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Research Article

Thrombospondin-1 Levels in ST-Elevation Myocardial Infarction Prior to Percutaneous Coronary Intervention: An Exploratory Study

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Abstract...

Thrombospondin-1 (TSP-1) is a highly expressed platelet-stored protein along the edge of the infarcted area, which limits tissue damage and the expansion of myocardial necrosis. In this study, we compared plasma levels of TSP-1 in patients with ST-segment elevation myocardial infarction (STEMI). A total of 30 STEMI patients were included. The determination of TSP-1 was performed together with Troponin I, N-terminal prohormone brain natriuretic peptide (NTproBNP), C-reactive protein, among others. An echocardiogram was performed and LVEF was calculated. STEMI patients were stratified according to GRACE. A positive correlation was found between TSP-1 levels and LVEF (R = 0.688, p = 0.020), as well as HBA1 (R = 0.496, p = 0.010). however, in GRACE score >126, significant differences were found in LVEF (R= -0.975, p=0.025, HBA1c (R=0.881, p= 0.002) and wall motion score index (R=-0.999, p=0.026). In addition, TSP-1 levels were significantly lower in our group of patients vs. controls (132 ± 19 vs. 253 ± 22, p<0.005). In addition, THBS1, THBS2, and THBS4 genotyping was performed, however, no differences were found. In conclusions, in STEMI patients, we found lower levels of TSP-1, associated with HBA1 and LVEF, which carry a higher risk of loss of function. However, multicenter studies are needed to confirm the importance of this biomarker.

Keywords: Thrombospondin-1; ST-elevation myocardial infarction; Coronary artery disease.

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Introduction

Thrombospondin-1 (TSP-1) is a 420-450 kDa platelet-stored protein that belongs to a family of multifunctional matricellular molecules [1]. TSP-1 is capable of binding over a wide range of ligands including integrin and non-integrin cellular domains, extracellular matrix components, and growth factors [2]. Moreover, this large homotrimeric protein has heparin and procollagen domains [3].

Evidence suggests that TSP-1 (and TSP family, in general) has a so-called "regulatory" role in inflammation [4.5]. In murine models, TSP-1 inhibits angiogenesis and tumor growth and promotes vascular smooth muscle cell migration [6]. Experimental ischemic injury models show that TSP-1 or CD47 (a TSP-1 receptor) blockage increases ischemic soft tissue survival [7]. Specifically, in myocardial infarction experimental models, TSP-1 is highly expressed along the border of the infarcted area, confining tissue damage and myocardial necrosis expansion [8]. In Apo $E^{-/-}$mice, TSP1 deficiency leads to high macrophage concentrations in the atheromatous plaque and increased intraplaque collagen composition [6]. All these findings seem to imply that, at least in murine models, TSP-1 inhibition may promote a greater infarct extension [4,9].

Despite all this extensive biological activity and pleiotropic effects in several molecular networks making TSP-1 a growing research target, the role of this molecule in the development of human Coronary Artery Disease (CAD) is scarcely described.

This study aimed to correlate TSP-1 plasma levels with ST-Elevation Myocardial Infarction (STEMI) patients. Plasma levels of TSP-1 were also assessed in different GRACE-based scoring groups. Secondarily, correlations between different risk markers were evaluated. In addition, we studied 3 genetic polymorphisms of thrombospondin, THBS1 (rs 2228262), THBS2 (rs 8089), and THBS4 (rs 1866389), with the purpose of knowing if any of these polymorphisms could be related to the disease.

Materials and methods

This was a prospective study carried out in the Coronary Care Unit and in the Department of Immunology both at the National Institute of Cardiology in Mexico City, during the period from January to November 2019.

Participants

A total of thirty adult STEMI patients were included. Exclusion criteria were prior infarction, pregnancy, autoimmune disease, cancer, hepatic failure, and sepsis. Patients with mechanical and/or electrical complications or with unsuccessful percutaneous coronary intervention (PCI) were also excluded. Healthy volunteers (blood bank donors), were matched by age and sex with STEMI patients and used as controls to compare TSP-1 levels only, the rest of the comparisons were made between TSP-1 with different variables and with two STEMI risk groups stratified by GRACE score (see below).

TSP-1 analysis

Blood was drawn at room temperature into appropriate tubes. Samples were obtained prior to PCI. The tubes were centrifuged at 10,000G/4°C during 10 min to remove cellular components. The supernatant was transferred into 5 ml Eppendorf Tubes and stored in aliquots at -80°C. Subsequent TSP-1 analysis was made using a TSP-1 determination kit: Human Quantikine TSP-1, R&D Systems Minneapolis, MN, USA, an ELISA-bassed assay [10].

Clinical markers

Troponin I (TnI), N-terminal of the prohormone brain natriuretic peptide (NTproBNP), C-reactive protein (CRP), glucose, glycated hemoglobin (HbA1c) and a lipid panel were obtained. In addition, a point-of-care echocardiogram was performed to exclude mechanical complications, assess ventricular motion, and calculate Left Ventricular Ejection Fraction (LVEF).

GRACE score

In all STEMI patients, GRACE score was calculated using the online MD calc© calculator (available at http://www.mdcalc.com/grace-acs-risk-and-mortality-calculator). According to previous studies (11,12) patients were stratified into low-risk (≤125 points) and high-risk (>126 points) groups.

DNA Preparation

Genomic DNA was extracted from whole blood containing EDTA by standard techniques. The THBS1 (rs 2228262), THBS2 (rs 8089), and THBS4 (rs 1866389) polymorphisms were genotyped using 5' exonuclease TaqMan genotyping assays on an ABI Prism 7900HT Fast Real-Time PCR system, according to manufacturer's instructions (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Continuous variables were expressed as mean or medians depending on their distribution. Normality of the variables was assessed using the Shapiro-Wilk test. Those variables with normal distribution were analyzed with the Student t-test. Non-parametric tests (Mann–Whitney U test *and* Kruskal–Wallis test*)* were used in those variables without normal distribution. Categorical variables were expressed as frequencies and percentages.

One-tailed statistical analysis was made to maximize the chances of finding an effect in a single direction while optimizing the sample size. This decision was made prior to sample collection.

A $p<0.05$ was considered as statistically significant. Statistical analysis was performed with SPSS software (V20.0, SPSS inc., Chicago IL, USA) and reviewed by two independent analysts.

Ethical standards

Institutional Ethics Committee approved the study protocol. Informed consent was obtained from all participants. This study was carried out according to the Declaration of Helsinki. (PT15-003).

Results

Table 1 shows the baseline population data in the total population (n=30) and divided by GRACE score into low (60%) and high risk (40%). We analyzed different parameters between

both GRACE score risks, we found significant differences in diabetes (p=0.040), left ventricular ejection fraction (LVEF) (0.014) and troponin I (p=0.025).

In order to know if the levels of thrombospondin had any influence on the parameters analyzed, a correlation analysis was carried out (Table 2). We found significant differences in HBA1c, with a positive correlation (R=0.496, p= 0.010). When we analyzed patients with HBA1c less than 5.7 and greater than 5.7, the patients with high values maintained this significance (R = 0.554, p = 0.011) (data not shown). Furthermore, a significant difference was found in LVEF and thrombospondin, but with a negative correlation (R= -0.688, p= 0.041). The same analysis was made, but with dyslipidemia parameters, however, did not find any significant correlation with TSP-1.

When we did the correlation analysis, according to our two groups, we found in the low-risk group, TSP-1 showed a significant negative correlation with LVEF (R -0.57). Moreover, patients in the high-risk category showed a significant negative correlation with NTproBNP (R -0.73), indicating that an increase of NTproBNP carries the opposite effect on TSP-1 (Table 3). TSP-1 showed no correlation with any other clinical markers.

Complementary to this, we studied TSP-1 plasma levels in our patients compared versus healthy volunteer's subjects (n=60) TSP-1 plasma levels were lower in STEMI patients than that in healthy subjects (132 ± 19 vs. 253 ± 22, p<0.005) (Data not shown).

A logistic regression model was made to predict the probability that a given patient could enter the high-risk GRACE category. For this analysis, we choose variables of known prognostic importance: LVEF, CRP, peak TnI, NTproBNP, and glucose at admission. We included TSP-1 as well. After the stepwise method, the only variable that remained significant in the equation was LVEF. The model indicates that for every decrease of a percentage point there is an 8.9% increase in the risk to fall into the high-risk category. TSP-1 did not show any predictive value in this study.

Finally, to know if the main polymorphisms reported for thrombospondin are associated with the disease, the analysis of 3 genetic polymorphisms of thrombospondin, THBS1, THBS2 and THBS3 was carried out (Table 4). However, in a model, both codominant, dominant and recessive, we did not find statistically significant differences in any model.

Table 1: Baseline characteristics of patients.

Total patients (n=30)		Low risk (n=18)	High risk (n=12)	p
Variable				
Men n (%)	27 (90)	16 (88.8)	11 (91.6)	0.812
Women n (%)	3 (10)	2 (11.1)	1 (8.3)	
Age (years)	59.3 ± 14.5	56.6 (±14.8)	71 (±17.4)	0.600
BMI (kg/m2)	28.67 ± 4.8	30 (±7.2)	28.9 (±4.6)	0.700
Glucose at admission (mg/dl)	133.1 (88.4-417.8)	122.2 (88.4-365.7)	159 (106.8-417.9)	0.130
HBA1c (%)	6.57 (5.3-11.9)	6 (5.39-9.9)	6.2 (5.44-11.9)	0.659
LDL-C (mg/dl)	98.5 (26.7-213.0)	115.8 (43.4-166.2)	58.2 (26.7-167.5)	0.567
HDL-C /mg/dl)	38.85 (5.1-68.3)	38.1 (21.3-50.7)	44.6 (5.2-49.4)	0.398
TOTAL Cholesterol (mg/dl	151.8 (74.6-266.9)	168.9 (96.4-216.5)	120 (74.7-209)	0.470
Diabetes n (%)	11 (37)	3 (2.7)	8 (66.7)	**0.040**
LVEF Eco (%)	49.5 ± 13.5	56.7 (±10.9)	43.4 (±13.1)	**0.014**
M-To-R Time (min)	263.1 (55-700)	244.5 (55-492)	275 (60-700)	0.653
D-To R- time (min)	85.2 (20-247)	86.5 (60-247)	61.5 (20-131)	0.754
Total-isch_time (min)	342.7 (85-770)	332.5 (85-674)	348 (100-770)	0.544
Trop-I	91.7 (11.10-150)	92.5 (15.7-150)	150	**0.025**
CRP (mg/dl)	38.76 (0.42-300)	4.5 (0.87-17.7)	7.5 (3.7-20)	0.066
NTproBNP (pg/ml)	1985.88 (10.52-18144)	773.2 (10.5-3127)	850.6 (518-1680)	0.178
Lactate	2.519 (1.00-5.70)	2.2 (1-5.7)	3.4 (1.4-4)	0.605
TSP-1	104.81 (15-525)	125.9 (28-228)	150.5 (15-525)	0.653

Hba1: Glycosylated Hemoglobin 1; Ldl-C: Low Density Lipoprotein Cholesterol, Hdl-C: High Density Lipoprotein Cholesterol, Lvef: Left Ventricular Ejection Fraction; M-To-R Time: Medical Contact–To-Reperfusion Time; D-To-R_time: Hospital Door-To-Reperfusion Time; Total-Isch_time: Total Ischemic Time; Trop_i: Troponin I; Crp: C-Reactive Protein (High Sensibility); Ntprobnp: N-Terminal Pro-Brain Natriuretic Peptide; Tsp-1: Thrombospondin 1. Grace: *Global Registry Of Acute Coronary Events.*

Table 2: Correlation between different clinical variables and TSP-1.

VARIABLE	R	P value
BMI (kg/m2)	-0.044	0.818
Glucose (mg/dL)	0.256	0.172
HBA1 (mg/dL)	0.496	**0.010**
LDL-C (mg/dL)	-0.158	0.412
HDL-C (mg/dL)	0.212	0.270
Total Cholesterol (mg/dL)	-0.121	0.532
LVEF (%)	-0.688	**0.041**
TnI	0.038	0.843
CRP	-0.182	0.335
NTproBNP (pg/ml)	-0.201	0.335
Lactate	0.235	0.364
BMI ≥25 kg/m2	-0.013	0.477
LDL-C ≥130 mg/dL	0.059	0.455
HDL-C< 40 mg/Dl	0.006	0.492
Total Cholesterol ≥150 mg/dL	-0.138	0.335

HBA1c: Glycated Hemoglobin; HDL: High-Density Lipoprotein Cholesterol; LDL: Low-Density Lipoprotein Cholesterol; LVEF: Left Ventricular Ejection Fraction; Tni: Troponin I; CRP: C-Reactive Protein; Ntprobnp: N-Terminal Pro-Brain Natriuretic Peptide; TSP-1: Thrombospondin 1; BMI: Body Mass Index.

Table 3: Correlation between different clinical variables and thrombospondin 1according to risk categories.

	Low risk (n = 17)		High risk (n = 11)	
	r	p	r	P
Total cholesterol (mg/dL)	0.10	NS	-0.28	NS
HDL (mg/dL)	0.35	NS	0.22	NS
LDL (mg/dL)	0.08	NS	-0.34	NS
Glucose at admission (mg/dL)	0.07	NS	0.10	NS
HbA1c (%)	-0.32	NS	0.20	NS
NTproBNP (pg/mL)	0.14	NS	-0.73	0.01
TnI (ng/mL)	0.43	NS	-0.43	NS
CRP (mg/L)	0.08	NS	-0.34	NS
LVEF (%)	-0.57	0.03	-0.33	NS

GRACE: Global Registry Of Acute Coronary Events; HDL: High-Density Lipoprotein Cholesterol; LDL: Low-Density Lipoprotein Cholesterol; HBA1c: Glycated Hemoglobin; Ntprobnp: N-Terminal Pro-Brain Natriuretic Peptide; Tni: Troponin I; CRP: C-Reactive Protein; LVEF: Left Ventricular Ejection Fraction; NS: Non-Statistically Significant.

Table 4: Allelic frequencies of thrombospondin-1.

THBSSA	controls n (%)	patients n (%)
Allele		
A	54 (90.0)	55 (91.6)
G	6 (10.0)	**5 (8.3)**
THBSCC		
Allele		
A	52 (86.6)	53 (88.3)
C	8 (13.3)	**7 (11.6)**
THBS4		
Allele		
C	3 (5.0)	2 (3.3)
G	57 (95.0)	58 (96.6)

Discussion

This study evaluated the role of thrombospondin-1 levels in ST-elevation myocardial infarction across different GRACE-based score groups and their correlations between different risk markers. Here, we found a positive correlation between TSP-1 with LVEF levels and HBA1 and significant differences in FEVI for GRACE score. Additionally, thrombospondin levels were higher in patients with respect to the control group.

Choi et al. [13] found that TSP-1 plasma levels were higher in patients with DM and stable CAD compared with controls. Similar findings were reported by Huang and coworkers [14], where higher TSP-1 levels were independently associated with the presence of CAD in hemodialysis patients (OR of 1.38). Another study [15] in individuals undergoing elective angioplasty and stenting found that TSP-1 (and platelet factor 4), measured before PCI was associated with monocyte and platelet aggregate formation and with thrombin generation.

However, studies in the acute context are scarce. Befekadu and colleagues [16] reported that, in STEMI patients with totally occluded culprit arteries, TSP-1 plasma levels were significantly elevated before PCI, decreasing after the procedure and even reaching lower levels than controls three months after PCI. They concluded that TSP-1 may be useful as an early marker of uncontrolled platelet activation in the acute but not late STEMI phase and attributed the low TSP-1 levels to anti-ischemic medication.

Notably, the different studies on TSP-1 show conflicting results and a simple mechanistic explanation seems insufficient. In our study, TSP-1 was lower in STEMI patients compared to controls *prior* Percutaneous Coronary Intervention (PCI). Our hypothesis indicates that, during stable CAD, higher TSP-1 levels may play a beneficial role. As those as levels decrease, this putative protective effect can be eventually lost and may promote coronary occlusion, meaning that, in STEMI patients, we could only be available to detect a low TSP-1 concentration after plaque rupture and coronary occlusion. Oscillations in TSP1 plasma levels may occur depending on the moment at which the sample is taken, that is, it before or after PCI.

The 'loss of protection' hypothesis is largely based on basic research. Data from murine models show that TSP-1 is significantly expressed over the border of the infarcted area [8] and that, in Apo $E^{-/-}$ mice, TSP-1 deficiency accelerates atherosclerotic plaque maturation [6]. Moreover, a decreased expression

of TSP-1 could favor ventricular remodeling [17]. In the early phase of postinfarction remodeling, inflammatory cells produce metalloproteinases (MMPs), especially MMP-2 and MMP-9 [18,19], which are capable of degrading ECM, rapidly increasing collagen content in the infarcted area [8]. After experimental infarction and early myocyte damage, TSP-1 may favor the healing process *via* TGF-β, selectively inducing the expression of specific fibroblast phenotypes [20]. In summary, TSP-1 seems to protect ECM against adverse remodeling [5] and limit the infarct size [8]. Low TSP-1 plasma levels in STEMI patients could imply a loss of those salutary effects.

Not surprisingly, we found a significant but discrete correlation (r -0.688) of TSP-1 with LVEF. The relationship between TSP 1 and heart failure with reduced ejection fraction (HFrEF) has been previously observed (20, 22–24). Batlle et al. [17] analyzed cardiac biopsies from patients with advanced HFrEF, showing reduced TSP-1 mRNA expression in myocardial tissue. The presence of ventricular dilation observed may be related to the key role of TSP-1 in cardiac remodeling [8,20,25,26]. Hypoxia inhibits the production of endogenous angiogenesis inhibitors such as TSP-1. The expression of NT-proBNP in the ventricle is associated with hypertrophy and fibrosis of the heart, and its elevation in plasma is used as a prognostic biomarker of heart failure and risk cardiovascular in humans. The finding in this study of such a significant inverse correlation of these two parameters, leads to evaluating the role and significance of both within the mechanisms of damage and remodeling of the infarction or if they can be useful markers during the evaluation of acute infarction.

On the other hand, an association was found between the levels of TSP-1 and HBA-1. Of our patients, 37% had diabetes mellitus. Some works have reported a higher expression of TSP-1 in murine cell models under culture conditions with a high glucose content [27-29]. Activation of TSP-1 transcription is mediated by the hexosamine glucose catabolism pathway, resulting in modulation of nuclear protein activity through glycosylation [30]. On the other hand, it has also been reported that high concentrations of TSP-1 in human plasma in patients with DM (+) CAD (+) compared with DM (-) CAD (+) and DM (-) and CAD (-) patients. A multivariate regression analysis of DM patients showed that male sex, low HDL-C level, high HbA1c level, and high plasma TSP-1 levels were independently and positively associated with CAD [31].

Finally, with respect to polymorphism analysis, despite having analyzed a few subjects, these results agree with other studies where they conclude that the presence of variants of thrombospondin-1 (rs2228262) and thrombospondin-2 (rs8089) should not be considered a risk of coronary artery disease or myocardial infarction [32], the same can be seen in a meta-analysis [33], that included 6388 (TSP-1), 4930 (TSP-2), and 6978 (TSP-4) cases, none of the polymorphisms was found to be linked with the risk of cardiovascular disease.

Limitations

The limitations of the study are that only the Mexican population has been included, the sample size is small because it is an exploratory study, which does not allow us to reach a robust conclusion. The SYNTAX score (an angiographic classification tool to assess the complexity of CAD) could not be incorporated into the final analysis.

Conclusions

In STEMI patients, TSP-1 levels are significantly lower than in controls, implying the loss of a presumed protective effect. There is also a correlation between TSP-1 and HBA1 and a negative correlation with LVEF and NTproBNP in high-risk patients. However, there is no association of TSP-1 with other clinical markers. According to the findings, the use of TSP-1 as a possible marker of myocardial damage in patients with STEMI is attractive, so scrutiny through multicenter studies can be proposed to confirm the importance of this biomarker.

Declarations

Data availability: Data used to support the findings of this study are available from the corresponding author upon request

Conflict of interest: The authors declare that they have no conflicts of interest.

Author contributions: J.A.V.R., M.E.S, R.G. designed the Thrombospondin 1 study and wrote the manuscript, J.A.V.R. recruited patients, R.M., revised the manuscript and made the laboratory determination, A.M.M and R.C.S Collected control patients with supervision and samples, R.G. and M.E.S statistical analysis. C.H.G and I.P.T. made laboratory determination. All authors have read and agreed to the published version of the manuscript.

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