Evaluation of plasma oxidative status in patients with slow coronary flow

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Abstract
Background: Slow coronary flow (SCF) is a coronary microvascular disorder characterised by delayed opacification of coronary vessels in a normal coronary angiogram. Coronary endothelial dysfunction plays an important pathogenetic role in patients with SCF. Oxidative stress is associated with cardiovascular diseases.

Aim: To assess the total antioxidant capacity (TAC) and total oxidative status (TOS) in patients with SCF.

Methods: The study included 36 patients with SCF. An age- and gender-matched control group was composed of 30 patients with normal coronary arteries and normal coronary flow. We measured plasma TAC and TOS levels and oxidative stress index (OSI) value in patients and control subjects. Linear regression analysis was performed to identify factors associated with the mean TIMI frame count (TFC).

Results: Plasma TOS level and OSI value were significantly higher in the SCF group compared to the control group (p = 0.005 and p = 0.004, respectively). However, there was no significant difference in plasma TAC levels between the groups (p = 0.104). Factors associated with mean TFC were plasma TOS levels (β = 0.425, p = 0.002) and fasting glucose levels (β = 0.099, p = 0.01) in linear regression analysis.

Conclusions: We found that plasma TOS and OSI were significantly higher in SCF compared to the control group and plasma TOS levels were independently associated with mean TFC.

Key words: total antioxidant capacity, total oxidative status, oxidative stress index, slow coronary flow

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INTRODUCTION
Slow coronary flow (SCF) is a slow antegrade progression of contrast agent to the distal branch of a coronary artery in the absence of obstructive coronary artery disease (CAD) [1]. The importance of SCF results from its association with angina pectoris, acute myocardial infarction, hypertension and sudden cardiac death [2]. Several mechanisms have been proposed for the aetiology of SCF, including microvascular and endothelial dysfunction, small-vessel disease, diffuse atherosclerosis and inflammation. However, its etiopathogenesis is still not clear [1–3].

Reactive oxygen species (ROS) such as hydroxyl radical and superoxide radical are reactive chemical species generated during normal metabolic processes and, in excess, can damage lipids and proteins [4]. ROS may cause peroxidation of cell membrane double-chain fatty acids and then cellular injury and also increase oxidative stress which has been determined to play a key role in the pathophysiology of cardiovascular diseases such as atherosclerosis, hypertension, and myocardial infarction [5, 6].

In the literature, there is no study about the plasma total oxidative status (TOS) and total antioxidant capacity (TAC) levels and oxidative stress index (OSI) in patients with SCF. In this study, we aimed to evaluate the oxidative status and factors associated with the mean thrombolysis in myocardial infarction frame count (TFC) in patients with SCF.
METHODS

Study group

The study group consisted of 36 patients with SCF (20 males, mean age 52 ± 10 years). From 100 consecutive patients with SCF, 22 patients with hypertension, 17 patients who had ≥ 30% diameter stenosis of major coronary arteries, 13 patients with diabetes mellitus, ten patients with coronary artery ectasia, one patient with hypothyroidism and one patient with systemic disease were excluded. The remaining 36 patients with SCF constituted the study group. An age- and gender-matched control group was composed of 30 patients with normal coronary arteries and normal coronary flow on coronary angiography (13 males, mean age 49 ± 8 years). The indications for coronary angiography in both groups were: presence of typical angina, positive or equivocal results of noninvasive screening tests for myocardial ischaemia, persistent limiting symptoms (angina or angina equivalent) despite optimal medical therapy, a proven significant ischaemic territory, serious ventricular arrhythmias, an inconclusive diagnosis on non-invasive testing, and conflicting results from different noninvasive screening tests.

Each patient was questioned about major cardiovascular risk factors including family history of CAD, current smoking status, hyperlipidaemia and obesity. Family history of CAD was defined as CAD in first-grade male relatives before 55 years and in female relatives before 65 years of age. Hyperlipidaemia was defined as fasting total cholesterol level > 200 mg/dL or being on lipid-lowering agents. Obesity was defined as body mass index > 30 kg/m². Patients who were smoking before hospitalisation were accepted as smokers.

Exclusion criteria were: prior myocardial infarction, acute coronary syndromes, moderate to severe valvular heart disease, New York Heart Association class II–IV chronic heart failure, acute heart failure, peripheral vascular disease, coronary artery ectasia, diabetes mellitus, hypertension, renal or hepatic dysfunction, haematological disorders, hypo- or hyperthyroidism, chronic obstructive pulmonary disease or cor pulmonale, history of malignancy, acute or chronic infection, systemic diseases such as collagenosis, stroke, recent use (within the previous 48 h) of any drug with anti-oxidant properties, such as acetylcysteine, vitamins E and C, and regular alcohol use or alcohol use within the previous 48 hours. Patients with SCF who had ≥ 30% diameter stenosis of major coronary arteries were excluded from the study. Patients using nebivolol [7, 8] and carvedilol [8, 9], which have greater anti-inflammatory and anti-oxidative properties than other beta-blockers, were also excluded from the study. The study was approved by the institutional review board, and informed consent was obtained from all the patients.

Coronary angiography

Selective coronary angiography was performed with the Judkins technique in multiple projections without the use of nitroglycerin. We used iopamidol (Iopamiro) as contrast agent. Coronary arteries were demonstrated at least four views of the left coronary system using 6 French left coronary catheters and two views of the right coronary artery using 6 French right coronary catheters by 15 fps rate in the same cardiac catheterisation laboratory. Coronary blood flow was measured quantitatively using the TFC. Initial frame count is defined as the frame in which concentrated dye occupies the full width of proximal coronary artery lumen, touching both borders of the lumen, and forward motion down the artery. The final frame is designated when the leading edge of the contrast column initially arrives at the distal end. Distal end was defined as distal bifurcation for the left anterior descending (LAD) artery, the distal bifurcation of the segment with the longest total distance for the circumflex artery (CX), and the first branch of the posterolateral artery for the right coronary artery (RCA). LAD coronary artery is usually longer than the other major coronary arteries; the TFC for this vessel is often higher. To obtain corrected TFC for LAD coronary artery, TFC was divided by 1.7 [10]. The mean TFC for each patient and control subject was calculated by adding the TFC for LAD, CX and RCA and then dividing the obtained value into three. Due to different durations required for normal visualisation of coronary arteries, the corrected cutoff values were 36.28 ± 2.6 frames for LAD, 22.28 ± 4.1 frames for CX, and 20.48 ± 3 frames for RCA, as has been reported earlier in the literature [10]. All participants with a TFC greater than the two standard deviations of the previously published range for the particular vessel were considered to have SCF. Any values obtained above these thresholds in one of three coronary arteries (not all three) were considered to be SCF in our study. Coronary angiograms and TFC were analysed by two experienced interventional cardiologists blinded to the clinical status and laboratory measurements of the subjects.

Blood sampling

All blood samples were obtained following an overnight fast, drawn from a large antecubital vein without interruption of venous flow, using a 19-gauge butterfly needle connected to a plastic syringe. Content of the syringe was transferred immediately to polypropylene tubes. These tubes then were centrifuged at 3,000 rpm for 10 min at 10–18°C. Supematant plasma samples were stored in plastic tubes at −30°C until assayed. All plasma samples for TAC and TOS were measured by the same and single assay.

Measurement of plasma total antioxidant capacity

The TAC of the plasma was determined using a novel automated measurement method developed by Erel [11]. In this method, hydroxyl radical, which is the most potent biological radical, is produced. The assay measures the antioxidative effect of the sample against potent free radical reactions that are initiated by the hydroxyl radical produced. The assay has...
excellent precision values, of greater than 97%. The results are expressed as μmol Trolox equivalent/L.

**Measurement of plasma total oxidative status**
The TOS of serum was determined using a novel automated measurement method by Erel [12]. Oxidants present in the sample oxidised the ferrous iron-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by the glycerol molecules present in the reaction medium. The ferric ion produced a coloured complex with xylenol orange in acidic solution. The colour intensity, which was measured spectrophotometrically, was proportional to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide, and the results were expressed in terms of micromolar hydrogen peroxide equivalents per litre (μmol H₂O₂ equivalent/L).

**Oxidative stress index**
The OSI is defined as the ratio of the TOS to TAC levels, expressed as a percentage. For the calculation, TAC units were changed to mmol/L, and the OSI value calculated according to the following formula: OSI (arbitrary unit) = TOS (mmol H₂O₂ equiv./L)/TAC (mmol Trolox equiv./L).

**Measurement of other biochemical markers**
Serum high-sensitivity C-reactive protein (hsCRP) level was measured by nephelometric method using available commercial kits according to the manufacturer’s instructions (Beckmann Assay 360, Bera, CA, USA). Other biochemical tests were also performed using original kits with Olympus auto-analysers.

**Statistical analysis**
Data was analysed with the SPSS software version 12.0 for Windows. Continuous variables from the study groups were reported as mean ± standard deviation, categorical variables as percentages. To compare continuous variables, the Student t-test or Mann-Whitney U test were used where appropriate. Categorical variables were compared using the χ² test. Spearman correlation analysis was performed for the analysis of factors correlated with mean TFC. Linear regression analysis was performed to identify the independent variables associated with mean TFC. The confounders which had importance at p ≤ 0.01 level were entered into the regression analysis (TOS, fasting glucose, creatinine and high density lipoprotein [HDL]-cholesterol). OSI was not entered because it was correlated with TOS. Statistical significance was defined as p < 0.05.

**RESULTS**
Demographic and clinical characteristics of the study population are listed in Table 1. There were no statistically significant differences between the two groups with respect to age, gender, body mass index, waist circumference, systolic and diastolic blood pressures, heart rate and the risk factors for CAD such as hyperlipidaemia, family history, obesity and smoking. The use of acetylsalicylic acid and beta-blockers was significantly higher in the SCF group than the control group, but there were no statistically significant differences between the two groups with respect to the use of other medications. Thirteen patients were using beta-blockers in the SCF group: ten of them were using metoprolol and three were using bisoprolol. Three patients were using beta-blockers in the control group, and those three were all using metoprolol.

Table 2 shows laboratory parameters of the study population. Fasting glucose level was significantly higher in the SCF group. There were no statistically significant differences between the groups with respect to levels of creatinine, aspartate transaminase, alanine transaminase, total cholesterol, triglyceride, low density lipoprotein-cholesterol, HDL-cholesterol, uric acid, haemoglobin, haematocrit, white blood cell and platelet count. Serum hsCRP levels were significantly higher in the SCF group than in the control group (p = 0.03).

The TFC for all the epicardial coronary arteries, and the mean TFC, were significantly higher in the SCF group than the control group. Mean TFC of all patients with SCF was higher than in the control group.

Oxidative/antioxidative parameters are presented in Table 2. In the SCF group, plasma TOS level and OSI value were significantly higher compared to the control group.

![Image of Table 1. Demographic and clinical characteristics](www.kardiologiapolska.pl)

<table>
<thead>
<tr>
<th></th>
<th>SCF group (n = 36)</th>
<th>Control group (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>51.7 ± 9.8</td>
<td>48.8 ± 7.9</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>20/16</td>
<td>13/17</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>12 (33%)</td>
<td>4 (13%)</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>8 (22%)</td>
<td>8 (27%)</td>
<td>NS</td>
</tr>
<tr>
<td>Obesity</td>
<td>14 (39%)</td>
<td>9 (30%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>9 (25%)</td>
<td>6 (20%)</td>
<td></td>
</tr>
<tr>
<td>Body mass index [kg/m²]</td>
<td>29.0 ± 4.5</td>
<td>28.2 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td>Waist circumference [cm]</td>
<td>98.6 ± 8</td>
<td>96.3 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP [mm Hg]</td>
<td>121 ± 11</td>
<td>120 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP [mm Hg]</td>
<td>75 ± 9</td>
<td>75 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate [bpm]</td>
<td>71.3 ± 8.3</td>
<td>71.6 ± 7.3</td>
<td>NS</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>18 (50%)</td>
<td>6 (20%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>13 (36%)</td>
<td>3 (10%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Statins</td>
<td>7 (19%)</td>
<td>3 (10%)</td>
<td>NS</td>
</tr>
<tr>
<td>ACEIs or ARBs</td>
<td>3 (8%)</td>
<td>1 (3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

ACEI — angiotensin converting enzyme inhibitors; ARB — angiotensin receptor blockers; BP — blood pressure; CAD — coronary artery disease; SCF — slow coronary flow; p value is for comparison between SCF and control population; NS — not significant.
However, compared to the control group, we did not find a significant difference in plasma TAC level.

The mean TFC was significantly and positively correlated with TOS (Fig. 1), OSI, fasting glucose, creatinine, and haematocrit; it was significantly and inversely correlated with HDL-cholesterol (Table 3), but it was not correlated with TAC, uric acid or hsCRP.

In linear regression analysis, when mean TFC was taken as dependent, we found that TOS and fasting glucose were independent significant predictors of SCF (Table 3).

**DISCUSSION**

The main findings of this study were increased levels of the TOS and OSI in patients with SCF when compared to the control group. To the best of our knowledge, this is the first study investigating plasma TOS levels and OSI in patients with SCF.

Oxidative stress is caused by an increased production of ROS and is linked with negative outcomes in cardiovascular diseases [13]. Clinical studies demonstrate that ROS production is increased in many cardiovascular diseases such as CAD, heart failure, essential hypertension, renovascular hypertension, malignant hypertension and atrial fibrillation [14–19]. Recently published studies have revealed oxidative stress to be
Endothelial dysfunction has been shown to be associated with an increase in ROS in atherosclerotic animal models and human subjects with atherosclerosis [24]. Although the pathophysiological mechanisms of SCF remain uncertain, it has recently been reported that coronary endothelial dysfunction plays an important pathogenetic role in patients with SCF [3]. The association between endothelial dysfunction and coronary blood flow has also been established by demonstrating the correlation between impaired brachial artery flow mediated dilatation and increased corrected TFC in patients with SCF [3, 25] in the presence of increased oxidative stress markers such as superoxide dismutase, reduced glutathione and malondialdehyde [25]. The relationship between mean TFC and both OSI and TOS revealed in our study supports the pathophysiology of SCF. However, increased oxidative stress may also be a result of pathological processes. Further prospective studies are needed to establish the pathophysiological and clinical significance of increased oxidative stress, and to investigate the effect of anti-oxidant agents in patients with SCF.

**Limitations of the study**

The sample size of this study was small. Angiographic diagnosis of normal coronary arteries was based on axial contrast angiograms of the vessel lumen, which underestimates the presence of atherosclerotic plaques.

**CONCLUSIONS**

Our present study suggests that oxidative stress is increased in patients with SCF when compared to a control group. Thus, increased oxidative stress may contribute to the pathogenesis of SCF. However, increased oxidative stress may also be a result of pathological processes. Future prospective studies are needed to establish the pathophysiological and clinical significance of increased oxidative stress, and to investigate the effect of anti-oxidant agents in patients with SCF.

**Conflict of interest:** none declared

**References**


Ocena stanu oksydacyjnego osocza u chorych z wolnym przepływem wieńcowym

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Streszczenie

Wstęp: Wolny przepływ wieńcowy (SCF) jest zaburzeniem dotyczącym mikrokrążenia wieńcowego polegającym na opóźnionej opacyfikacji naczyń wieńcowych w prawidłowym angiogramie wieńcowym. Dysfunkcja śródbłonka tętnic wieńcowych stanowi ważny czynnik patogenetyczny u pacjentów z SCF. Stres oksydacyjny wpływa na rozwój chorób układu sercowo-naczyniowego.

Cel: Celem badania była ocena całkowitej zdolności antyoksydacyjnej (TAC) i całkowitego stanu oksydacyjnego (TOS) osocza u chorych z SCF.

Metody: Badaniem objęto 36 chorych z SCF. Dobrana pod względem płci i wieku grupa kontrolna składała się z 30 osób z prawidłowym obrazem tętnic wieńcowych i prawidłowym przepływem wieńcowym. Autorzy zmierzyli TAC i TOS osocza oraz wskaźnik stresu oksydacyjnego (OSI) u chorych oraz u osób z grupy kontrolnej. Przeprowadzono analizę regresji liniowej w celu określenia czynników związanych ze średnią liczbą klatek TIMI (TFC).

Wyniki: Wartości TOS osocza i OSI były istotnie wyższe w grupie z SCF w porównaniu z grupą kontrolną (odpowiednio p = 0,005 i p = 0,004). Jednak nie stwierdzono istotnych różnic między grupami w wartościach TAC osocza (p = 0,104). Czynniki związanymi ze średnią TFC w analizie regresji liniowej były TOS osocza (β = 0,425; p = 0,002) i glikemia na czczo (β = 0,099; p = 0,01).

Wnioski: Autorzy wykazały, że TOS osocza i OSI były istotnie wyższe u chorych z SCF niż w grupie kontrolnej oraz że wartości TOS osocza były niezależnie związane ze średnią TFC.

Słowa kluczowe: całkowita pojemność antyoksydacyjna, całkowity stan oksydacyjny, wskaźnik stresu oksydacyjnego, wolny przepływ wieńcowy

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