Clin. Lab. 2018;64:XXX-XXX

ORIGINAL ARTICLE

STAT1 and STAT6 Act as Antagonistic Regulators of PPARγ in Diabetic Patients with and without Cardiovascular Diseases

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SUMMARY

Background: **The processes that mediate an inflammatory environment and increase atherosclerosis in diabetes are not well understood. Peroxisome proliferator-activated receptors (PPARs) are a subgroup of the nuclear hormone receptor superfamily of ligand-activated transcription factors which play an important role in the pathogenesis of type 2 diabetes mellitus (T2DM) and atherosclerosis. PPARγ promotes changes in lipid metabolism, especially in fatty acid (FA) trafficking, and the activity of PPARγ could be modulated by diabetes phenotype patients. Fatty acid translocase CD36 is one of the advanced PPARγ targets to arbitrate this action. In the current study, we investigated the potential role of signal transducer and activator of transcription STAT1 and STAT6 signaling linked to PPARγ and its implication in the modulation of lipid metabolism.**

Methods: **Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) was used to quantify target genes in Peripheral Blood Mononuclear Cells (PBMCs) isolated from two diabetic groups: diabetic patients with cardiovascular diseases (D.CVD) and without cardiovascular diseases (D).**

Results: **We demonstrated that PPARγ and CD36 mRNA expressions were downregulated along D.CVD compared to D ($p = 0.002$; $p = 0.04$; respectively). Decreased CD36 was accompanied by elevated levels of plasma triglyceride (TG) concentrations, 0.83 ± 0.29 vs. 2.46 ± 0.22), respectively. Furthermore, STAT1 was significantly more expressed in D.CVD ($p = 0.01$). On the other hand, we demonstrated that STAT6 induces a significant level of PPARγ mRNA expression in D patients ($p = 0.01$).**

Conclusions: **Our results suggest that the expression and activity of PPARγ mediates CD36 in PBMCs and varies with respect to STAT6 and STAT1 trafficking in diabetic patients with and without cardiovascular diseases.**
(Clin. Lab. 2018;64:xx-xx. DOI: 10.7754/Clin.Lab.2017.171013)

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Manuscript accepted November 10, 2017

KEY WORDS

T2DM, CVD, PBMC, PPARγ, CD36, STAT1, STAT6, triglycerides

INTRODUCTION

Three hundred sixty six million people had T2DM in 2011, and half of these (183 million people) are undiagnosed [1]. Patients with diabetes are responsible for a two- to four-fold rise in the risk of developing CVD compared to non-diabetic patients and constitute a heightened part of the population [2]. Therefore, new targeted strategies are urgently needed [3]. Diabetes

mellitus (DM) commonly refers to the pathologic processes arising when the pancreas does not make enough insulin or requires the use of insulin [4]. This pathology evolved progressively in many different ways. Compared with healthy individuals, patients with type 2 diabetes mellitus have a significantly higher risk of cardiovascular morbidity and mortality and are affected by cardiovascular disease [5]. The advantage of PBMCs above other cells is that blood is the most readily accessible cellular material in humans, and PBMCs can be isolated from whole blood relatively easy [6]. Circulating PBMCs are open to the systemic environment, including metabolic factors like dyslipidemia and inflammatory molecules produced by other organs and tissues, and may be directly involved in the chronic inflammation associated with the development of atherosclerosis [7]. Moreover, the expression of the PPARγ has been demonstrated in human peripheral blood mononuclear cells (hPBMC), such as macrophages and monocytes [8], and seems to play a pivotal role in the regulation of gene expression of diverse diseases including obesity, diabetes and cancer [9]. PPARγ is a lipid-activated transcription factor that acts as an important regulator of lipids, glucose metabolism, and energy balance [10,11]. It also has advanced potent anti-inflammatory properties that modulate immune inflammatory responses [12]. By regulating cellular metabolism, PPARγ could affect the balance of immune activities. This chronic effect plays a prominent role in the pathogenesis of atherosclerosis and type 2 diabetes mellitus [13].

Many studies have shown that PPARγ mediates CD36 expression in the genes participating in the release, transport, and storage of fatty acids [14] and its role may influence the progress of atherosclerosis [15]. CD36 is a transmembrane protein of the class B scavenger receptor family and it has been recently implicated in various aspects linked to fatty acid (FA) metabolism containing fat taste perception, fat intake, fat absorption, absorption-related peptide secretion, and FA utilization by muscle and adipose tissues [16]. CD36 is expressed in various cell types including monocytes [17] and endothelial cells [18], it recognizes a variety of ligands including oxLDL [19]. CD36 expression is induced in human monocytes following exposure to IL-13 associated with the PPARγ pathway [20]. It also has been demonstrated that CD36 deficiency decreases FA uptake by heart, skeletal muscle, and adipose tissue [21]. Fasting plasma TG and FA levels are elevated. The decline in muscle FA utilization improves insulin sensitivity of the tissue [22]. It should be noted, that among risk factors for CVD estimated by the NHANES 1999 to 2008, the overall prevalence of hypertriglyceridemia in the adult population in the United States is 31% [23].

STAT6 interacts with PPARγ to favour fatty acid β-oxidation [24]. On the other hand, an interaction between STAT1 and PPARγ is required for CD36 and foam cell formation, creating critical events that regulate pathogenesis of atherosclerosis [25].

Our study was conducted with two groups, diabetic patients with cardiovascular disease (D.CVD) and diabetic patients without CVD (D). We explore the impact of STAT1 and STAT6 phenotypes on PPARγ activity linked to CD36 expression in PBMCs and the lipid glyceride profile of the two groups.

MATERIALS AND METHODS

Patient selection

A total of 40 patients aged between 47 and 69 years, all with type 2 diabetes mellitus (20 D.CVD and 20 D) were recruited at the University Hospital of Bizerte, and 5 healthy volunteers were studied as controls. All control subjects have a negative history of CVD, T2DM, and had a normal resting ECG (electrocardiogram). The research protocol was approved by the local Ethical Committee of Bizerte Hospital and informed written consent was obtained from all participants. The diagnosis of diabetes mellitus was based on a previous history of diabetes according to the American Diabetes Association (ADA) criteria [26]. Diabetic subjects were treated with sulfonylurea alone or in combination with metformin. The diagnosis of CAD was assessed by a cardiologist, and it was based on symptoms and clear ischemic changes in an electrocardiogram, either at rest or during an exercise test, or on findings from coronary angiography. Patients' recruitment satisfied the following criteria: age 45 to 69 years, body mass index (BMI) less than 35 kg/m^2, glycosylated hemoglobin (HbA1c) less than 12%, and absence of kidney failure and liver or thyroid disease. Fasting total cholesterol concentrations > 5.17 mmol/L and fasting plasma triglyceride concentrations > 1.17 mmol/L were considered to be elevated. Hypertension in the diabetic population was diagnosed according to predetermined blood pressure level (systolic blood pressure > 130 mm Hg and/or diastolic blood pressure > 85 mm Hg). Clinical characteristics of patients are shown in Table 2.

Laboratory procedures

Blood samples were collected after 10 to 12 hours of fasting via peripheral venipuncture to assess the levels of fasting glucose and triglycerides (TG). Plasma glucose concentrations were determined by using an enzymatic kit (glucose oxidase). HbAlc (glycated hemoglobin A1c) values were determined using an ion-exchange microcolumn chromatographic procedure (DS5 HbA1c Analyzer) where normal values are 4.1 - 5.9%. TG concentrations were measured using reference enzymatic techniques on a COBAS 6000 analyzer (Roche Diagnostics).

Preparation of human peripheral blood mononuclear cells (PBMCs)

For each participant, 5 mL of blood were collected from a peripheral vein into EDTA tubes (BD Vacutainer® Tubes) using standard phlebotomy procedures. Samples were processed within 2 hours of collection. The blood

was applied carefully to a Ficoll 1077 gradient (Eurobio, Les Ulis, France) in 15 mL collection tubes and centrifuged at 2400 rpm for 20 minutes at room temperature. To obtain PBMCs, the cells were aspirated from the interface, rinsed three times with PBS, pH 7.4, and stored at -80°C until RNA extraction [27].

RNA extraction

Total RNA was extracted with the RNeasy Mini Kit (Qiagen) following the manufacturer's protocol. Purified RNA quality control for quantity and purity was assessed using an ND-1000 NanoDrop spectrophotometer (NanoDrop Technologies).

Reverse transcription and qPCR conditions

Equal amounts of total RNA (1 µg) were reverse transcribed. cDNA synthesis was carried out using Prime Script TM103 1st strand cDNA Synthesis Kit (Takara). All PCR reactions were performed using an ABI Prism 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems). cDNA was amplified by the SYBR Premix Ex Taq Kit (Takara) according to the manufacturer's protocol.

For each reaction, 1 µL of each primer (10 mM), 0.4 µL of Rox reference dye II (50x), and 10 µL of SYBR Primer Ex Taq were added, in a total volume of 20 µL. Thermal cycler parameters included 10 minutes at 95°C and then 40 cycles of 95°C for 10 seconds and 62°C for 20 seconds. The relative expression of each gene was calculated by the ΔCt method, where ΔCt is the value obtained by subtracting the Ct (threshold cycle) value of β-actin mRNA from the Ct value of the target gene. The amount of target relative to the β-actin mRNA was expressed as $2^{-(\Delta Ct)}$. Primers used throughout this study are listed in Table 1.

Statistical analysis

Statistical analysis was performed with Statistica_8por 1 (version 8.0.360.0, STATISTICA, USA). The data were expressed as a mean ± standard deviation. The intergroup differences were determined by the two independent samples *t*-test. A $p < 0.05$ was considered statistically significant.

RESULTS

Clinical and biological characteristics

Firstly, we note that there were no significant differences in the distributions of age in diabetics with or without CVD (respectively, 59.60 ± 6.67 vs. 58.60 ± 4.32 years). BMI was not significantly different between groups (34.4 ± 5.8 vs. 32.3 ± 5.2 kg/m^2, respectively) and like fasting blood sugar levels, HbA1c showed no significant differences in both patient groups (Table 2). Triglyceride levels were increased significantly in the D.CVD compared to the D group (2.46 ± 0.22 vs. 2.05 ± 0.1 g/L respectively; p = 0.0003).

Expression of PPARγ and CD36 in PBMC

To evaluate the potential of PPARγ to regulate CD36, relative quantitative RT-PCR was used. As shown in Figure 1, relative PPARγ expression presented a significantly lower constitutive expression in the D.CVD with regard to the D group (respectively, 0.76 ± 1.62 vs. 2.07 ± 0.8; p = 0. 003). Furthermore, relative CD36 expression in D.CVD reported a significant decrease (respectively, 0.83 ± 0.29 vs. 1.06 ± 0.4; p = 0.04) compared to D patients. No statistically significant differences were found between relative PPARγ and CD36 expressions in D.CVD (respectively, 0.83 ± 0.29 vs. 0.76 ± 1.62; p = 0.74). In contrast, in D patients there was a higher significant difference between relative PPARγ and CD36 expressions (2.07 ± 0.8 vs. 1.06 ± 0.4, respectively; p = 0.01).

The decrease of CD36 expression upregulated TG levels in PBMCs from D.CVD

The relative decrease of CD36 expression in D.CVD was followed with increased plasma triglyceride concentrations (respectively, 0.83 ± 0.29 vs. 2.46 ± 0.22; p < 0.0001) (Figure 2). However, the relative increased expression of CD36 in D patients was accompanied by lower plasma triglyceride concentrations (1.06 ± 0.4 vs. 2.05 ± 0.1). Hence, the increase of TG was more noticeable in D.CVD compared to D patients (2.46 ± 0.22 vs. 2.05 ± 0.1 g/L, respectively; p = 0.0003).

Elevated STAT1 expression is associated with reduced PPARγ levels in D.CVD patients

To determine whether there was a possible regulation of PPARγ by STAT1 in PBMCs from D.CVD patients, we measured the level of STAT1 expression in both PBMC cases of diabetic patients. As shown in Figure 3A, STAT1 increased significantly in D.CVD with regard to the D group (respectively, 0.12 ± 0.4 vs. 0.81 ± 0.08; p = 0.01). The relatively higher level was accompanied by a significant decrease of PPARγ in the D.CVD compared to D group (respectively 0.81 ± 0.08 vs. 0.76 ± 1.62).

Increased STAT6 leads to enhanced expression and activation of PPARγ in the D group

In the same context, to investigate the influence of STAT6 on PPARγ dynamics, we studied its expression in PBMCs of both groups of diabetic patients (Figure 3B). Our results demonstrated a significant decrease of PPARγ expression in the group characterised by a down level of STAT6 (D.CVD vs. D respectively, 0.76 ± 1.62 vs. 0.35 ± 0.24; p = 0.01). The elevated STAT6 level was associated with a higher PPARγ expression in D patients (respectively, 1.04 ± 0.32 vs. 2.07 ± 0.8).

PPARγ and TG levels with respect of STAT1 and STAT6 expressions

Given that STAT1 level was increased in D.CVD compared to D group, we also demonstrated that this elevation was accompanied with reduced PPARγ expression.

Table 1. Sequences of primers used for real-time qPCR.

Gene	Base sequences
PPARγ	(f) 5'-ATAAAGTCCTTCCCGCTGACCAAAGC-3' (r) 5'-CGGTCTCCACTGAGAATAATGACAGC-3'
CD36	(f) 5'-AAGTCACTGCGACATGATTAATGG-3' (r) 5'-GAACTGCAATACCTGGCTTTTCTC-3'
STAT6	(f) 5'-CCTCGTCACCAGTTGCTT-3' (r) 5'-TCCAGTGCTTTCTGCTCC-3'
STAT1	(f) 5'-CCGTTTTCATGACCTCCTGT-3 (r) 5'-TGAATATTCCCCGACTGAGC-3'
β ACTIN	(f) 5'-CCTGGCACCCAGCACAAT-3' (r) 5'-GCCGATCCACACGGAGTACTT-3'

Table 2. Clinical characteristics of diabetic patients with and without cardiovascular diseases.

Variable	Diabetics with CVD	Diabetics without CVD	p-value
Number of subjects	20	20	
Age (years)	59.60 ± 6.67	58.60 ± 4.32	NS
Gender (F/M)	10/10	10/10	NS
BMI (kg/m^2)	34.4 ± 5.8	32.3 ± 5.2	NS
HbA1c (%)	10.01 ± 0.4	10 ± 0.1	NS
Fasting blood sugar (g/L)	2.1 ± 5.56	1.98 ± 0.8	NS
Total TG (g/L)	2.46 ± 0.22	2.05 ± 0.1	0.0003

P < 0.05 was considered statistically significant. Data are expressed as mean standard deviation (S.D.) or numbers. P-values were obtained using Student's *t*-test.

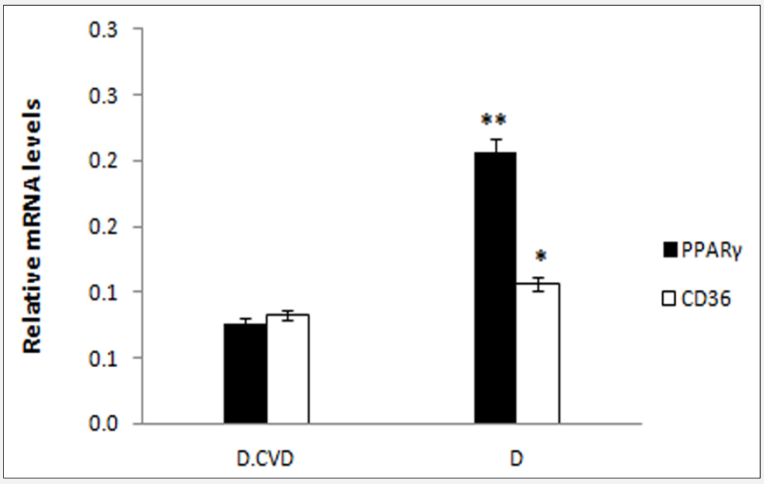

Figure 1. Expression of PPARγ and CD36 in PBMCs of diabetic patients with and without CVD.

PBMCs were isolated from D.CVD and D patients. mRNA levels of PPARγ and CD36 were measured by Q-PCR. The relative expression of each gene was calculated as described above, normalised to β-actin mRNA, and expressed as means ± SD. * - p < 0.05 versus D.CVD patients, ** - p < 0.01 versus D.CVD patients.

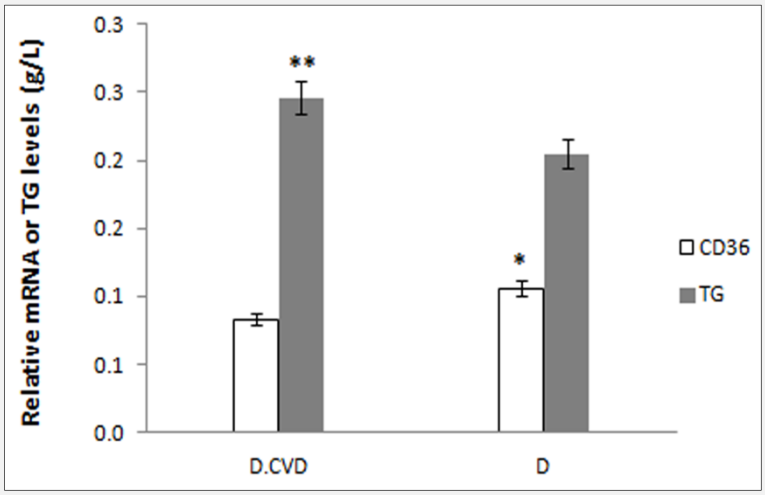

Figure 2. Expression of CD36 and TG levels in PBMCs of D.CVD and D patients.

PBMCs were isolated from D.CVD and D patients. mRNA levels of CD36 were measured by Q-PCR, TG levels were measured by using reference enzymatic techniques on a COBAS 6000 analyzer. The relative expression of each gene was calculated as described above, normalised to β-actin mRNA, and expressed as means ± SD. * $p < 0.05$ versus D.CVD group; ** $p < 0.001$ versus D group.

Otherwise, STAT6 level was increased in the group of patients who did not have cardiovascular disease compared to D.CVD (respectively, 1.04 ± 0.32 vs. 0.35 ± 0.24; $p = 0.002$), and this increase was followed by an elevation of PPARγ expression. In this sense, we showed that elevated STAT1 in the D.CVD group was linked with hyperglyceridemia. In fact, the TG level was significantly increased in D.CVD compared to D patients (respectively, 2.46 ± 0.22 vs. 2.05 ± 0.1 g/L; $p = 0.0003$) (Figure 4).

DISCUSSION

Our study demonstrates that PPARγ induced TG deposition by down-regulating CD36 expression in diabetic patients with CVD, suggesting a probable act mediated by STAT1 and STAT6. First, we have demonstrated that PPARγ expression was significantly lower in D.CVD patients as compared with D patients, and a similar trend was observed for CD36 expression. In fact, the mechanism by which oxidized LDL or linoleic acid metabolites up-regulate CD36 requires activation of the transcription factor PPARγ [10]. Previous studies indicate that both natural and synthetic PPARγ ligands, like prostaglandin J2 (PGJ2) and thiazolidinediones can increase CD36 expression. Regulation of CD36 expression by few cytokines is also related to PPARγ activity [28]. Second, we have demonstrated that TG levels were significantly higher in the lower CD36 expression group related to the D group. Our result is consistent with previous studies which have reported that the increase of TG may be generated in the diabetic lower CD36-deficient group [29]. Other work reported that inflammation increases, pro-inflammatory cytokines, stimulate the expression of LOX-1 and decrease CD36: it then ensures up to 40% of the internalization of ox-LDL. It therefore takes an increasingly important part in the formation of foam cells as the disease progresses [30].

PPARγ is a lipid-activated transcription factor regulating lipid metabolism and inflammatory response [31]. In the present study, we report a mechanism by which inflammatory molecules like STAT1 and STAT6 modulate PPARγ signaling in PBMCs from diabetic patients with and without CVD, and it would be reasonable to hypothesize that the higher expression of triglyceride levels in the D.CVD patients was associated with a lower PPARγ expression.

Indeed, our results show that in D.CVD patients, level of STAT1 was significantly higher than in the D group. Indeed, IFNγ can activate signal STAT1 factor and increases the production of pro-inflammatory cytokines, like tumor necrosis factor α (TNFα), IL-1β, chemotactic factors, reactive oxygen species, and nitrogen monoxide (NO) [32]. Moreover, recent studies demonstrate that an interaction between STAT1, p300, and PPARγ is appropriate for 15(*S*)-HETE-induced CD36 expression, oxidized low density lipoprotein uptake, and foam cell formation, main events in atherosclerosis. In fact, PPARγ

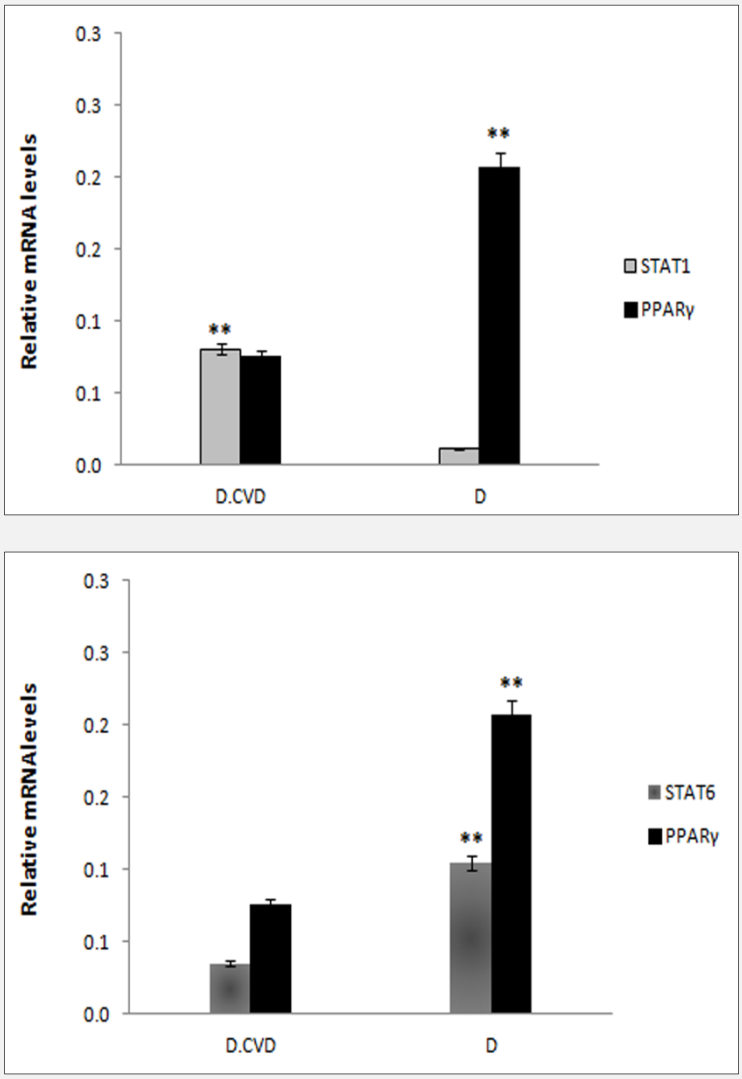

Figure 3. Quantification of PPARγ, STAT1, and STAT6 in PBMCs from D.CVD and D patients.

PBMCs were isolated from D.CVD and D patients. mRNA levels of STAT1, PPARγ, and STAT6 were measured by Q-PCR. The relative expression of each gene was calculated as described above, normalised to β-actin mRNA and expressed as means ± SD. (A) STAT1 and PPARγ levels in both diabetic patients with and without CVD; ** - p = 0.01 STAT1 versus D patients, p < 0.01 PPARγ vs. D.CVD patients. (B) STAT6 and PPARγ levels in both diabetic patients, ** - p < 0.01 versus D.CVD patients.

advances uptake of oxLDL by transcriptional induction of the scavenger receptor CD36 [33]. These findings suggest a potentially important role for STAT1 in atherogenesis [34], and it is phenotypically demonstrated in our study by an elevation of triglycerides in the D.CVD group.

In contrast, STAT6 level appears elevated in D patients compared to D.CVD. It is interesting to note that the anti-inflammatory cytokine IL-4 is identified as an important negative regulator of proinflammatory gene expression in mononuclear cells [35]. In addition, STAT6 is required for mediating responses to IL-4 to activate peroxisome-proliferator-activated receptor γ (PPARγ) [36]. The circulating monocytes penetrate and reside in the atherosclerotic plaques and transform to macrophages. Recognized changes in the microenvironment lead to macrophages switching between pro-inflammatory (M1) and anti-inflammatory (M2) functional phenotypes [37]. Other authors have recently reported that M2 phenotype macrophages induced by STAT6 advance

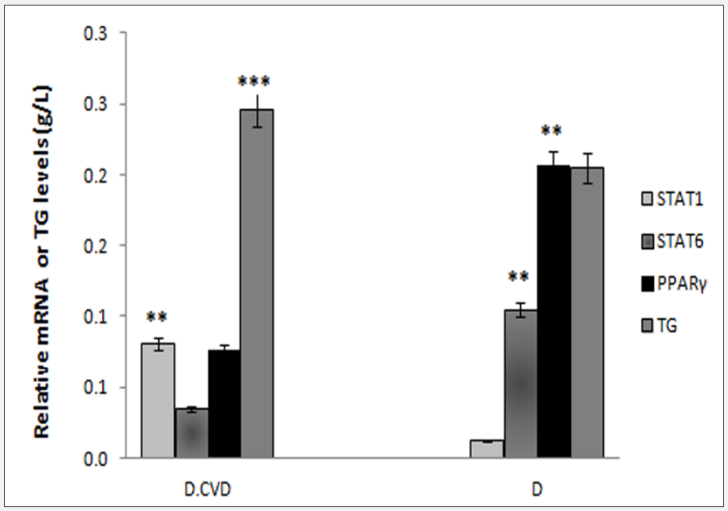

Figure 4. Quantification of STAT1, STAT6, PPARγ, and TG in PBMCs from D.CVD and D patients.

The relative expression of each gene was calculated as described above, normalised to β-actin mRNA, and expressed as means ± SD. ** - p < 0.01 STAT1 versus D, STAT6 versus D.CVD, and PPARγ patients versus D.CVD patients, * - p < 0.001 TG versus D patients.**

tissue repair and inhibit the progression of atherosclerosis [38]. It is possible that the origin of ROS followed by PPARγ ligand released during this type of inflammation positively influences the dynamic of CD36 expression and reflects negatively on the triglyceride in the serum as shown in our work.

Other work is necessary to better clarify this dynamic metabolic regulation between STAT1and STAT6 signaling phenotype and PPARγ.

Acknowledgement:

The present study was funded by the IMEC unit, Faculty of Sciences of Bizerte, University of Carthage and Tunisian Ministry of Higher Education.

Author's Contributions:

Imen Bendaya performed sampling, analysis, interpretations, data handling and writing of manuscript. Aouatef Riahi and Maher Kharat contributed in the development of the practical part (qRT-PCR). Saloua Kahla contributed to manuscript writing. Wissem Sdiri, the chief, Division of Cardiology directed the sampling and monitoring of clinical data. Professor Ridha Oueslati is the director and supervisor of the work. All authors read and approved the final manuscript.

Declaration of Interest:

We have no conflict of interest to declare.

References:

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 2011;94:311-21 (PMID: 22079683).

2. Armstrong EJ, Waltenberger J, Rogers JH. Percutaneous coronary intervention in patients with diabetes: current concepts and future directions. J Diabetes Sci Technol 2014;8:581-9 (PMID: 24876623).

3. Zellweger MJ, Maraun M, Osterhues HH, et al. Progression to overt or silent CAD in asymptomatic patients with diabetes mellitus at high coronary risk: main findings of the prospective multicenter BARDOT trial with a pilot randomized treatment substudy. JACC Cardiovasc Imaging 2014;7:1001-10 (PMID: 25240 454).

4. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2014;37 (Supplement 1):S81-90 (PMID: 24357215).

5. Martín-Timón I, Sevillano-Collantes C, Segura-Galindo A, del Cañizo-Gómez FJ. Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? World J Diabetes 2014; 15:444-70 (PMID: 25126392).

6. Bouwens M, Afman LA, Müller M. Fasting induces changes in peripheral blood mononuclear cell gene expression profiles related to increases in fatty acid beta-oxidation: functional role of peroxisome proliferator activated receptor alpha in human peripheral blood mononuclear cells. Am J Clin Nutr 2007;86:1515-23 (PMID: 17991667).

7. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999;340:115-26 (PMID: 9887164).

8. Klotz L, Schmidt M, Giese T, et al. Proinflammatory stimulation and pioglitazone treatment regulate peroxisome proliferator-activated receptor gamma levels in peripheral blood mononuclear cells from healthy controls and multiple sclerosis patients. J Immunol 2005;175:4948-55 (PMID: 16210596).

9. Janani C, Ranjitha Kumari BD. PPAR gamma gene--a review. Diabetes Metab Syndr 2015;9:46–50 (PMID: 25450819).

10. Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. Cell 1998;93:229–40 (PMID: 9568715).

11. Lehrke M, Lazar MA. The many faces of PPARgamma. Cell 2005;123:993-9 (PMID: 16360030).

12. Azhar S. Peroxisome proliferator-activated receptors, metabolic syndrome and cardiovascular disease. Future Cardiol 2010;6:657-91 (PMID: 20932114).

13. Ivanova EA, Parolari A, Myasoedova V, Melnichenko AA, Bobryshev YV, Orekhov AN. Peroxisome proliferator-activated receptor (PPAR) gamma in cardiovascular disorders and cardiovascular surgery. J Cardiol 2015;66:271-8 (PMID: 26072262).

14. Yu M, Jiang M, Chen Y, Duan Y, Han J. Inhibition of Macrophage CD36 Expression by Tamoxifen-A PPARgamma Dependent Mechanism. FASEB J 2016;30 S1:1134.2-1134.2. http://www.fasebj.org/content/30/1_Supplement/1134.2.

15. Hsueh WA, Law RE. PPARgamma and atherosclerosis: effects on cell growth and movement. Arterioscler Thromb Vasc Biol 2001;21:1891-5 (PMID: 11742860).

16. Pepino MY, Kuda O, Samovski D, Abumrad NA. Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism. Annu Rev Nutr 2014;34:281-303 (PMID: 24850 384).

17. Nicholson AC, Frieda S, Pearce A, Silverstein RL. Oxidized LDL binds to CD36 on human monocyte-derived macrophages and transfected cell lines. Evidence implicating the lipid moiety of the lipoprotein as the binding site. Arterioscler Thromb Vasc Biol 1995;15:269-75 (PMID: 7538425).

18. Cho S. CD36 as a therapeutic target for endothelial dysfunction in stroke. Curr Pharm Des 2012;18:3721-30 (PMID: 22574985).

19. Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. CD36 is a receptor for oxidized low density lipoprotein. J Biol Chem 1993;268:11811-6 (PMID: 7685021).

20. Berry A, Balard P, Coste A, et al. IL-13 induces expression of CD36 in human monocytes through PPARgamma activation. Eur J Immunol 2007;37:1642-52 (PMID: 17458857).

21. Coburn CT, Knapp FF, Febbraio M, Beets AL, Silverstein RL, Abumrad NA. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. J Biol Chem 2000;275:32523-9 (PMID: 10913136).

22. Hajri T, Han XX, Bonen A, Abumrad NA. Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. J Clin Invest 2002;109:1381-9 (PMID: 12021254).

23. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-421 (PMID: 12485966).

24. Szanto A, Balint BL, Nagy ZS, et al. STAT6 transcription factor is a facilitator of the nuclear receptor PPARγ-regulated gene expression in macrophages and dendritic cells. Immunity 2010;33: 699-712 (PMID: 21093321).

25. Kotla S, Rao GN. Reactive Oxygen Species (ROS) Mediate p300-dependent STAT1 Protein Interaction with Peroxisome Proliferator-activated Receptor (PPAR)-γ in CD36 Protein Expression and Foam Cell Formation. J Biol Chem 2015;290:30306-20 (PMID: 26504087).

26. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97 (PMID: 9203460).

27. Bennett S, Breit SN. Variables in the isolation and culture of human monocytes that are of particular relevance to studies of HIV. J Leukoc Biol 1994;56:236-40 (PMID: 8083595).

28. Huang JT, Welch JS, Ricote M, et al. Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. Nature 1999;400:378-82 (PMID: 10432118).

29. Furuhashi M, Ura N, Nakata T, Shimamoto K. Insulin sensitivity and lipid metabolism in human CD36 deficiency. Diabetes Care 2003;26:471–4 (PMID: 12547883).

30. Schaeffer DF, Riazy M, Parhar KS, et al. LOX-1 augments oxLDL uptake by lysoPC-stimulated murine macrophages but is not required for oxLDL clearance from plasma. J Lipid Res 2009;50: 1676-84 (PMID: 19359704).

31. Ohshima K, Mogi M, Horiuchi M. Role of Peroxisome Proliferator-Activated Receptor-γ in Vascular Inflammation. Int J Vasc Med 2012;2012:508416 (PMID: 22888436).

32. Ivashkiv LB, Hu X. Signaling by STATs. Arthritis Res Ther 2004;6:159-68 (PMID: 15225360).

33. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 1998;93:241-52 (PMID: 9568716).

34. Leopold JA, Loscalzo J. Oxidative risk for atherothrombotic cardiovascular disease. Free Radic Biol Med 2009;47:1673-706 (PMID: 19751821).

35. Donnelly RP, Fenton MJ, Kaufman JD, Gerrard TL. IL-1 expression in human monocytes is transcriptionally and posttranscriptionally regulated by IL-4. J Immunol 1991;146:3431-6 (PMID: 2026872).

36. Wang N, Liang H, Zen K. Molecular mechanisms that influence the macrophage m1-m2 polarization balance. Front Immunol 2014;5:614 (PMID: 25506346).

37. Liberale L, Dallegri F, Montecucco F, Carbone F. Pathophysiological relevance of macrophage subsets in atherogenesis. Thromb Haemost 2017;117:7-18 (PMID: 27683760).

38. Zhao XN, Li YN, Wang YT. Interleukin-4 regulates macrophage polarization via the MAPK signaling pathway to protect against atherosclerosis. Genet Mol Res 2016 Feb 22;15(1) (PMID: 26910 000).