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Research article

HEMOGLOBIN A_1C INDUCED DOWN-REGULATION OF CD36 OF *PLASMODIUM FALCIPARUM* PARASITIZED RED CELL

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ABSTRACT

Objective: High values of glycosylated hemoglobin have been found to correlate with decreased deformability of erythrocyte. CD36 (Cluster of Differentiation 36) is an integral membrane protein found on the surface of many cell types of class B scavenger receptor family. *Plasmodium falciparum* and diabetes mellitus is associated many complications. Aim of this study to investigate the down-regulation of HbA1c to CD36 on *P. falciparum* parasitized red blood cells Diabetes mellitus patients. **Methods:** This is cross section study conducted among diabetic patients attending in Jabir Abo Eleiz diabetic center in Khartoum state. Venous blood samples were collected in heparin containers for *Plasmodium falciparum* culture, and random blood sugar. For HbA1c in 0.04 mg EDTA anticoagulant, 2-5 ml of blood was collected. Sample size was 45 samples and was collected from known diabetic patients with HbA1c more than 8%. All data were analyzed by using Statistical Package for Social Science (SPSS). **Results:** show the mean difference between CD36 negative control and CD36 positive control was found to be statistically significant increasing of CD36 presence at P. value =0.001 ($P \leq 0.001$). The mean difference between CD36 positive control and diabetic patients with HbA1C more 8% was found to be statistically significant reduction of CD36 expression at p=0.001. **Conclusion:** Hyperglycemia (HbA1c) leads to decrease of CD36 expression and interfere with innate and active immunity. In this study HbA1c participates in increasing of *P. falciparum* malaria complications.

Keywords: HbA1c, CD36, *Plasmodium falciparum*, Diabetes mellitus.

INTRODUCTION

Glycation of proteins is a frequent occurrence, but in the case of hemoglobin, a non-enzymatic reaction occurs between glucose and the N-end of the beta chain. Abnormal glycation, which can adversely affect hemoglobin and membrane proteins in erythrocytes, has been shown to correlate with reduced membrane fluidity[1] separately, high values of glycosylated hemoglobin have been found to correlate with decreased deformability of erythrocyte.[2]

CD36 is a multi-functional 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[3], falciparum malaria infected erythrocytes.[4,5]

CD36 also functions as an adhesion molecule, it has been identified CD36 as the receptor that helps in cytoadherence of *Plasmodium Falciparum* parasitized erythrocytes[6], It has been reported that CD36 on platelet mediates clumping of *P. falciparum* infected erythrocytes is strongly associated with severe malaria.[7] 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.[8] Malaria culture is the method to grow malaria parasite outside the body i.e. in an in vitro environment. P. falciparum is currently the only human malaria parasite that has been successfully cultured continuously in vitro. Although attempts for propagation of the parasites outside of humans or animal models.[9]

METHODOLOGY

Ethical approval: Ethical clearance obtained from the Ethical Committee Board of the Tropical Medicine Research Institute. The consent was taken from patients and taken 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 is cross section study was conducted in Khartoum state among diabetic patients attending in Jabir Abo Eleiz diabetic Centre. In an aseptic conditions ml venous blood samples were collected in heparin containers for culture, Ox LDL, and Random Blood Sugar (RBS). For HbA1c in 0.04 mg EDTA anticoagulant, 2-5 ml of blood was collected. The samples were mixed well and tested within 6 hour. Samples were classified into three groups:

Group I: 15 samples were collected from apparently health People free from any disease as negative control.

Group II: 15 samples were cultured with *Plasmodium falciparum* as CD36 positive control. Culture technique as following: Erythrocytes were washed 3 times in Roswell Park Memorial Institute medium (RPMI) 1640 to remove citrate phosphate dextrose (CPD), serum, and leukocytes if present. Dilute to 5% hematocrit with cMCM in small flasks of 25cm2 (0.2 mL of packed cells to 4 mL of malaria culture media (MCM) or in 75-cm2 flasks (1.0 mL to 20 mL). Parasites were added to an appropriate parasitemia. Flask was put in a candle jar and loosens the screw cap. Produce low oxygen by burnt out candle and place the jar at 37 °C. MCM was replace every day (not necessary the day after sub cultivation). Subculture was cultured 2 times / week.

Group III: 15 samples from known diabetic patients (HbA_1c more than 8%) and were tested for CD36.

CD36 was measured by Flow cytometer uses the principles of light scattering, light excitation, and emission of fluorochrome molecules to generate specific multi-parameter data from particles and cells in the size range of 0.5um to 40um diameter. Cells are hydro-dynamically focused in a sheath of phosphate buffer saline (PBS) before intercepting an optimally focused light source; Lasers are most often used as a light source in flow cytometer properties of single particles (e.g. cells, nuclei, chromosomes) during their passage within a narrow, precisely defined liquid stream.

The hemolysate, where the labile fraction is eliminated hemoglobin's are retained by a cationic exchange resin. Hemoglobin A1C (HbA1c) is specifically eluted after washing away the hemoglobin A1a+b fraction1 (HbA1a+b), and is quantified by direct photometric reading at 415 nm.

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.

Data analysis: Data were analyzed by using Statistical Package for Social Science (SPSS) version 21 and Microsoft Excel 2013. Results were obtained by using student T .test.

RESULTS

Table 1: Shows the mean difference of CD36 amount in negative control and positive control:

Group	N	Mean ±	SD	DF	T	P Value
CD36 negative control	15	0.3600 ±	0.12923	28	10.513	0.001**
CD36 positive control	15	26.1000 ±	9.48194			

P *≤ 0.05, P **≤ 0.01

Table 2: Shows the mean difference of CD36 amount in negative control and diabetic patients with $HbA_{1C} \geq 8\%$

Group	N	Mean ± SD	DF	T	P Value
CD36 negative control	15	0.3600 ± 0.12923	28	12.943	0.001**
CD36 in diabetic patient with $HbA_{1C} \geq 8\%$	15	5.9460 ± 1.66648			

Table 3: Shows the mean difference of CD36 amount in positive control and diabetic patient with $HbA_{1C} \geq 8\%$

Group	N	Mean ± SD	DF	T	Sign
CD36 positive control	15	26.1000 ± 9.48194	28	8.108	0.001**
CD36 in diabetic patient with $HbA_{1C} \geq 8\%$	15	5.9460 ± 1.66648			

Table 4: Shows mean difference of RBCs percentage containing (CD36); between CD36 negative control and positive control:

Group	N	Mean ± SD	DF	T-value	P-value
CD36 negative control	15	1.7600 ± 0.64454	28	3.29	0.003**
CD36 positive control	15	11.1800 ± 11.05740			

Table 5: Shows mean difference of RBCs percentage containing (CD36) between CD36 positive control and diabetic patients with $HbA_{1C} \geq 8\%$

Group	N	Mean ± SD	DF	T-value	P-value
CD36 positive control	15	11.1800 ± 11.05740	28	2.64	0.013*
CD36 in diabetics patients with $HbA_{1C} \geq 8\%$	15	3.6067 ± 1.11257			

Forty five (45) individuals participated in the present study.

In table: 1 the mean difference between CD36 negative control and CD36 positive control was found to be statistically significant at P. value =0.001 ($P \leq 0.001$).

The mean difference between CD36 negative control and CD36 in diabetic patients with $HbA_{1C} \geq 8\%$ was highly significant at P. value = 0.001 as shows in table 2.

The mean difference between CD36 positive control and diabetic patient with HbA_{1C} more 8% was found to be statistically significant at p=0.001 as shows in table 3.

The mean difference percentage of RBCs containing (CD36) between CD36 negative control and CD36 positive control was found to be statistically significant at p = 0.003 as shown in table 4.

The mean difference of percentage of RBCs containing (CD36) between CD36 Positive control and CD36 in diabetics patients with $HbA_{1C} > 8\%$ was found to be statistically significant at p = 0.013 as shown in table 5.

DISCUSSION

HbA1c occurs when hemoglobin joins with glucose in the blood. Hemoglobin molecules make up the red blood cells in the blood stream. When glucose sticks to these molecules it forms a glycosylated hemoglobin molecule, also known as A1c and HbA1c. The more glucose found in the blood the more glycated hemoglobin (HbA1c) will be present.[10]

CD36 is a broadly expressed membrane glycoprotein that acts as a facilitator of fatty acid uptake, a receptor for low density lipoprotein, 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 impact of hemoglobin A1c on CD36 of *Plasmodium falciparum* infection in diabetic patients.

In present study when data of red blood cells infected with *P. falciparum* (P.F) was compared with normal healthy RBCs there was significant ($P < 0.001$) expression of CD36 in the red blood cell in addition, comparison between the same groups also found significant ($P < 0.003$) increasing of RBCs percentage containing CD36 that means plasmodium falciparum could increases the expression of CD36 on the surface of the parasitized red cells. This agreed with Ho, M. and White, N.J. 1999,[11] who were proposed CD36 a major receptor for *P. falciparum*-infected red blood

cells (IRBCs). Moreover, comparison of the group that contains parasitized red blood cell (*P. falciparum*) with high concentration of glycosylated hemoglobin (HbA1c) more than 8% with normal healthy RBCs (negative control) there was significant ($P < 0.001$) expression of CD36, and the CD36 expression was expressed in the presence of HbA1c in small amount which indicates the P.F plays a role in IRBCs cell membrane leads to CD36 expression, this is has no conflict with previous study that represented .These abnormal circulatory properties of erythrocytes involve parasite-induced alterations in their biomechanical and adhesive properties and are important for survival and pathogenicity of *P falciparum.*[12] Cytoadhesion is mediated by the antigenically variant *P. falciparum* erythrocyte membrane protein-1 (PfEMP1), which can bind to host receptors including CD36 and chondroitin sulfate A „CSA"[13] PfEMP1 is concentrated on electron-dense elevations of the membrane referred to as knobs.[14,15] providing a platform for adherence under physiologic flow conditions.[16] Increased erythrocyte rigidity and adhesiveness result in dramatically augmented hemodynamic resistance observed in microvasculature perfused with *P falciparum*–infected erythrocytes.[17]
However, the group that contains CD36 with high concentration of glycosylated hemoglobin (HbA1c) more than 8% has been compared with the group which contains CD36 in patient with *P. falciparum* malaria only (positive control) there was significant reduction of CD36 expression ($P < 0.001$), also this study agreed with Van Nieuwenhoven *et al*, 1998[18] was proposed that hyperglycemia might play an important role in the regulation of CD36 expression .The result in this study shows the significant effect of HbA1c on CD36 expression reduction. Thus, these results was agreed with the previous study shown that hyperglycemia is also associated with increased levels of reactive oxygen species in diabetic red blood cell and rise in the levels of the reactive carbonyl compounds that worsen their compromised functions, leading to diminished lifespan, accelerated non-enzymatic modification of proteins, and loss of protein function.[19,20] A result of increased protein glycosylation could participate in the mechanism, whereby diabetic erythrocytes may acquire membrane abnormalities.[21] Spectrin is a very important protein of erythrocyte membrane and a target for glycosylation and further oxidation, which might be responsible for increased number of poorly deformable erythrocytes found among diabetic erythrocytes.[22] Abnormal glycation, which can adversely affect hemoglobin and membrane proteins in erythrocytes, has been shown to correlate with reduced membrane fluidity[1] separately, high values of glycosylated hemoglobin have been found to correlate with decreased deformability of erythrocyte.[2] The reduction of CD36 expression may have remarkable in the development of severity *P. falciparum* malaria in diabetic patients.

CONCLUSION

We conclude that *Plasmodium falciparum* might increase the density and amount of CD36 in parasitized red blood cells. Hyperglycemia (HbA1c) leads to down-regulate of CD36 expression and interfere with innate and active immunity. In this study HbA1c participates in increasing of *P. falciparum* malaria complications.

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Conflict of Interest Statement: The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/ or affiliations relevant to the subject matter or materials included.

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