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ARE INSULIN RESISTANCE AND NON-ALCOHOLIC FATTY LIVER DISEASE ASSOCIATED WITH PEYRONIE'S DISEASE? A PILOT STUDY

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Risk factors for Peyronie's disease (PD) are serum lipid abnormalities, hypertension and type 2 *diabetes mellitus* (T2DM). Oxidative stress and inflammation are key-players in the pathogenesis of arterial diseases, leading to insulin resistance (IR), which is a major determinant of non-alcoholic fatty liver disease (NAFLD). We studied the potential relationship between PD, IR, and NAFLD. Forty-nine male patients were enrolled, fulfilling the well-accepted diagnostic criteria of stable PD. Fifty male individuals without PD, well-matched for age and BMI, were selected as the control group. Comorbidities (T2DM and hypertension), as well as the lipid profile and the glucometabolic asset, were evaluated. The triglycerides/HDL ratio (TG/HDL-C ratio) with a cut-off of ≥3 and the triglycerides-glucose index (TyG) with an optimal cut-point of 8.5 were used for diagnosis of IR and NAFLD, respectively. NAFLD diagnosis was confirmed by the presence of bright liver at ultrasonography. Hypertension was found more frequently in PD patients than in no-PD subjects (P=0.017), independently of age (P=0.99). Both IR and NAFLD were significantly associated with the presence of PD in our population of men (P=0.043 and 0.0001, respectively), no matter how old (P=0.11 and 0.74, respectively). At logistic regression, NAFLD was the only predictor of the PD presence (p=0.021). The AUROC of TyG to predict PD was 0.7437 (sensitivity 67.35% and specificity 80%) with a percentage of correctly classified patients of 73.74%. Oxidative stress markers were significantly associated with NAFLD. Testosterone level was significantly low in the subjects with NAFLD in cross-sectional analyses. Both factors, *i.e.*, oxidative stress and hypogonadism, are central to PD pathogenesis. In conclusion, NAFLD and IR are strongly associated with PD. The pathogenic link between these conditions and the underlying mechanisms are only hypothetical and thoroughly summarized in the discussion.

Key words: *Peyronie's disease, non-alcoholic fatty liver disease, insulin resistance, oxidative stress, erectile dysfunction, inflammation, hypogonadism*

INTRODUCTION

Peyronie's disease (PD) is a non-malignant disorder characterized by pain and penile curvature caused by the formation of abnormal sclerotic tissue (fibrous plaque) in the tunica albuginea of *corpora cavernosa* (1). Its diffusion is more widespread than previously thought, reaching a prevalence of 3.2% in the general population (2). Different risk factors, such as serum lipid abnormalities, hypertension, and type 2 *diabetes mellitus* (T2DM), seem to have a significant impact on the severity of symptoms and outcome of PD (3-5). Arterial flow along the cavernous arteries has been found to be, in some cases, altered in PD patients, while corporeal venous-occlusive dysfunction seems to be the main hemodynamic abnormality (6). Significant associations between PD, erectile dysfunction (ED), and ED duration were detected, confirming the high prevalence of PD among diabetic patients with ED (7-9).

Oxidative stress and inflammation are key players in the pathogenesis of arterial diseases, both of them ultimately leading to insulin resistance (IR) (10, 11). Interestingly, endothelium-dependent flow-mediated dilation was impaired in PD patients compared to controls (12). IR is frequently associated with endothelial dysfunction and has been proposed to play a major role in cardiovascular diseases (13). In a very recent study, PD has been shown to be associated to obesity among a population comprehending 656 individuals seeking urological care in nearly 27% of the cases (14).

Non-alcoholic fatty liver disease (NAFLD) is the predominant form of liver disease worldwide as well as the leading cause of liver cirrhosis, hepatocellular carcinoma (HCC), and organ transplant (15). NAFLD pathogenesis is multifactorial with endogenous (genetic background and epigenetic modifications, immunity activation status, gut microbiota) and exogenous elements (lifestyle, in terms of high-

fat diet and sedentary life) interacting and contributing to its onset and progression (16, 17). NAFLD is part of the metabolic syndrome (MS) and is associated to a number of non-metabolic extrahepatic manifestations, including also urological diseases such as hypogonadism, male sexual dysfunction, and urolithiasis (18). Hyperinsulinemia has a critical role also in the pathophysiology of hypertension with IR being the likely predominant mechanism and hypertension is among the risk factors for PD together with hypercholesterolemia and T2DN (19, 20). Whether IR and NAFLD are involved in the pathogenesis of PD is not clear. This cross-sectional, observational study was therefore designed to assess the potential role of IR and NAFLD in the pathogenesis of PD by determining the triglycerides/HDL cholesterol ratio (TG/HDL-C ratio) and the triglycerides-glucose index (TyG) in patients with PD. TG/HDL-C ratio and TyG have been shown to be accurate surrogate markers of IR and NAFLD, respectively (21-24).

MATERIAL AND METHODS

Patients

We carried out a cross-sectional, observational study where at a particular point of time we described some characteristics of PD patients, without follow-up, compared to controls in accordance with the guidelines for the care. This piece of research does not report on primary research and analysed data was gathered during the hospital stay. Our analysis looked at data of these cohorts, respecting complete anonymity, and was performed internally as part of an evaluation to improve our quality of care.

This study is in compliance with ethical guidelines of the Declaration of Helsinki (1975).

Inclusion and exclusion criteria

Written informed consent was obtained before proceeding to the study by all patients involved. Fifty male individuals without PD, well-matched for age and BMI, were selected as the control group. PD patients who were suffering from different grades of obesity were on a calorie-reduced, low-fat diet. In presence of co-morbidities, such as T2DM and hypertension, PD patients and controls were maintained on previously prescribed drugs obtaining a metabolic and hemodynamic control.

Patients were excluded if they had an increase of weight loss in the past months (*i.e.* ±10% initial body weight) or recent acute illness (infection) that might have affected gluco-lipid metabolism.

Furthermore, any viral, autoimmune, metabolic liver disease (Wilson disease, hemochromatosis, or antitrypsin deficiency) was ruled out by using appropriate testing, according to well-accepted diagnostic guidelines. Alcohol abuse was similarly excluded, following the DSM-IV diagnostic criteria, utilizing screening tests such as MAST (Michigan Alcohol Screening Test) and CAGE (Cut down, Annoyed, Guilty, and Eyeopener) as well as random tests for blood alcohol concentration and the use of a surrogate marker, *e.g.*, mean corpuscular volume.

To avoid misinterpretation of the liver enzyme elevation, celiac disease was excluded by evaluating IgA anti-tissue transglutaminase antibodies.

Patient evaluation

1. Diagnostic criteria for Peyronie's disease

First of all, medical history from patients with suspected PD (based on physical examination) was collected. Subsequently, patients underwent a dynamic penile color-Doppler ultrasound scan (US), in order to evaluate penile curvature and the ultrasonographic appearance of tunica albuginea. Finally, the Index of Erectile Function questionnaire was submitted to the patients.

2. Anthropometric evaluation

Normal weight was considered a body mass index (BMI) between 18.5 and 24.9, overweight a BMI between 25 and 29.9, while obesity was characterized by a BMI of 30 or more.

3. Metabolic profile

T2DM was diagnosed in presence of fasting plasma glucose concentrations ≥126 mg dL^{-1}, or on antidiabetic agents. Insulin resistance was studied by the TG/HDL-C using the cut-off ≥3. IR was defined by a TG/HDL-C ratio equal or higher than 3 (22). TG values of subjects who had fasted at least 12/14 h before the blood draw were evaluated averaging the results of at least two determinations, made on different days.

3. Non-alcoholic fatty liver disease

In our study, NAFLD was indirectly diagnosed by calculating the TyG, selecting as optimal cut-point 8.5 (AUROC = 0.78, sensitivity = 72.2% and specificity = 70.5%), and then confirmed by the presence of bright liver at ultrasonography (21). Other known causes of liver damage, including viral infection, alcohol or drug abuse, celiac disease, autoimmunity, liver or copper storage diseases were excluded.

4. Alcohol consumption

Enrolled individuals were categorised as non-drinkers or moderate drinkers if they have limited intake to 2 drinks or less in a day according to https://www.dietaryguidelines.gov /resources/2020-2025-dietary-guidelines-online-materials.

5. Exercise

Patients selected for the study were categorised as having a sedentary lifestyle or doing at least 150 minutes of moderate-intensity aerobic physical activity throughout the week or at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week or an equivalent combination of moderate- and vigorous-intensity activity, according to the WHO guidelines on physical activity and sedentary behaviour ISBN 978-92-4-001512-8 (electronic version) ISBN 978-92-4-001513-5 (print edition).

6. Laboratory assessment at entry

Fasting plasma glucose (FPG, n.v. 70–100 mg/dL), the lipid profile comprehending serum levels of TG (n.v.<150 mg/dL), total cholesterol (TC, n.v.<200 mg/dL), HDL (n.v.>40 mg/dL), low density lipoprotein (LDL, n.v.<100 mg/dL) and serum creatinine (n.v. 0.72–1.25 mg/dL) were measured according to in-house procedures.

7. Ultrasonography features

As previously mentioned, the diagnosis of PD was made by an experienced urologist *via* physical examination, performed examining the penis in order to identify a palpable penile plaque in the flaccid and stretched state. Successively, the US analysis was carried out utilizing an ultrasound scan equipped with a 7 – 12 MHz multi-frequency linear probe (BK Flex Focus 800, BK medical System Inc, United States), with the patient in the

supine position. B-mode US study was performed in transversal and longitudinal planes starting at the level of the glans and moving down to the base of the penis, with the penis placed toward the abdomen and transducer placed at the ventral surface of the penis. Ten micrograms of prostaglandin E1 (PGE1) were injected into the left *corpus cavernosum via* a 25-gauge insulin injector in order to perform the Doppler study and evaluate the presence of non-palpable plaques with erected penis. In particular, calcified penile plaques were detected as focal hyperechoic thickening of the tunica albuginea with attenuation of the acoustic beam while non-calcified plaques were isoechoic or slightly hyperechoic compared with the surrounding *tunica albuginea*.

Statistical analysis

Data, derived from a normally distributed population were given as mean plus SD. Variables not normally distributed or ordinals are expressed as median (25–75 IQR). The difference in medians was assessed by the Wilcoxon rank-sum test (Mann-Whitney test).

For assessing frequencies we applied a two-way table with measures of association, calculating the Fisher's exact test or G-test of independence as appropriate (25). The extended Mantel-Haenszel with ANOVA (https://www.statology.org/friedman-test-stata/) was used when frequencies were adjusted for age. Correlation between TyG and TG/HDL-C ratio was assessed by the determination of Spearman's coefficient of rank correlation (rho). Logistic regression was used to predict the presence/absence of a dependent variable and R-square statistics were used for assessing the predictive strength of the logistic regression model. To avoid concomitant imbalance between the two groups propensity score matching was used. The bootstrap method was used for estimating quantities about a population by averaging estimates from multiple small data samples. Collinearity was defined by a variance inflation factor (VIF) >2.5 and a value of tolerance <1.0. ROC analysis was used to correctly classify patients as having or not PD. The area under the receiver operating characteristic (AUROC/AUC) was performed to evaluate the most appropriate models (with the highest specificity and sensitivity), under the nonparametric assumption. The correct classification with related sensitivity and specificity was performed using the Probit model by the >estat class< command. The test equality of more ROC areas was performed to compare the performance of several variables. The concordance correlation coefficient (ρ_c) was adopted to evaluate the degree of observations in the US. Stata 17.0, Copyright 1985-2021, 4905 Lakeway Drive, College Station, Texas 7784, was used for statistics.

RESULTS

Forty-nine male patients were enrolled in this study fulfilling the diagnostic criteria of stable PD (*i.e*., palpable plaque, no pain and symptoms unchanged in the past three months) (26). The main characteristics of the whole population are presented in *Table 1*.

Prevalence

Age and BMI of the two groups, *i.e*., with and without PD, were similar, proving that all subjects under study were well-matched.

The presence of hypertension was significantly different between the two groups, with a clear tendency to an increase in the PD cohort compared to controls, (P=0.017) independently from the age of patients (P=0.99). Meanwhile, the T2DM presence resulted to be slightly increased in PD patients, but without reaching statistical significance.

Neither alcohol use nor physical activity was different between the groups (P=0.50 and 0.07, respectively).

The median values of the FPG and the total cholesterol, as well as of the triglycerides registered in the PD group, were significantly higher compared to the group without PD. The presence of hypercholesterolemia (>200 mg/dL) was statistically more relevant in the PD population than the no PD one (P=0.0001). The median of International Normalized Ratio (INR) for prothrombin time belonging to patients with PD was lower, compared to subjects without PD, but both values were within the range of normality. The PD cohort did not show a major prevalence of T2DM compared to controls. Interestingly, the prevalence of IR and mainly of NAFLD in the cohort of patients suffering from PD was significantly higher compared to that of the no PD cohort (P=0.043 and 0.0001, respectively). Interestingly, both the prevalences were independent of age (P=0.11 and 0.74, respectively). The median TG/HDL ratio was superior to 3, a value considered a reliable cut-off for diagnosing the IR presence, in the PD group, even though it was not significantly higher than that of the no PD group. The mean TyG of the PD group was significantly higher than that of no PD, with a value superior to that considered a valid cut-off for the NAFLD presence, *i.e*., 8.5. All these data are shown in *Table 1*.

Correlations and predictions

The correlation between TG/HDL-C ratio and TyG on 99 observations (both groups) gave a Spearman's rho of 0.53; Prob >|t|=0.0000, indicating that the two parameters were likely to measure the same glucose and lipid dysmetabolism.

Dealing with predictions, *Table 2* evidences the main variables studied to assess the PD presence at univariate analysis. TyG, hypertension, and FPG (with only a scarce tendency) resulted to be predictors.

To test whether the PD presence predicted by hypertension could be mediated by lipid dysmetabolism a multiple logistic regression was performed. This analysis found that hypercholesterolemia could play a key role, as the main covariate, in this prediction (*Table 3*).

Constructing an artificial control group aiming at matching each unit (PD patient) with a non-unit of similar characteristics (no PD patient), as might be expected, the principal and unique prediction were given by NAFLD, among age, IR as well as hypertension and NAFLD, confirming the previous statistical finding (*Table 4*).

Sensitivity/specificity analysis

The AUROC of TyG to predict the PD presence was = 0.7437, with 95% CI= 0.6501–0.8294 (*Table 5* and *Fig. 1*).

The AUROC of the TG/HDL-C ratio in diagnosing PD was significantly inferior (P=0.0025) to that of TyG, *i.e*., 0.5784, with 95% CI= 0.4723–0.6745 (*Fig. 1*).

The concordance correlation coefficient (ρ_c), to measure the agreement of US observation was high, *i.e*., 0.87.

DISCUSSION

This study shows that hypertension was found more frequently in PD patients than in no PD subjects. NAFLD and, to a lesser degree, IR were associated with the presence of PD in our male population of men independently of age. Interestingly, NAFLD was the only predictor of PD.

The AUROC of TyG, surrogate marker of NAFLD, to predict the PD presence was superior to that of TG/HDL ratio (a surrogate marker of IR) and was able to correctly classify 73.74% of patients.

Considering that the variable age is always taken into account in studies concerning PD, we enrolled in our PD population also patients of age higher than that of patients enrolled in other studies not in literature (27). Precisely, our patients below 50 years old represented only the ten-percentile.

The pathogenic mechanism/s whereby NAFLD is associated with PD are largely speculative. The PD pathogenesis is composite, but the hypothesis that repetitive microvascular injury (with fibrin deposition and its trapping in the tissue space) does not recover during the normal remodelling and repair of the

Table 1. The characteristics of the studied population comprehending lifestyles, anthropometric features, clinical presentation, and laboratory data.

Variables	**Peyronie's disease group** (n = 49)	**Control group** (n = 50)	**P**
Age (years); (mean±SD)	61.4±9.8	60.2±8.1	0.48
BMI (mean±SD)	26.4 ±3	25.6±2.3	0.15
Normoweigth/Overweigth/Obese; (n) *	15/27/7	20/28/2	0.17
T2DM (yes/not); (n) *	7/42	3/47	0.18
Hypertension (yes/not); (n) *	28/121	17/33	0.017
Alcohol consumption (yes/not); (n) *	12/37	16/34	0.50
Exercise (yes/not); (n) *	29/20	20/30	0.07
Fasting plasma glucose (mg/mL); median (IQR)	101 (85–115)	87 (77–98)	0.0088
Hypercholesterolemia >200 mg/dL; (n) *	25 (24)	7/43	0.0001
Total cholesterol (mg/dL); median (IQR)	187 (162–203)	159 (136–192)	0.0018
HDL-cholesterol (mg/dL); median (IQR)	45 (35–59)	40.5 (32–51)	0.053
LDL-cholesterol (mg/dl); median (IQR)	95 (85–131)	89.7 (61.8–131.6)	0.11
Creatinine (mg/dL); median (IQR)	0.9 (0.8–1.3)	0.975 (0.82–1.16)	0.08
INR median (IQR)	0.94 (0.92–0.95)	1.01 (0.97–1.08)	0.0001
Triglycerides (mg/dL); median (IQR)	133 (122–170)	103.5 (75–127)	0.0001
Triglycerides/HDL ratio (mean±SD)	3.08 (1.97–3.72)	2.63 (1.81–3.56)	0.18
Triglycerides glucose index (mean±SD)	8.8±0.39	8.4 0±0.51	0.0001
IR (n)*	25/24	16/34	0.043
NAFLD (n)*	39/10	19/31	0.0001

* Two-way table with measures of association, calculating the Fisher's exact.
IQR, 25–75 interquartile range; n, number; SD, standard deviation; BMI, body mass index; T2DM, type 2 diabetes mellitus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; INR, international normalized ratio; IR, insulin resistance; NAFLD, non-alcoholic fatty liver disease.
The IR presence was ascertained by using a cut-off of the triglycerides/HDL ratio ≥3.
The diagnosis of NAFLD was determined by setting as cut-point of the triglycerides glucose index >8.5.

Table 2. Prediction of the Peyronie's disease presence by the main variables taken into account.

Logistic regression: Number of observations = 99. Pseudo R2 = 0.0056						
d.v. PD	**Odds ratio**	**Std. err.**	**z**	**P>|z**	**[95% C.I.]**	
i.v. TG/HDL ratio	1.11845	0.14377	0.87	0.384	0.86935	1.4389
Logistic regression: Number of observations = 99. Pseudo R2 = 0.124						
d.v. PD	**Odds ratio**	**Std. err.**	**z**	**P>|z**	**[95% C.I.]**	
i.v. TyG	6.934	3.6701	3.66	0.000	2.457	19.566
Logistic regression: Number of observations = 99. Pseudo R2 = 0.036						
d.v. PD	**Odds ratio**	**Std. err.**	**z**	**P>|z**	**[95% C.I.]**	
i.v. FPG	1.0185	0.00939	1.99	0.047	1.0002	1.0371
Logistic regression: Number of observations = 99. Pseudo R2 = 0.039						
d.v. PD	**Odds ratio**	**Std. err.**	**z**	**P>|z**	**[95% C.I.]**	
i.v. Hypertension	2.5882	1.074	2.29	0.022	1.146	5.8410

PD, Peyronie's disease; TG/HDL ratio, triglycerides HDL-cholesterol ratio; TyG, triglycerides glucose index; FPG, fasting plasma glucose, d.v., dependent variable; i.v., independent variable; std.err., standard error; C.I., confidence interval.

Table 3. Prediction of the Peyronie's disease presence by lipid dysmetabolism and by hypertension.

Multiple logistic regression: Number of observations = 99. Pseudo R2 = 0.117						
d.v. PD	**Odds ratio**	**Standard error**	**z**	**P>|z**	**[95%**	**C.I.]**
i.v. Hypertension	2.2150	0.97519	1.81	0.071	0.93460	5.2496
i.v. Hypercholesterolemia	3.5389	1.93355	2.31	0.021	1.2128	10.32
i.v. Triglycerides	1.0081	0.00480	1.71	0.087	0.9988	1.0176

PD, Peyronie's disease; C.I., confidence interval. It is evident the mediation effect of hypercholesterolemia due to the clear decrease of z value of hypertension from significant 2.29 in the logistic regression of *Table 4* and the no significant presented in this Table, *i.e*., 1.81.

Table 4. Prediction of Peyronie's disease by the age, the metabolic condition and co-morbidities.

Logistic regression: Number of observations = 99 Bootstrap Replications = 50: Pseudo R2 = 0.148						
	Observed Bootstrap			Normal-based		
d.v. PD	**odds ratio**	**standard error**	**z**	**P>|z|**	**[95%**	**C.I.]**
i.v. Age	0.99658	0.02630	–0.13	0.897	0.94634	1.0494
i.v. Hypertension	1.84	0.86082	1.32	0.187	0.74172	4.6047
i.v. NAFLD	5.1657	2.869	2.96	0.003	1.7391	15.343
i.v. IR	1.2661	0.75358	0.40	0.692	0.39435	4.0653
Collinearity Diagnostics						
	Square Root					
Variable	**VIF**	**VIF**	**Tolerance**	**R-Squared**		
Age	1.04	1.02	0.9577	0.0423		
Hypertension	1.08	1.04	0.9228	0.0772		
NAFLD	1.28	1.13	0.7795	0.2205		
IR	1.17	1.08	0.8531	0.1469		
Mean VIF:	1.15					

PD, Peyronie's disease; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; IR, insulin resistance. VIF, variance inflation factor. Among the metabolic condition, *i.e*., IR and co-morbidities (NAFLD and hypertension) associated with PD only NAFLD predicted its presence constructing an artificial control group. Noteworthily, age was unrelated to the PD presence. This analysis was performed by using propensity score matching. There was no-collinearity among the examined variables as evident by a mean value of VIF inferior to 2.5 and tolerance inferior to 0.10. It is noteworthy that the best R-square is shown by the variable NAFLD.

Table 5. Sensitivity and specificity of triglycerides glucose index to confirm or refuse the Peyronie's disease.

Sensitivity	Pr (+| D)	67.35%
Specificity	**Pr (–|~D)**	**80.00%**
Positive predictive value	Pr (D |+)	76.74%
Negative predictive value	**Pr (~D|–)**	**71.43%**
False + rate for true ~D	Pr (+|~D)	20.00%
False - rate for true D	**Pr (–| D)**	**32.65%**
False + rate for classified +	Pr (~D|+)	23.26%
False - rate for classified -	Pr (D|–)	28.57%
Correctly classified	73.74%	

Statistical output of the probit model with the post estimation command >estat class< showing an overall rate of correct diagnosis of 73.74%. Specifically, 80% of the no Peyronie's disease (PD) group were correctly classified and 67.35% of the PD group were correctly classified; D, disease; ~D, no disease; Pr, probability.

tear in the tunica finds vast consensus among researchers. Moreover, fibroblast activation and proliferation, increased vessel permeability, and generation of neutrophil chemotactic factors are activated by fibrin deposited in the normal process of wound healing (28). The inflammatory process, following vascular trauma, stimulates smooth muscle cells and macrophages to produce nitric oxide synthase (29). Up-regulation of nitric oxide synthase causes the generation of high levels of nitric oxide, a potent free radical leading to oxidative stress, leads to subsequent excessive fibrous tissue generation, as

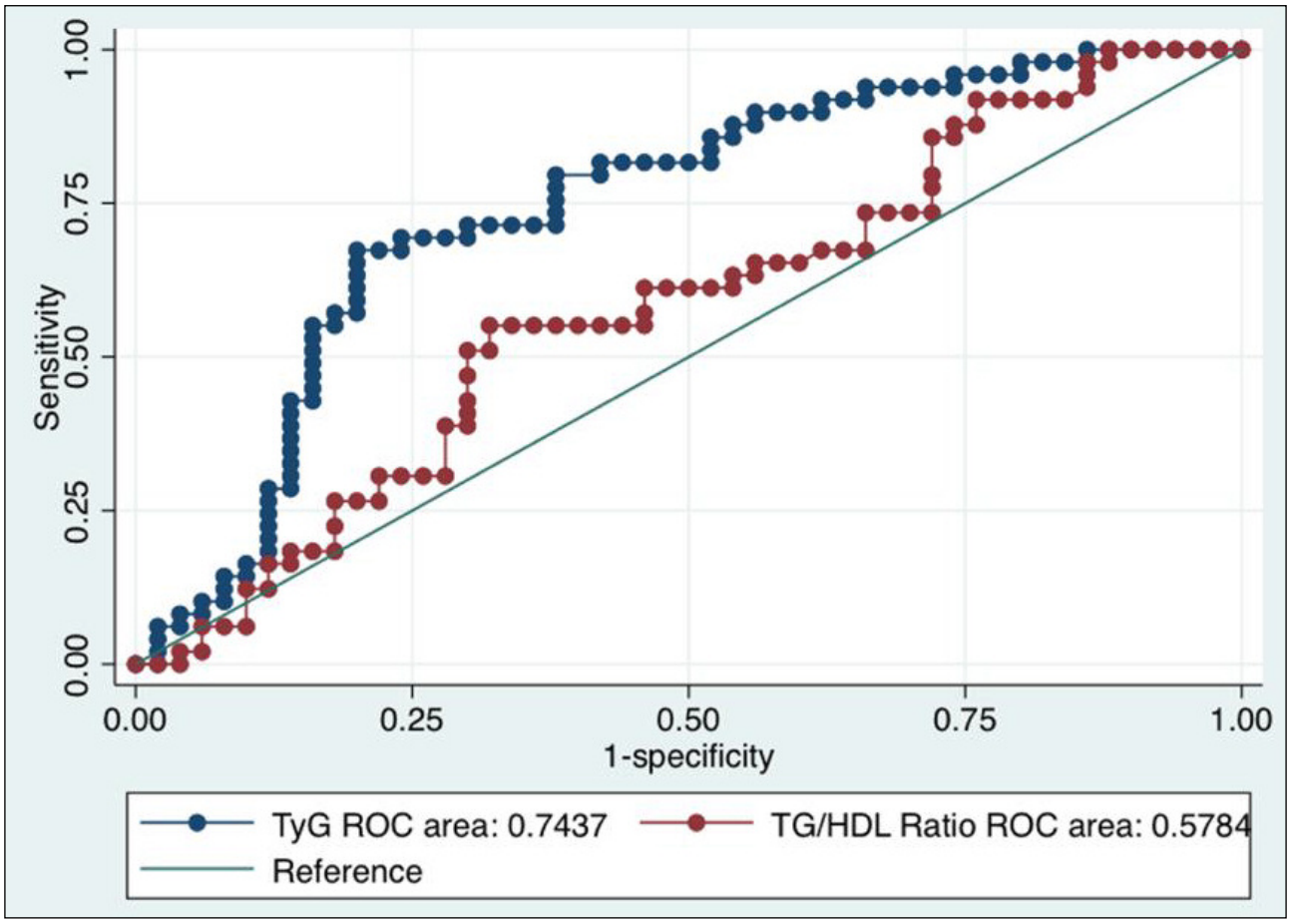

Fig. 1. The AUROCs of the triglycerides glucose index and the triglycerides/HDL ratio in diagnosing Peyronie's disease. These AUROCs show a reduced diagnostic power of the triglycerides /HDL ratio confronted with the AUROC of the triglycerides glucose index.

evidenced in a recently proposed animal model of PD (30, 31). On the other hand, inflammation is one manifestation of oxidative stress, thus a vicious circle in the progression of PD can develop (32). Therefore, oxidative stress plays an important role in the pathogenesis of PD, and this might represent the pathogenic link with NAFLD. In particular, the NAFLD association with PD could be contextualized within a picture in which the IR-linked obesity, inflammation, and oxidative stress, as well as the hypogonadism, may represent the vertices of triangles symmetrically fuelling the genesis and worsening of both diseases (33) (*Fig. 2*). Several evidence suggests hepatic steatosis's relationship with systemic IR, in the presence of which, the liver and the adipose tissues become sites of oxidative stress and inflammation (34, 35). Indeed, oxidative stress markers were significantly associated with NAFLD even after adjusting for age, gender, BMI, and glycemic status in Asian Indians without and with T2DM (36). In addition to oxidative stress, inflammation (detected by higher hs-CRP) is tightly and independently related to NAFLD status, thus lending credence to their strict link (37).

Pathogenically, IR contributes to the hepatic fat accumulation through different mechanisms including the reduced inhibition of lipolysis at the level of adipose tissue causing an increased free fatty acids afflux to the liver, the exalted hepatic *de novo* lipogenesis, and the impairment of beta-oxidation mechanisms (35). The free fatty acids overloading promotes the oxidative stress by representing a substrate for the genesis of lipotoxic substances (especially by mitochondria and cytochrome P activities), as well as by favouring reactive oxygen species production through the direct interaction with the electron transport chain (ETC) components or interference with membrane fluidity which provokes proton leakage (38, 39).

On one side, this metabolic stress induces proinflammatory cytokine release, on the other, it leads to the cells damage and death whose degradation products represent damage-associated molecular patterns (DAMPs) able in turn to sustain the vicious circle of oxidative stress and inflammation (38, 39). Furthermore, in IR conditions, the adipose tissue's infiltration (and polarization) by the M1 macrophages specialized in the production of pro-inflammatory cytokines as tumor necrosis factor-alpha (TNF-α), interleukin 1 (IL-1), IL-6, and chemokines as the monocyte chemoattractant protein-1 (MCP-1) is also observed (34). In support of this, the MCP-1 levels were shown positively related to the levels of IL-6 and homeostasis model assessment of IR (HOMA-IR) index in a group of obese patients (40).

All of these mechanisms have repercussions at a systemic level including the local penis microenvironment, thereby contributing to a state of chronic, low-grade inflammation responsible for the arterial disease and, in particular, for the endothelial dysfunction status featured by the disruption of intercellular junctions, increased vessel permeability, and significant enhancement in the reactive oxygen species production and fibrosis (41, 42). These appear pathogenetic elements shared both by NAFLD and PD, reinforced thus the concept of IR, inflammation, and oxidative stress as potential contact points (*Fig. 2*).

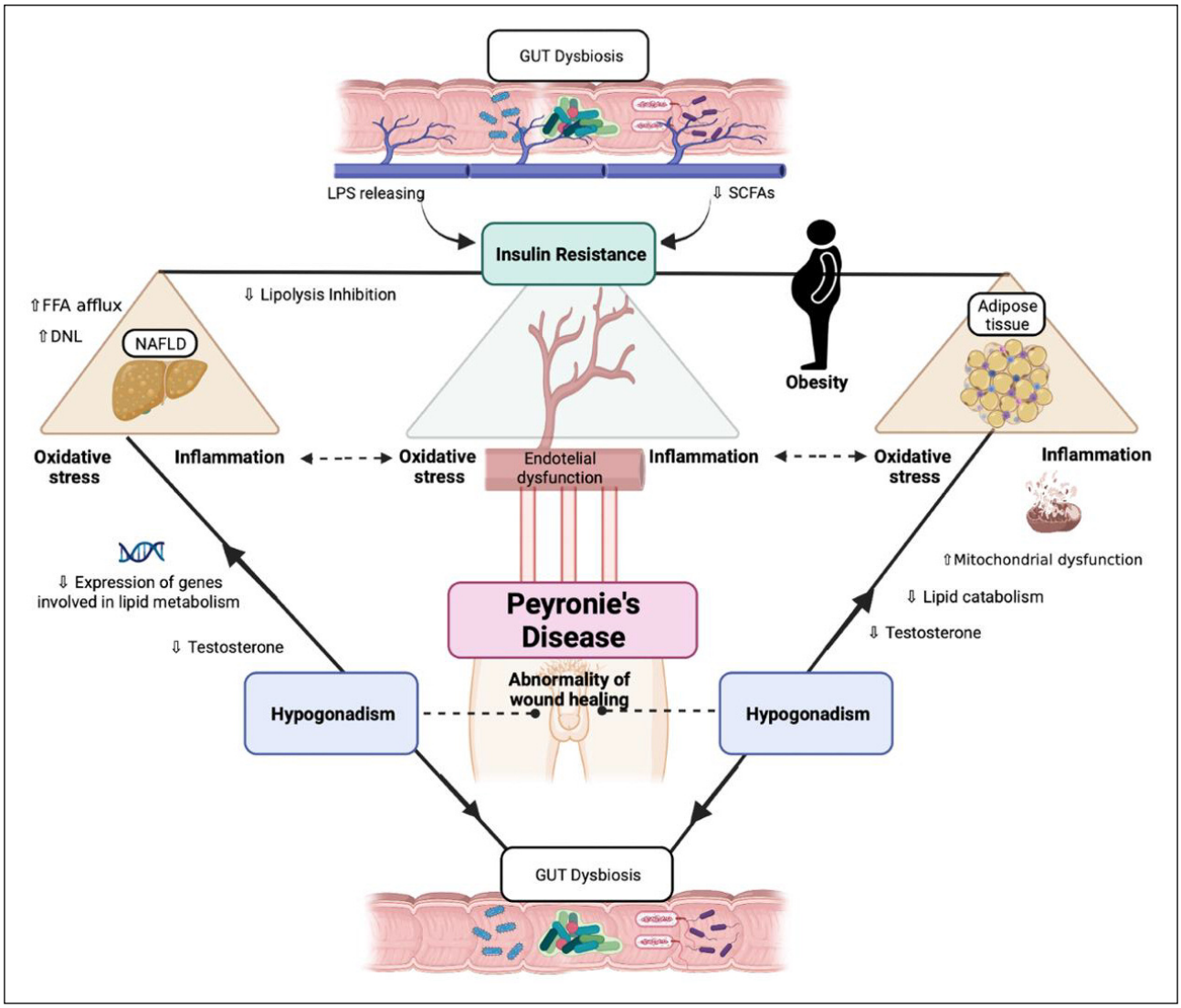

Fig. 2. Non-alcoholic fatty liver disease (NAFLD)-Peyronie's disease (PD) pathogenetic links. Insulin resistance (IR)-linked obesity, inflammation, and oxidative stress on one side, and the hypogonadism on one other may represent the vertices of triangles symmetrically and systemically fuelling the genesis and worsening of both NAFLD and PD. FFA, free fatty acids; SCFAs, short-chain fatty acids; LPS, lipopolysaccharide.

Moreover, there is some controversy as to the role of hormonal factors in PD and NAFLD, testosterone being the main impute (43, 44). Regarding NAFLD, some evidence suggests a potentially protective role for testosterone by improving hepatic steatosis and reducing intrahepatic triglycerides as well as the correlation between low testosterone levels and non-alcoholic steatohepatitis (NASH)/fibrosis severity; contrariwise, cross-sectional studies carried out in NAFLD patients testosterone levels have been demonstrated to be significantly lower than normal even though baseline testosterone levels did not influence the development or regression of fatty liver at the median 4.2 years follow-up (45-47). Testosterone is an anabolic hormone having several positive pleiotropic effects such as the regulation of the wound healing process by influencing the collagen metabolism and the transforming growth factor-beta1 (TGF-β1) expression in the fibrosis, as well as the promotion of anti-inflammatory dynamics (48).

Therefore, in the case of low testosterone levels, it is conceivable a worsening of phlogistic and oxidative stress mechanisms involved in PD genesis and progression both systemically and locally. In particular, low levels of this hormone may contribute to the worsening of systemic IR given the reported associations in preadipocytes with a decreased lipid catabolism and mitochondrial dysfunction, in the hepatocytes with a reduced expression of genes involved in the lipid metabolism (45).

Furthermore, emerging evidence suggests the recurrence in the IR-related disorders of alterations in the gut microbiota composition (dysbiosis) and its metabolites, which translocate from the gut across an impaired intestinal barrier to affect various organs, such as the liver and adipose tissue, thereby contributing to the perpetuation of metabolic inflammation status (49, 50). The lipopolysaccharide (LPS) released from the intestinal flora to the systemic circulation may act as a pathogen associated molecular pattern (PAMP), inducing a chronic subclinical inflammatory process through the activation of toll-like receptor 4 (TLR4) pathways with the worsening of IR to which also the reduction in circulating short-chain fatty acids (SCFAs) may contribute (51). At the same time, hypogonadism may induce alterations in the GUT microbiota composition as

suggested by evidence in animals revealing modifications in cecal microbiota (*e.g*., an increased Firmicutes/Bacteroidetes ratio and the number of Lactobacillus species) consistent with changes in feces after the androgen deprivation *via* castration in male mice model (52). In the light of this, the role of the gut microbiota appears crucial in this complex scenario, impacting on all of the above-described NAFLD-PD pathogenic elements.

In further support of a pathogenic link between NAFLD and PD, ED, a condition frequently observed in PD patients, has been found to be significantly associated with NAFLD (53). Also, it has been suggested that liver injury may contribute to ED (53). The role of alcohol use in our population was negligible, and this is in contrast with a previously reported study that emphasized the risk of alcohol consumption for PD (54). Finally, in our cohort of PD patients, there was a statistically significant association between IR and PD. This further supports the pathogenic link between PD and NAFLD. In fact, it is well established that IR plays a crucial causative role in the development of NAFLD (55). In this regard, TyG, a surrogate marker for NAFLD is also a marker of IR, and shows a good level of agreement with hyperglycemic clamp, performing better than HOMA (56-58).

Looking to the clinical practice implications, the herein proposed NAFLD-PD pathogenetic shared mechanisms may represent potential therapeutic targets for both diseases. Regarding IR, a first-line-not-pharmacological approach based on the measures of lifestyle changes, in terms of adequate physical exercise and compliance to the Mediterranean diet regimen, may be proposed (59).

A second step may be represented by the administration of insulin-sensitizing, anti-oxidant agents, tailored pre-/pro-biotics as well as periodical testosterone injections.

However, since no evidence about their efficiency, benefits, and risks in NAFLD/PD patients exist, as well as no data have been evaluated by consistent clinical trials, these represent only a conceivable interesting frontier. Certainly, in the era of Precision Medicine in which research efforts focus on the designation of patient-tailored therapeutic approaches, the evidenced impact of genetic individual background on the potential response to a specific treatment cannot be ignored (60).

Finally, the early identification of patients who are at risk of PD could be crucial in an effort to improve diagnostic and therapeutic alternatives of this disease. Briefly, current therapies for PD could be divided in non-surgical and surgical, with the first that encompasses the utilization of oral drugs (vitamin E, tamoxifen, L-carnitine) or intralesional injections (collagenase Clostridium histolyticum, interferon-a-2b and verapamil) while the second includes incision of the plaque (in order to pair the curvature) up to more invasive procedures (as the excision of the plaque and the application of a graft) (26, 61, 62). Considering the possible morbidities of surgical therapies (erectile dysfunction, shortening of the penis, recurrence of the curvature and loss of penile sensation), the surgery is indicated for patients who presents a deformity that impairs sexual activity, prior to a thorough examination of nature and location of deformity, baseline erectile functionality and penile dimensions (63).

This study has a few limitations. Firstly, the relatively reduced sample size of the PD population could jeopardise the precision of results, but it is necessary to point out that it was well-matched with a cohort of subjects without PD. Second, there was no follow-up of our PD patients in order to compare whether worsening of NAFLD was associated with increased severity of lesions reported in PD patients.

In conclusion, this study demonstrates that NAFLD, and, to a lesser degree, IR are significantly associated with PD even though this is far from establishing a cause-effect relationship. We hypothesize that oxidative stress and/or IR may represent the main pathogenic link between these conditions. Whether NAFLD patients are at greater risk of developing PD cannot be established solely based on this study. However, should our observation be confirmed in further studies involving a greater number of patients, one may hypothesize routine assessment of sexual function in NAFLD patients for early diagnosis and treatment of PD, an increasingly disabling disease with severe psychological consequences (9).

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