36 negative control                      | 0.3600   | 0.12923  | 28 | 10.385| 0.001**|
| CD36 in diabetic patient with oxLDL ≥170 mg/dL | 6.3593  | 2.3363   |    |       |        |
| CD36 positive control                      | 26.1000  | 9.48194  |    |       |        |
| CD36 in diabetic patients with oxLDL ≥170 mg/dL | 6.3593  | 2.23363  |    |       |        |
| CD36 negative control                      | 1.7600   | 0.64454  | 28 | 3.29  | 0.003**|
| CD36 positive control                      | 11.1800  | 11.05740 |    |       |        |
| CD36 positive control                      | 11.1800  | 11.05740 |    | 2.67  | 0.013* |

SD: Standard deviation; DF: Degree of freedom; $T$: $T$ value; Sign: Significant CD: Cluster of differentiation; oxLDL: Oxidative low density lipoprotein; *$P$$\leq0.05$, **$P$$\leq0.01$
infected red blood, this process believed to contribute to parasite survival by avoiding phagocytic clearance in the spleen, and also in adaptive immunity such as IgG antibody “opsonization” might be hindered and participates in increasing of the severity of malaria PF and its complications. In this study, PF malaria participates in increasing of complications of systemic diseases such as diabetes mellitus, hypertension, and cardiovascular diseases.

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