Uniwersytet Medyczny w Łodzi

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Poziom stresu oksydacyjnego u chorych z kamicą moczową leczonych zabiegami małoinwazyjnymi

Rozprawa na stopień doktora nauk medycznych

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Łódź 2020

Wstęp

Kamica moczowa jest jedną z częściej występujących chorób. Najstarszy kamień układu moczowego odkrył Eliot Smith w mumii egipskiej w 1901 roku. Wiek znaleziska oszacowano na 4800 r. p.n.e.[1] W Stanach Zjednoczonych Ameryki na kamicę moczową choruje 10,6% mężczyzn i 7,1 % kobiet[2]. Obserwowany jest stały wzrost zachorowalności w ostatnich dekadach.[3] Kamica moczowa to choroba, której leczenie na przestrzeni wieków ewaluowało od procedur kończących się śmiercią leczonego do mało inwazyjnych zabiegów obarczonych minimalną liczbą powikłań.

Pierwsze opisy objawów kamicy moczowej pochodzą z Mezopotamii i datowane są na 1200 lat przed naszą erą. Teksty medyczne z tego okresu podają skład leku rozpuszczającego kamień moczowy.[4] Podana przez rzymskiego lekarza Corneliusa Celsusa (25 r. p.n.e. – 40 r. n.e.) technika operacji kamicy pęcherza moczowego przetrwała do XVIII wieku.[5] Dziewiętnasty wiek to czas rozwoju kruszenia i usuwania kamieni z pęcherza na drodze endoskopowej. Rozwój ten zawdzięczamy takim lekarzom jak Jean Civiale, Leroy d'Etoilles, oraz Henry J. Bigelow, który opracował system kruszenia i ewakuacji drobnych fragmentów kamienia z pęcherza moczowego co pozwoliło na zmniejszenie śmiertelności z około 24% do 2,4%.[6,7] W XX wieku następuje bardzo szybki rozwój technik litotrypsji kamieni w drogach moczowych. W 1955 r. Goodwin wytwarza pierwszą nefrostomię przezskórną.[8] W 1977 roku Kurth jako pierwszy kruszy kamienie w nerce z zastosowaniem fal ultradźwiękowych.[9] W 1980 rozpoczęto leczenie kamicy moczowej z zastosowaniem litotryptora Dornier HM-1, a w 1983 litotryptorem Dornier HM-3.[10] Metoda ESWL nie pozwala wyleczyć wszystkich chorych. Od tego czasu obserwujemy gwałtowny rozwój bardzo nowoczesnych metod leczenia kamicy dróg moczowych na drodze endoskopowej. Wprowadzenie do leczenia kamicy moczowej lasera Nd:YAG oraz nefroskopów i ureterorenoskopów pozwala na bardzo skuteczne leczenia kamicy moczowej w całym układzie moczowym.[11] Od początku XXI wieku obserwujemy intensywny rozwój miniaturyzacji sprzętu do PCNL oraz wprowadzenie odpowiednio cienkich oraz giętkich ureterorenoskopów pozwalających na mniejszą traumatyzację tkanek w trakcie zabiegów.

Zastosowanie w leczeniu kamicy nerkowej dostępu do układu kielichowo-miedniczkowego przetoki skórno-nerkowej oraz stała miniaturyzacja sprzętu do PCNL w połączeniu z laserem holmowym sprawia, że zabiegi stają się bardziej skuteczne i mniej inwazyjne. Zgodnie z wytycznymi Europejskiego Towarzystwa Urologicznej do PCNL kwalifikowani są chorzy z kamieniem w miedniczce nerkowej powyżej 2 cm, a w kielichu dolnym 1,5 cm, chorzy z kamicą odlewową nerki, pacjenci, u których zabieg ESWL nie był skuteczny oraz chorzy z wadami układu moczowego[12]. W 1998 roku Jackman wykonał pierwszy zabieg usunięcia kamienia z nerki na drodze mini PCNL. Obecnie ten sposób leczenia stosowany jest w odlewowej kamicy nerki bez konieczności powtarzania zabiegu[13].

Ureterorenoskopia (URSL) jest zabiegiem usuwania kamieni z moczowodu pod kontrolą wzroku za pomocą urządzenia zwanego ureterorenoskopem. Pierwszą ureterorenoskopię wykonał za pomocą giętkiego endoskopu Marshall w 1964 roku. Gwałtowny rozwój tej metody nastąpił od roku 1980, po skonstruowaniu przez Pereza-Castro sztywnego ureterorenoskopu o średnicy 9 F.

Ureterorenoskopia giętkim ureteroskopem URSL (flexible ureteroscopy) jest zabiegiem nowoczesnym, jednak na jego pełne i skuteczne zastosowanie czekaliśmy do momentu

wprowadzenia nowoczesnych giętkich endoskopów oraz odpowiednio cienkich włókien pozwalających na przenoszenie energii lasera o dużej mocy. Kruszenie kamienia na drodze giętkiej ureterorenoskopii jest zabiegiem bezpiecznym i obarczonym małą liczbą powikłań[14].

Zabiegi giętkimi ureterorenoskopami mogą być alternatywą dla mini PCNL w leczeniu kamicy górnych dróg moczowych[15]. Wprowadzenie urządzeń o większych możliwościach penetrowania układu kielichowo-miedniczkowego zwiększy skuteczność leczenia kamieni dolnego kielicha[16].

Mini PCNL jest również nowoczesną techniką kruszenia kamieni moczowych u wybranych chorych. Pozwala ona na usuniecie małego kamienia na drodze bezpośredniego wprowadzenia do układu kielichowo-miedniczkowego nerki zminiaturyzowanych nefroskopów. Jest to metoda wspomagająca kruszenie kamienia na drodze ureterorenoskopii[17].

Obecnie w leczeniu kamicy moczowej mamy szeroki wybór urządzeń od maszyn wykorzystujących fale uderzeniową generowaną poza ciałem pacjenta przez urządzenia do PCNL oraz URSL z ich giętkimi odmianami. Powszechne stosowanie maszyn do ESWL w leczeniu kamicy moczowej pokazało że nie jest to zabieg w pełni bezpieczny. Obarczony jest on różnego stopnia urazem w zależności od lokalizacji kamienia w drogach moczowych. Kruszenie kamienia w nerce obarczone jest miejscowym uszkodzeniem miąższu, powoduje zwiększone wydzielanie endoteliny, czego następstwem jest wzrost ciśnienia tętniczego krwi[18]. Bardzo częstym objawem urazu dróg moczowych po zabiegu kruszenia kamienia w układzie moczowym jest krwinkomocz lub krwiomocz, a w skrajnych przypadkach objawowe krwiaki nerki[19].

Pamiętając, że fala uderzeniowa powoduje uraz tkanki nerki, a wiązka lasera może powodować uraz nabłonka urotelialnego dróg moczowych dokonaliśmy porównania wielkości urazu jaki powstaje w drogach moczowych po zastosowaniu obu tych metod. Za wspólny parametr dla różnego rodzaju urazu dróg moczowych podczas zabiegu kruszenia kamieni przyjęliśmy oznaczenie poziomu stresu oksydacyjnego.

Cel pracy

Celem pracy jest ocena poziomu stresu oksydacyjnego u chorych na kamicę moczową leczonych zabiegami małoinwazyjnymi (ESWL i URSL) i ich wpływ na hemostazę. Zwiększenie stresu może oddziaływać na parametry krzepnięcia krwi[20] i powodować większe krwawienie po zabiegu. Może też negatywnie wpływać na funkcje nerek i przyczyniać się do rozwoju przewlekłej choroby nerek, czy nadciśnienia tętniczego[21].

Analiza otrzymanych wyników pozwoli na dobór odpowiedniej metody leczenia, potwierdzenie słuszności takiego postępowania, bądź wskaże mniej inwazyjne sposoby eliminacji kamicy układu moczowego. Uzyskane wyniki mogą w znaczący sposób przyspieszyć rekonwalescencję i poprawić proces leczenia, a także zminimalizować ryzyko powikłań około i pooperacyjnych.

PLOS ONE

RESEARCH ARTICLE

Evaluation of hemostasis parameters and the role of the oxidative damage to plasma proteins in the modulation of hemostasis in patients with nephrolithiasis before and after extracorporeal shock wave lithotripsy

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Citation: Woźniak P, Kontek B, Różański W, Olas B (2017) Evaluation of hemostasis parameters and the role of the oxidative damage to plasma proteins in the modulation of hemostasis in patients with nephrolithiasis before and after extracorporeal shock wave lithotripsy. PLoS ONE 12(10): e0185157. https://doi.org/10.1371/journal.pone.0185157

Editor: Reza Khodarahmi, Kermanshah University of Medical Sciences, ISLAMIC REPUBLIC OF IRAN

Received: March 20, 2017

Accepted: September 7, 2017

Published: October 2, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by grant 506/1136 from the University of Lodz to BO.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Purpose

Extracorporeal shock wave lithotripsy (ESWL) is a commonly-used method in urology, which may modulate hemostasis and may induce lipid peroxidation in patients with nephrolithiasis. However, previous studies only examine changes occurring in patients 30–240 min after ESWL. The main aim of the present study was to determine whether oxidative stress may modulate the hemostatic activity of plasma in patients with nephrolithiasis before ESWL and the day after treatment ESWL. This will be performed by measuring selected parameters of hemostasis in these patients, both before ESWL and the following day, and assessing the level of oxidative damage to plasma proteins in these patients by measuring two biomarkers.

Methods

Twelve patients with nephrolithiasis and 10 healthy participants were included. The following parameters of hemostasis were measured: the activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) of plasma, the level of fibrinogen, the level of D-dimer and blood platelet count. In addition, two selected biomarkers of oxidative stress were measured: protein carbonylation level and the number of protein thiol groups.

Results

No difference was observed between patients with nephrolithiasis before and after ESWL and healthy controls with regard to PT, TT or APTT. Fibrinogen concentration and blood platelet count were lower in the nephrolithiasis patients in the period after ESWL than before ESWL. The nephrolithiasis patients demonstrated elevated D-dimer concentration after ESWL. However, although oxidative damage was observed in the plasma proteins in the nephrolithiasis patients, this was not influenced by ESWL.

Abbreviations: APTT, activated partial thromboplastin time of plasma; ESWL, extracorporeal shock wave lithotripsy; PT, prothrombin time of plasma; SWL, shock wave lithotripsy; TT, thrombin time of plasma.

Conclusion

Oxidative stress may induce changes of hemostasis in patients with nephrolithiasis, both before and after ESWL. In addition, changes of hemostasis parameters such as fibrinogen, blood platelet count and D-dimer level can be observed in these patients, especially after ESWL, and this may suggest that ESWL modulates hemostasis. By having a better understanding of the influence of ESWL on hemostasis, this could lead to modifying patient care for those patients at increased risk of bleeding.

Introduction

Hemostasis is the term given to a group of mechanisms which prevent the outflow of blood from blood vessels. It is also defined as a state of dynamic equilibrium between anti- and procoagulation reactions, which may be modulated by various factors, including oxidative stress. Many systems take part in hemostasis, including the wall of the blood vessel, the clotting process with its various factors, including fibrinogen, and the fibrinolytic and phagocyte systems [1]. Blood platelets are also very important element of hemostasis. Some papers note the presence of various hemostatic complications are observed in patients with nephrolithiasis after extracorporeal shock wave lithotripsy (ESWL) [2,3]. Moreover, other complications associated with ESWL (i.e. infection and sepsis) may exist, but ESWL is generally considered as a safe treatment. In addition, other methods (i.e. surgery and Flexible Ureterorenoscopy) for treatment of kidney stones also have effect on changes in hemostasis [4,5]. Hughes et al. [3] report changes in the levels of specific biomarkers of hemostasis, i.e. plasma fibrinogen, in patients after shock wave lithotripsy (SWL); however, the effect of SWL was measured on hemostasis parameters 30–240 minutes after SWL treatment.

It is very important that the ESWL results are dependent on several technical factors, including type of lithotripsy device, energy, and frequency of pulses, coupling of the patient to the lithotripter, location of calculus, and type of anesthesia. In addition, other factors related to the patients, stone size and density, skin to stone distance, anatomy of the excretory path, and kidney anomalies are also relevant. Moreover, ESWL treatment caused an increase in the free radical generation, and a decrease in the activity of antioxidant enzymes (i.e. superoxide dismutase and catalase) in parotid glands of ESWL-treated rats [6]. Gecit et al. [7] also observed oxidative stress in the levers and diaphragm muscles of ESWL-treated rats.

The aim of our present study was to determine whether oxidative stress may modulate the hemostatic activity of plasma in patients during ESWL (before ESWL and the day after treatment). To this end, selected hemostasis parameters were measured in patients with nephrolithiasis before ESWL and the day after treatment: the activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) of plasma, the level of fibrinogen, the level of D-dimer and blood platelet count. In addition, the levels of two biomarkers of oxidative damage to plasma proteins, protein carbonylation and the level of thiol groups in proteins, were also determined.

Materials and methods

Patients and samples

The blood samples were collected from 12 patients (eight men and four women; median age = 46) with similar socio-economic backgrounds who had been referred to the 2nd Department of Urology, Medical University of Lodz, Poland, for extracorporeal shock wave lithotripsy. Treatment

was given as per standardized protocol using SYSTEM Sonolith ® i-move device, with the mean energy 348 kV, frequency of pulses 2 Hz and 2000 pulses per procedure. The stones that were crushed were localized in the kidney and their size were from 10 to 20 mm. Before ESWL anatomy of the kidney and excretory path for all patients were investigated by intravenous urography. No anomalies were found. No kind of anesthesia was used. Demographic data and medical history were obtained at the entry of each patient to the study. A group of ten healthy individuals (six men and four women; median age = 48) who were present in the hospital for routine health checkups and used as controls. All controls were randomly-selected, non-related men and women who had never been diagnosed with nephrolithiasis nor chronic disease and were frequency matched to the cases on age. The blood and plasma samples were taken from patients and healthy participants who were eating a balanced diet of meat and vegetables and using no antioxidant supplementation. They had not taken any medications (i.e. aspirin or any other anti-platelet drugs or anti-inflammatory agents) or addictive substances (including tobacco, alcohol, antioxidant supplementation and aspirin or any other anti-platelet drugs).

The blood samples were collected from the patients before ESWL, and one day after ESWL. The plasma samples obtained from the participants, used for measuring the biomarkers of oxidative stress, were stored at -80˚C within two hours of being drawn. The protein concentration in the tested samples was calculated according to Bradford [8].

Ethical approval for this study was received from the Committee for Research on Human Subjects, Medical University of Lodz (RNN/101/13/KE).

Markers of oxidative stress

Carbonyl group measurement. The detection of carbonyl groups in proteins was carried out according to Levine et al. [9] and Bartosz [10]. The carbonyl group concentration was calculated using a molar extinction coefficient ($\varepsilon = 22{,}000\ M^{-1}cm^{-1}$), and the level of carbonyl groups was expressed as nmol carbonyl groups/mg of protein. Carbonyl content was determined by taking the SPECTROstar Nano Microplate Reader- BMG LABTECH Germany.

Thiol group determination. The thiol group content was measured spectrophotometrically (the SPECTROstar Nano Microplate Reader- BMG LABTECH Germany) by absorbance at 412 nm with Ellman's reagent: 5,5'-dithio-bis-(2-nitrobenzoic acid). The thiol group concentration was calculated using a molar extinction coefficient ($\varepsilon = 13{,}600\ M^{-1}cm^{-1}$) [10–12]. The level of thiol groups was expressed as nmol thiol groups/mg of plasma protein.

Parameters of hemostasis

The measurement of prothrombin time. The PT (seconds) was determined coagulometrically (BCS XP Healthcare Diagnostics Siemens, Germany) in citrated samples.

The measurement of thrombin time. The TT (seconds) was determined coagulometrically (BCS XP Healthcare Diagnostics Siemens, Germany) in citrated samples.

The measurement of APTT. The APTT (seconds) was determined coagulometrically (BCS XP Healthcare Diagnostics Siemens, Germany) in citrated samples.

The measurement of blood platelet concentration. Blood platelet count was performed using an automated cell counter (Sysmex XN-2000, Sysmex, Japan) in citrated samples. The platelets were measured in units x 10^9/l.

The measurement of fibrinogen. Fibrinogen (g/l) concentration (in citrated samples) was measured using an analyser (BCS XP Healthcare Diagnostics Siemens, Germany).

The measurement of D-dimer. D-dimer (ng/ml) concentration was determined by an analyser (BCS XP Healthcare Diagnostics Siemens, Germany) in citrated samples.

Statistical analysis

All the values in this study were expressed as mean ± SD and median. In order to eliminate uncertain data, the Q-Dixon test was performed. The distribution of the data was tested using the Kolmogorov-Smirnov test. Since the selected hemostasis parameters and biomarkers of oxidative stress were not normally distributed, the non-parametrical Mann-Whitney U-test was used for their analysis. The reported p-values were two-sided. Probabilities were considered significant when the p-value was lower than 0.05. All analyses were completed using Statistica software.

Results

Figs 1–4 present selected parameters of hemostasis in patients with nephrolithiasis before or after ESWL. Neither APTT, PT nor TT were influenced by the presence of nephrolithiasis, nor by treatment with ESWL ($p>0.05$) (Fig 1, S1 Table). However, fibrinogen concentration fell, with the plasma level from the nephrolithiasis patients before ESWL reaching about 25% of control group–healthy subjects levels ($p<0.05$) (Fig 2). The same trend was observed for blood platelet count ($p<0.05$) (Fig 4). On the other hand, the concentration of D-dimer in plasma from nephrolithiasis patients after ESWL was higher than that seen in plasma obtained from healthy volunteers ($p<0.01$) and from patients before ESWL ($p<0.01$) (Fig 3). In addition, blood platelet counts were about 20% lower in patients after ESWL than in those currently before ESWL ($p<0.05$) (Fig 4, S2 Table).

The concentration of thiol groups in plasma proteins from patients (before and after ESWL) was also found to be lower than the concentration of thiol groups in plasma obtained from healthy volunteers ($p<0.05$) (Fig 5). The concentration of thiol groups in plasma proteins from patients with nephrolithiasis (after ESWL) was also lower than in patients with nephrolithiasis (before ESWL); however, these changes were not statistically significant ($p>0.05$) (Fig 5). Contrary to the concentration of thiol groups, the carbonylation of plasma proteins from

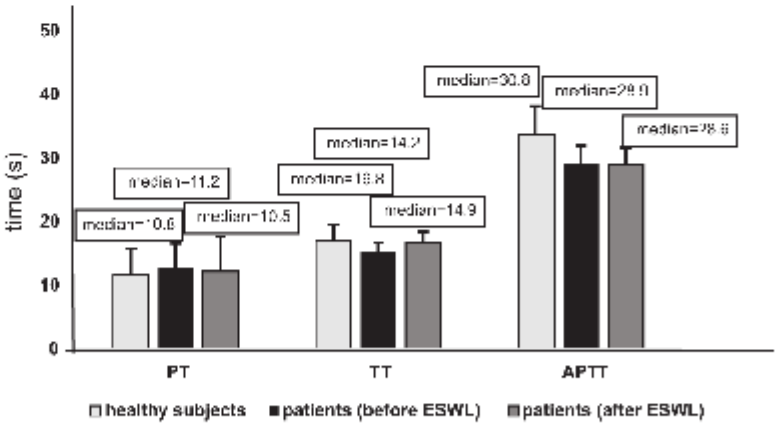

Fig 1. The activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) of plasma in patients with nephrolithiasis (before and after ESWL), and in control plasma obtained from healthy volunteers. Results are given as means ± SD, and median. The statistical analysis was performed using the Mann-Whitney test (for all times: $p>0.05$ –patients [(before/after ESWL] *versus* healthy subjects; $p>0.05$ patients [after ESWL] *versus* patients [(before ESWL]).

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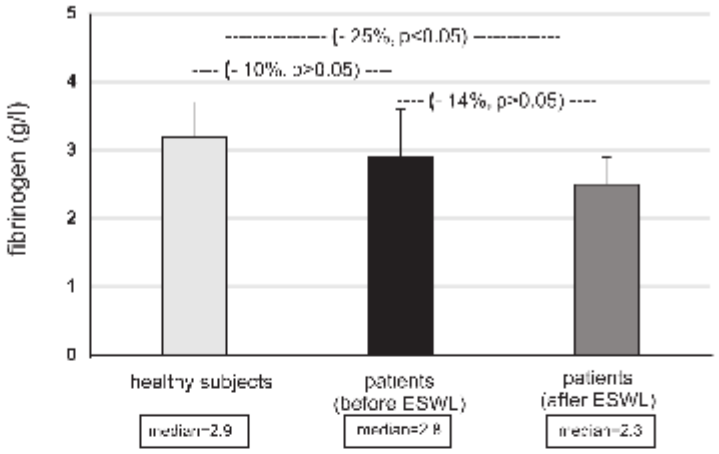

Fig 2. The level of fibrinogen in plasma from patients with nephrolithiasis (before and after ESWL), and in control plasma obtained from healthy volunteers. Results are given as means ± SD, and median. The statistical analysis was performed using the Mann-Whitney test.

https://doi.org/10.1371/journal.pone.0185157.g002

patients with nephrolithiasis (before and after ESWL) was significantly higher than in plasma proteins obtained from healthy volunteers ($p<0.02$) (Fig 6). However, the level of carbonyl groups in plasma proteins from patients after ESWL was not changed, compared with patients before ESWL ($p>0.05$) (Fig 6, S3 Table).

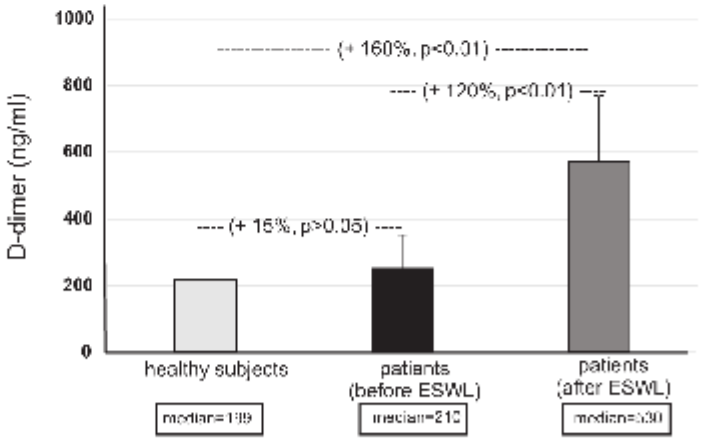

Fig 3. The level of D-dimer in plasma in patients with nephrolithiasis (before and after ESWL), and in control plasma obtained from healthy volunteers. Results are given as means ± SD, and median. The statistical analysis was performed using the Mann-Whitney test.

https://doi.org/10.1371/journal.pone.0185157.g003

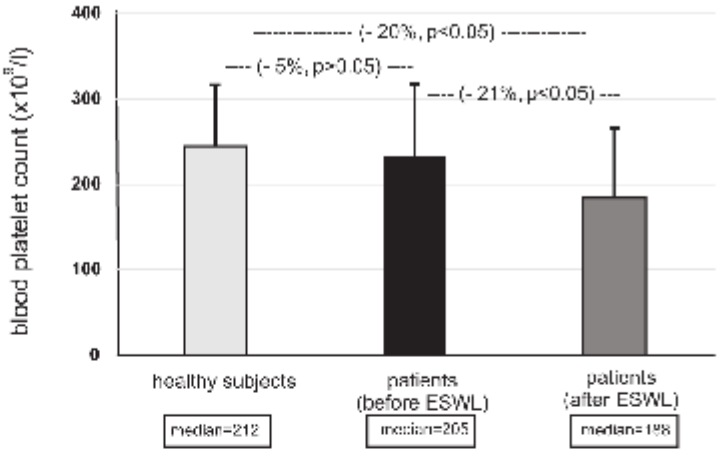

Fig 4. The level of blood platelets in patients with nephrolithiasis (before and after ESWL), and in healthy volunteers. Results are given as means ± SD, and median. The statistical analysis was performed using the Mann-Whitney test.

https://doi.org/10.1371/journal.pone.0185157.g004

Discussion

A few papers indicate that patients with nephrolithiasis are at increased risk of hemostatic complications, especially increased risk of coagulopathy, following SWL [2,3]. However, the pathogenesis of hemostasis in patients with nephrolithiasis, particularly after forms of

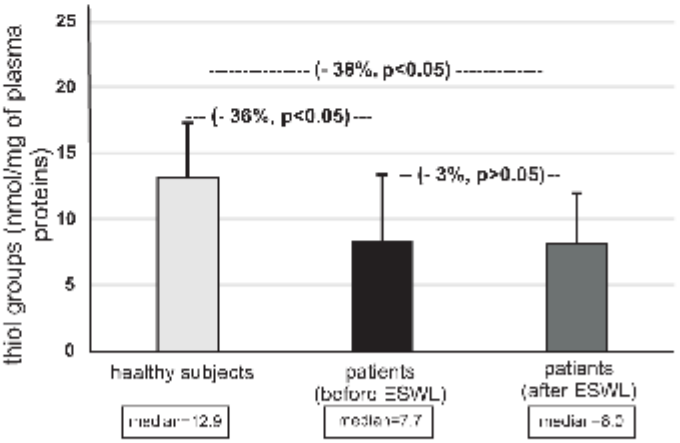

Fig 5. The level of thiol groups in plasma proteins in patients with nephrolithiasis (before and after ESWL), and in control plasma proteins obtained from healthy volunteers. Results are given as means ± SD, and median. The statistical analysis was performed using the Mann-Whitney test.

https://doi.org/10.1371/journal.pone.0185157.g005

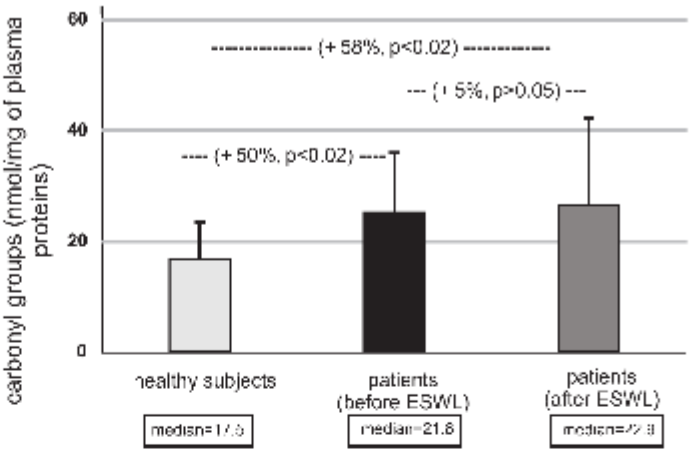

Fig 6. The level of carbonyl groups in plasma proteins in patients with nephrolithiasis (before and after ESWL), and in control plasma proteins obtained from healthy volunteers. Results are given as means ± SD, and median. The statistical analysis was performed using the Mann-Whitney test.

https://doi.org/10.1371/journal.pone.0185157.g006

treatment such as SWL, is not completely understood. Moreover, oxidative modifications of plasma proteins such as fibrinogen or other coagulation factors may lead to changes in hemostasis [1,13,14].

In vitro and *in vivo* experiments demonstrate that increased oxidative stress is correlated with kidney stone development [15–22]. Ma et al. [21] indicate that erythrocyte oxidative stress in patients with calcium oxalate stones correlates with stone size and renal tubular damage. A key novel finding of this study is that patients with nephrolithiasis undergoing ESWL experience an increase in oxidative stress, manifesting as oxidative damage to plasma proteins, as compared to healthy volunteers both before and after ESWL. However, no changes in the oxidative stress were found between patients before ESWL and those who had completed treatment, as measured by the levels of thiol groups and carbonyl groups in plasma proteins. It should be emphasized that the level of oxidative damage to plasma proteins was not found to be dependent on patient age. A recent study by Ceban et al. [22] reports an increased intensity of oxidative stress in patients with renal lithiasis compared to healthy individuals, demonstrated by various biomarkers, including the lowering of thiol groups in proteins. In addition, they note an improvement of these biomarkers after surgical treatment.

Various papers indicate that fibrinogen, a very important protein in the coagulation system, is much more vulnerable to oxidative modifications than others, such as albumin. In addition, oxidized fibrinogen may inhibit thrombin-catalyzed clot formation [1,13,14]. The present study demonstrates not only that oxidative damage is present in the plasma proteins (including fibrinogen) of patients with nephrolithiasis following ESWL, compared to healthy subjects, but also that fibrinogen concentration decreases.

Our present findings indicate a significant increase of another important marker of plasma hemostatic activity, i.e. D-dimer concentration, in nephrolithiasis patients following ESWL, compared to healthy subjects and patients undergoing ESWL. These observations were similar to those of Umekawa et al. [2] and Hughes et al. [3]. Umekawa et al. [2] report a correlation

between fibrin degradation products (FDP) and D-dimer concentration following SWL. Interestingly, our present findings do not include any significant changes in PT, TT or APTT in patients with nephrolithiasis, either before or after ESWL. Other authors [3] describe the same observations for PT and APPT in patients after SWL.

Together with the coagulation system and fibrinolysis, blood platelets are a very important element of hemostasis, whose main role is to form mechanical plugs during normal hemostasis. Our study demonstrated a significant decrease in blood platelet count in patients with nephrolithiasis both before and after ESWL. Our findings compliment those of Dedej et al. [4] and Hughes et al. [3]. Hughes et al. [3] report a decreased blood platelet count at 30, 120 and 240 min following SWL. They suggest that the changes of blood platelet count and fibrinogen observed when taking readings 30–240 min after SWL may be due to their redistribution in the body following renal trauma caused by SWL: blood platelet counts and fibrinogen will be consequentially increased in the kidney immediately after SWL, and decreased at the peripheral blood. It is also possible that a similar process exists in our present experimental model. Moreover, considering the data presented in this study, it is likely that the oxidative stress present in patients with nephrolithiasis, both before and after ESWL, may induce changes of hemostasis in these patients. Results of Moyes et al. [5] also demonstrated significant changes in haematology (i.e. the decrease of blood platelet count and fibrinogen concentration) and in biochemistry parameters, including the increase of APTT, following Flexible Ureterorenoscopy.

Further experiments based on larger groups of patients will be needed to determine the effect of oxidative modifications of plasma proteins on hemostatic abnormalities observed in patients with nephrolithiasis before and after ESWL. In addition, a deeper analysis of specific parameters of hemostasis, i.e. fibrinogen, D-dimer and blood platelet count, may provide valuable data on the hemostatic response following ESWL, and may decrease the risk of coagulopathy in patients with nephrolithiasis after ESWL. By having a better understanding of the influence of ESWL on hemostasis, this could lead to modifying patient care for those patients at increased risk of bleeding.

Supporting information

S1 Table. The activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) of plasma in patients with nephrolithiasis (before and after ESWL), and in control plasma obtained from healthy volunteers.
(TIF)

S2 Table. The level of fibrinogen and D-dimer in plasma and the level of blood platelets in patients with nephrolithiasis (before and after ESWL), and in control obtained from healthy volunteers.
(TIF)

S3 Table. The level of thiol groups and the level of carbonyl groups in plasma proteins in patients with nephrolithiasis (before and after ESWL), and in control plasma proteins obtained from healthy volunteers.
(TIF)

Acknowledgments

Special thanks goes to Igor Sokolovski (student, Faculty of Biology and Environmental Protection, University of Lodz) for skilled technical assistance.

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Research Article

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Future Medicinal Chemistry

The lipid peroxidation in patients with nephrolithiasis before and after extracorporeal shock wave lithotripsy

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Aim: To evaluate the level of lipid peroxidation in patients with nephrolithiasis before and after extracorporeal shock wave lithotripsy (ESWL). **Materials & methods:** Isoprostane concentration (8-isoPGF$_{2\alpha}$) was measured in urine, and thiobarbituric acid reactive substance production in serum and erythrocytes. In addition, the concentrations of selected compounds (uric acid, glucose and creatinine) were measured in serum. **Results:** The patients (before and after ESWL) demonstrated significantly higher levels of two different biomarkers of lipid peroxidation compared with the control group. A correlation was identified between increased amounts of uric acid and biomarkers of lipid peroxidation in patients with nephrolithiasis, both before and after ESWL. **Conclusion:** Uric acid may be associated with lipid peroxidation in patients with nephrolithiasis.

First draft submitted: 27 April 2018; Accepted for publication: 10 October 2018; Published online: 6 December 2018

Keywords: lipid peroxidation • nephrolithiasis • oxidative stress • uric acid

Some studies suggest that oxidative events may have great significance in patients with nephrolithiasis [1–8]; however, the mechanisms behind this process are not well known. Oxidative stress is correlated with lipid peroxidation, which can disrupt the organization of membranes, including mitochondrial membranes. Damage to mitochondria stimulated by lipid peroxidation can lead to further reactive oxygen species (ROS) production. In the presence of ROS, the double bonds of the unsaturated fatty acids constituting phospholipids are oxidized and various phospholipid aldehydes, including malonyldialdehyde and 4-hydroxy nonenal are formed [9,10]. Moreover, during ROS-induced oxidation of arachidonic acid, isoprostanes are formed [11–20]. It is important to note that isoprostanes are specific and reliable markers of lipid peroxidation *in vivo*. The aim of the present study was to determine the concentration of two biomarkers of lipid peroxidation, that is, 8-isoprostaglandin F_2 (8-isoPGF$_{2\alpha}$) and thiobarbituric acid reactive substances (TBARS), and selected compounds (uric acid, glucose and creatinine) in patients with nephrolithiasis, both before and after extracorporeal shock wave lithotripsy (ESWL). ESWL is one of the most popular and useful methods for the treatment of urolithiasis and may be employed for all types of stones. The treatment has been reported to have an 85–100% success rate [21]. In addition, extracorporeal shock wave lithotripsy is generally considered a safe form of treatment. However, adverse effects (such as reduced renal function, hypertension or sepsis) can occur [22–25]. Complication rates range from 0 to 20% [21]. Moreover, the oxidative stress mediated by ischemia reperfusion may contribute to kidney injury after ESWL [26,27]. In our present studies, the concentration of 8-isoPGF$_{2\alpha}$ was estimated in urine samples and measurement of these compounds in single sample represents daily secretion of isoprostanes. The concentration of selected compounds (uric acid, glucose and creatinine) was determined in serum. Moreover, we measured concentration of TBARS in serum and erythrocytes.

newlands press

10.4155/fmc-2018-0149 © 2018 Newlands Press *Future Med. Chem.* (Epub ahead of print) ISSN 1756-8919

Table 1. Demographic and medical characteristics of study subjects.

Characteristic of control and patients	Patients	Control
Simply size:	n = 30	n = 15
– Men	n = 13	n = 7
– Women	n = 17	n = 8
Age, years:	53.6 ± 11.4	44.6 ± 13.7
– Men	52.1 ± 10.4	42.7 ± 14.1
– Women	50.9 ± 9.8	45.8 ± 17.2

Materials & methods

Patients & samples

Thirty patients (13 men and 17 women; age: 53.6 ± 11.4) were recruited to the study. All patients had been referred to the II Department of Urology, Medical University of Lodz, Poland for ESWL, between 2016 and 2017. Samples of urine and blood were taken from all patients.

The inclusion criteria were as follows: the kidney stone formers were the first stone former (investigated by ultrasound and intravenous urography); nephrolithiasis visible on x-ray (calcium oxalate monohydrate, calcium oxalate dehydrate, calcium phosphates); the size of the stone from 8 to 20 mm (located in the upper urinary tract); and sterile urine culture. Moreover, the stone former subjects were on any medications for kidney stones, including uric acid-lowering drug.

The exclusion criteria were as follows: the presence of urinary tract infection; pregnant women and children; up-to-date taking oral anticoagulants or antiplatelet agents, and a positive interview in the direction of the bleeding diatheses; patients with cholelithiasis invisible in x-ray (radiolucent stones: uric acid, ammonium urate, xantine, drug-stones 2,8-dihydroxyadenine); obesity (severe obesity or BMI >30), severe skeletal malformations, arterial aneurysm and patients with diabetes.

Treatment (ESWL) was administered using Sonolith® i-move lithotripter, EDAP, France, according to standard procedure. The device had the following specifications: mean energy of 348 kV, pulse frequency 2 Hz and 2000 pulses per procedure. Anesthesia was not required. Data regarding patient demographics and medical history were provided by patients on entry to the study (Table 1).

To form a set of controls, a group of 15 healthy individuals (seven men and eight women; age: 44.6 ± 13.7) was formed (patients attending hospital for routine health examinations) (Table 1). All patients were unrelated, selected at random and were frequency matched to the cases with regard to age; in addition, none of the patients had any previous diagnosis of nephrolithiasis or chronic disease. The patients selected for urine, serum and erythrocyte sample collection maintained a balanced diet of meat and vegetables; all had a similar socio-economic background and used no antioxidant supplementation.

The blood samples were collected from the patients both before and 1 day after ESWL. Urine was only collected from the patients before ESWL (at the same time – at 8 am). The erythrocytes were separated from plasma by centrifugation (3300 × *g* for 10 min). The concentration of hemoglobin was determined by the cyano-hemoglobin method using Drabkin's reagent. Serum samples obtained from the participants were stored at -80°C within 2 h of removal. Participants also provided first morning void urine samples (50–100 ml), which was kept on ice and processed within 4 h.

The protocol was approved by the Committee for Research on Human Subjects of the Medical University of Łódź, Poland (RNN/101/13/KE).

Measurement of lipid peroxidation – the level of 8-isoPGF$_{2\alpha}$

The urine samples obtained from both patients and controls were assayed for 8-isoPGF$_{2\alpha}$ level using a Cayman Chemical immunoassay kit (Cayman Chemical, MI, USA) according to the manufacturer's instructions.

Measurement of lipid peroxidation – the level of TBARS

The serum and erythrocytes samples were added to an equal volume of cold 20% (v/v) trichloroacetic acid in 0.6 M HCl. Following this, the mixture was centrifuged at 6000 × *g* for 5 min. Five volumes of clear supernatant were then mixed with one volume of 0.12 M thiobarbituric acid in 0.26 M Tris (pH 7.0). The resulting mixture was then placed in a boiling water bath for 15 min. The absorbance at 535 nm was then measured (SPECTROstar

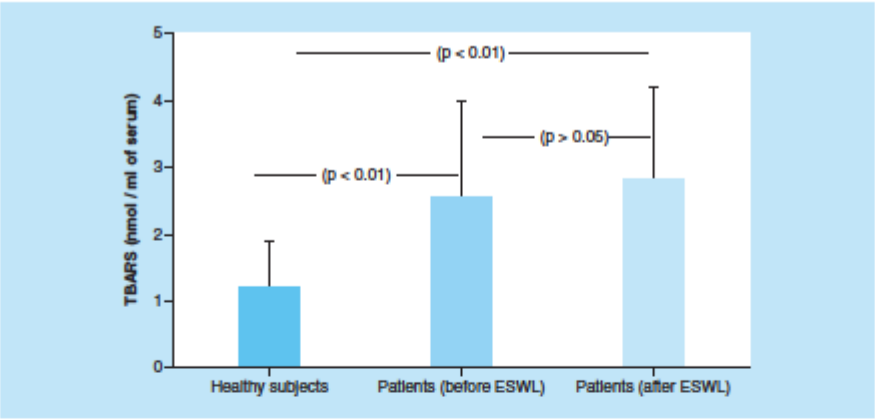

Figure 1. The level of thiobarbituric acid reactive substance in serum in patients with nephrolithiasis (before and after extracorporeal shock wave lithotripsy), and in control serum obtained from healthy volunteers. Results are mean ± standard deviation. The statistical analysis was performed with the Mann–Whitney test.
TBARS: Thiobarbituric acid reactive substances; ESWL: Extracorporeal shock wave lithotripsy.

Nano Microplate Reader, BMG LABTECH, Germany) [9,28]. The TBARS concentration was calculated using the molar extinction coefficient (ϵ = 156,000 $M^{-1}cm^{-1}$).

Measurement of uric acid, glucose & creatinine in serum

Uric acid, glucose and creatinine levels were measured using Abcam assay kits (Abcam, Cambridge, UK), according to the manufacturer's instructions.

Statistical analysis

All values are expressed as mean ± SD. To eliminate uncertain data, the Q-Dixon test was performed. The Kolmogorov–Smirnov test was used to determine the distribution of the levels of 8-iso$PGF_{2\alpha}$,TBARS, uric acid, glucose and creatinine; as they did not follow a normal distribution, nonparametrical tests (Mann–Whitney U-test, and the Spearman's rank correlation test) were applied. The Mann–Whitney U test was used to identify significant differences between sexes for both objectives. All p-values were two-sided. A p-value <0.05 was considered to indicate significance. Statistical software was used for all analyses.

Results

The concentrations of 8-iso-$PGF_{2\alpha}$ in urine and TBARS in serum and erythrocytes were found to be higher in patients with nephrolithiasis (before ESWL) than the control subjects (Figure 1, Tables 2 & 3). The concentration of TBARS was also higher in patients with nephrolithiasis (after ESWL) than control. However, no difference in TBARS concentration was observed between patients before ESWL and patients after ESWL (Figure 1). In addition, no differences were observed between men and women with regard to the concentrations of biomarkers of lipid peroxidation (8-iso-$PGF_{2\alpha}$ and TBARS) (Tables 2 & 3).

Higher levels of glucose, uric acid and creatinine were found in the serum of patients with nephrolithiasis (before ESWL) than of healthy subjects (Table 4). However, no such difference was observed between patients before ESWL and patients after ESWL (data not shown).

A correlation was found between an increased level of uric acid and changes in isoprostane or TBARS concentration in patients before and after ESWL (Table 5). However, no statistically significant correlation was found between patient age and the concentrations of other tested compounds or tested biomarkers of oxidative stress (data not shown).

Table 2. The level of 8-isoPGF$_{2\alpha}$ in urine in patients with nephrolithiasis (before extracorporeal shock wave lithotripsy), and in control urine obtained from healthy volunteers.

Characteristic of control and patients	Urine
	Level of 8-isoPGF$_{2\alpha}$ (pg/mg creatinine)
Control (A)	239.7 ± 45.2
Patients with nephrolithiasis (before ESWL) (B)	475.8 ± 111.7 (B vs A – $p < 0.05$)
Patients with nephrolithiasis (before ESWL)	
Sex:	
– Male (C)	434.9 ± 239.7
– Female (D)	483.9 ± 207.6 (D vs C – $p > 0.05$)

Results are mean ± standard deviation. The statistical analysis was done by Mann–Whitney test.
ESWL: Extracorporeal shock wave lithotripsy.

Table 3. The level of thiobarbituric acid reactive substance in serum and erythrocytes in patients with nephrolithiasis (before extracorporeal shock wave lithotripsy), and in control serum and erythrocytes obtained from healthy volunteers.

Characteristic of control and patients	Erythrocytes	Serum
	Level of TBARS (nmol/g Hb)	Level of TBARS (nmol/ml of serum)
Control (A)	26.4 ± 4.0	0.121 ± 0.069
Patients with nephrolithiasis (before ESWL) (B)	41.0 ± 7.5 (B vs. A – $p < 0.05$)	0.255 ± 0.142 (B vs. A – $p < 0.01$)
Patients with nephrolithiasis (before ESWL)		
Sex:		
– Male (C)	39.6 ± 6.8	0.276 ± 0.124
– Female (D)	42.4 ± 5.9 (D vs C – $p > 0.05$)	0.209 ± 0.199 (D vs C – $p > 0.05$)

Results are mean ± standard deviation. The statistical analysis was done by Mann–Whitney test.
ESWL: Extracorporeal shock wave lithotripsy; Hb: Hemoglobin; TBARS: Thiobarbituric acid reactive substance.

Table 4. The level of selected compounds (creatinine, glucose and uric acid) in serum from patients with nephrolithiasis [before extracorporeal shock wave lithotripsy]), and in control serum obtained from healthy volunteers.

Characteristic of control and patients	Level of creatinine (mg/dl)	Level of glucose (mg/dl)	Level of uric acid (mg/dl)
Control (A)	0.85 ± 0.16	85.9 ± 20.9	4.5 ± 2.0
Patients with nephrolithiasis (before ESWL) (B)	1.67 ± 1.23 (B vs A – $p < 0.05$)	116.7 ± 43.9 (B vs A – $p < 0.05$)	8.5 ± 4.3 (B vs A – $p < 0.05$)
Patients with nephrolithiasis (before ESWL)			
Sex:			
– Male (C)	2.22 ± 1.89	125.8 ± 47.9	8.7 ± 3.8
– Female (D)	2.78 ± 0.99 (D vs C – $p > 0.05$)	108.7 ± 30.4 (D vs C – $p > 0.05$)	7.7 ± 3.6 (D vs C – $p > 0.05$)

Results are mean ± standard deviation. The statistical analysis was done by Mann–Whitney test.
ESWL: Extracorporeal shock wave lithotripsy.

Table 5. The correlation between concentration of biomarkers of lipid peroxidation (8-isoPGF$_{2\alpha}$ and thiobarbituric acid reactive substance) and uric acid in patients with nephrolithiasis (before and after extracorporeal shock wave lithotripsy).

Patients with nephrolithiasis (before ESWL)
The correlation between the level of isoprostanes (pg/mg creatinine) and the level of uric acid (mg/dl)
$r = 0.532$ ($p < 0.05$)
The correlation between the level of TBARS (nmol/ml of serum) and the level of uric acid (mg/dl)
$r = 0.499$ ($p < 0.01$)
Patients with nephrolithiasis (after ESWL)
The correlation between the level of TBARS (nmol/ml of serum) and the level of uric acid (mg/dl)
$r = 0.478$ ($p < 0.05$)

ESWL: Extracorporeal shock wave lithotripsy; TBARS: Thiobarbituric acid reactive substance.

Discussion

The cumulative generation of ROS is known as oxidative stress. It may be indicated by different biomarkers, these being markers of lipid oxidative modification. One of the best-known manifestations of oxidative stress in cells and tissues is lipid peroxidation. A major final product of lipid peroxidation found in cells and tissues is TBARS, which can be used to evaluate the extent of oxidative stress [9,10,29–31].

Recent years have seen a number of studies which have used various biomarkers to investigate the role of oxidative stress in nephrolithiasis [1–8,32]. Their findings indicate that renal cellular injury and inflammation caused by ROS may play a role in idiopathic nephrolithiasis, as demonstrated by higher than normal levels of TBARS and other markers of oxidative stress [3,4,33]. Huang *et al.* [34] have indicated that lipid peroxidation correlates with urinary levels of oxalate, citric acid and osteopontin in patients with renal calcium oxalate stones. Moreover, increased oxidative stress has been found to be associated with kidney stone development, both *in vivo* and *in vitro* [6,33,35]. In addition, oxidative stress in erythrocytes (lipid peroxidation measured by malondialdehyde) in patients with calcium oxalate stones correlates with stone size and renal tubular damage [7]. Moreover, erythrocyte oxidative stress also correlated with urinary glutathione S-transferases.

Our present findings indicate that patients with nephrolithiasis exhibited oxidative stress in urine, serum and erythrocytes. In the present study, the urine level of isoprostanes, which is correlated as a reliable marker of oxidative stress in patients with nephrolithiasis was found to be over two-times higher than in control subjects. The TBARS generation assay found lipid peroxidation, in both serum and erythrocytes, to be significantly higher in patients with nephrolithiasis than in healthy control, suggesting that the serum and erythrocytes of patients with nephrolithiasis have a poor ability to scavenge the excess circulating ROS.

Treatment of renal stones relies on various surgical techniques. Ceban *et al.* [8] have demonstrated that the surgical treatment of complicated urolithiasis results in a decrease of oxidative stress and an increase in the potential of antiradical and antiperoxidative protection. In these studies, oxidative stress and antioxidant system changes were examined by different specific biochemical indices, including the concentration of TBARS, the concentration of thiol groups of proteins and the activity of various antioxidant enzymes, including glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase.

Our findings demonstrate for the first time that no difference in TBARS concentration exists between patients before ESWL and patients after ESWL. It should be underlined that in our present study, the level of oxidative stress in patients with nephrolithiasis was not dependent on the age of the patient. In addition, it has been previously demonstrated that oxidative damage to the plasma protein, measured by the degree of thiol groups and protein carbonylation, occurs in patients with nephrolithiasis, and ESWL does not appear to have any effect on this damage [36]. In addition, some authors have reported that ESWL causes oxidative stress in various rat models [37,38].

Preclinical and clinical experiments have demonstrated link between kidney stone formation and cardiovascular diseases. Cardiovascular disease risk factors, especially cholesterol, phospholipids and uric acid contribute to kidney stone formation [39]. Kovacevic *et al.* [40] have also observed association between nephrolithiasis and cardiovascular disease in children. Moreover, the patients with nephrolithiasis were found to display altered hemostasis parameters, including blood platelet count, fibrinogen and D-dimer concentration, especially after ESWL [36]. On the other hand, our earlier results have demonstrated that other hemostatic parameters (the activated partial thromboplastin time, the prothrombin time and the thrombin time of plasma) are not changed in patients with nephrolithiasis before and after ESWL, compared with healthy group.

Other experiments have shown that patients with nephrolithiasis not only demonstrate lipid peroxidation, but also oxidation of other biomolecules, including nucleic acids. Boonla *et al.* [6] have reported elevated excretion of urinary 8-hydroxydeoxyguanine, a marker of oxidative DNA damage, in nephrolithiasis patients. Urinary 8-hydroxydeoxyguanine was determined by competitive ELISA. The level of malondialdehyde, a biomarker of lipid peroxidation, was also evaluated; a greater level of renal tubular damage was found to be independently associated with elevated 8-hydroxydeoxyguanine in the urine. In this experiment, 30 healthy controls and 36 nephrolithiasis patients were recruited.

Our findings indicate that various tested compounds, including the serum level of uric acid are to be higher in patients with nephrolithiasis than the control group. Humbert and Stucker [41] and other authors [42,43] have reported that uric acid may play an important role in development and progression in chronic kidney diseases, opening a way for new therapeutic targets. However, uric acid may induce oxidative stress [44,45]. For example, Ahmadnezhad *et al.* [46] have noted that serum uric acid is associated with the pro-oxidant–antioxidant balance in

patients with metabolic syndrome. Recently, Cheng *et al.* [47] have studied the effect of different concentrations of uric acid on oxidative stress (using some biomarkers, including the activity of superoxide dismutase, the level of reduced glutathione and the level of TBARS) in cell model (*in vitro*). Model cells were treated with uric acid (5, 10, 20 and 30 mg/dl) for 24, 72 and 96 h. These authors have observed that uric acid has antioxidant properties in the L-02 hepatocyte cell line *in vitro* when applied at concentrations of 5 and 10 mg/dl, but increases the level of oxidative stress at the higher concentration of 30 mg/dl (for 48 h). Fabbrini *et al.* [48] have demonstrated that the level of serum uric acid (about 4.5 mg/dl) is associated with an increase in oxidative stress, assessed by measuring urinary isoprostanes (about 680 pg/mg creatinine) in obese subjects. It is also possible that uric acid may play an important role in the level of oxidative stress in patients with nephrolithiasis, as the nephrolithiasis patients examined in the present study displayed a correlation between an elevated concentration of uric acid and a greater degree of lipid peroxidation, expressed as urinary 8-iso-$PGF_{2\alpha}$ (the end product of peroxidation of arachidonic acid) and TBARS level; this was true for both patients before and after ESWL.

In addition, glucose is very unlikely to function as antioxidant, because it induces glycation, for example, from serum albumin, and the products of this process serve as oxidative markers. Therefore, we hypothesized that changes of glucose level may be associated with the oxidative stress in patients with nephrolithiasis. Our findings show that the concentration of glucose in patients with nephrolithiasis is also increased. However, no correlation was observed between increased amounts of glucose and changes in the concentration of biomarkers of oxidative stress.

Different studies demonstrate that diets with a higher intake of fruits and vegetables may play an important role in the prevention of kidney stones [49,50]. It is believed that the administration of exogenous antioxidants may be useful in preventing and treating nephrolithiasis [21]. Especially dietary phyto-phenolic compounds as antioxidants were found to be effective for the prevention of urolithiasis. Tracy *et al.* [33] have shown that daily pomegranate extract supplementation with 1000 mg phenolic compound extract (for 90 days) may reduce oxidative stress levels (including lipid peroxidation [measured by TBARS] and oxidation of nucleic acids [measured by urinary 8-hydroxydeoxyguanine]) in recurrent stone formers. Other authors also have demonstrated beneficial effect of pomegranate juice in prevention of nephrolithiasis [51–54]. Grases *et al.* [55] have indicated that phenolic compounds from grape seeds have the same effects. In this experiment, rats were treated with pure phenolic compounds (for example epicatechin) and grape seed extract (200 mg/l per day, for 16 days). Moreover, Amin *et al.* [56] have reported that animals receiving aqueous extract of saffron (25, 50 and 100 mg/kg, daily) exhibited lower lipid peroxidation, measured by TBARS level, as compared with lithiatic control group. Recently, results of Ahmad *et al.* [57] have shown that *Bergenia ciliata* possesses antioxidant and antiulcer properties. Phytochemical screening of *B. ciliate* demonstrated the presence of various compounds, including tannins, terpenoids, flavonoids and steroids, which may decide about therapeutic potential [57–59]. In addition, Azaryan *et al.* [60] have demonstrated that aqueous extract of *Cerasus avium* has a therapeutic action in rats with nephrolithiasis. In this experiment, two doses of aqueous extract (low dose and high dose: 200 and 400 mg/kg) were used. However, recently, Rodgers *et al.* [61] have reported that green tea from Japan and a Rooibos tea from South Africa, both rich in antioxidants, do not reduce the risk factor for stone formation, but this effect was observed in small sample sizes. Each subject drank two cups per day (125 ml each: the first at mid morning and the second at mid afternoon) for 30 days.

Qin *et al.* [62] have observed that losartan ameliorated renal crystallization by inhibiting oxidative stress and NADPH oxidase. Oxidative stress was evaluated by various biomarkers: urinary 8-hydroxydeoxyguanine, malonyldialdehyde and antioxidant enzymes: superoxide dismutase and catalase.

It is important that antioxidants may prevent shock wave-induced renal tubular oxidative stress [63,64].

These findings may suggest some clues for new treatment strategies with various food supplements or medications, which reduce not only the level of oxidative stress, but also the level of uric acid in patients with nephrolithiasis, before and after ESWL. In addition, considering the data presented in this study, it is likely that ESWL does not induce an increase of lipid peroxidation in patients with nephrolithiasis. Further studies based on larger groups of patients with nephrolithiasis, either before or after ESWL, or another form of treatment, will be needed to determine the effect of uric acid on the levels of various biomarkers of oxidative stress.

Conclusion & future perspective

The obtained results may help to better understand mechanism of oxidative stress, especially lipid peroxidation and the role of uric acid in this process, in patients with nephrolithiasis. In addition, the obtained results may also help monitoring side-effects of ESWL in patients with nephrolithiasis.

Summary points

- In patients with nephrolithiasis, dysregulation of reactive oxygen species metabolism is observed.
- Our results show an increased concentration of biomarkers of lipid peroxidation and uric acid in patients with nephrolithiasis in comparison with healthy group.
- Our results demonstrate there is no difference in lipid peroxidation between patients with nephrolithiasis before extracorporeal shock wave lithotripsy, and the day after extracorporeal shock wave lithotripsy.
- We suggest that the increased amount of uric acid may be associated with lipid peroxidation in patients with nephrolithiasis.

Acknowledgements

Special thanks goes to I Sokolovski (student, Faculty of Biology and Environmental Protection, University of Lodz, Poland) for skilled technical assistance.

Financial & competing interests disclosure

This work was supported by grant 506/1136 from the University of Lodz, Poland. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional Review Board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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ORIGINAL RESEARCH
published: 21 June 2019
doi: 10.3389/fphys.2019.00799

Oxidative Stress and Hemostatic Parameters in Patients With Nephrolithiasis Before and After Ureteroscopic Lithotripsy

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Purpose: In patients with nephrolithiasis, oxidative stress, especially lipid peroxidation is observed. Moreover, various invasive methods [including extracorporeal shock wave lithotripsy (ESWL)] for treatment of nephrolithiasis may induce not only the oxidative stress, but they may modulate hemostasis. The study was aimed to evaluate the oxidative damages of lipids and proteins in patients with nephrolithiasis (before and after ureteroscopic lithotripsy – URSL). The aim of the present study was also determine selected parameters of hemostasis in these patients.

Methods: 56 patients with nephrolithiasis and 49 healthy participants were included: 30 men and 26 women (for patient group); 27 men and 22 women (for healthy group). We measured the level of selected typical two biomarkers of oxidative modification of lipids [such as the production of thiobarbituric acid reactive substances (TBARS) and isoprostane concentration (8-isoPGF$_{2\alpha}$)] and two biomarkers of oxidative damages of proteins (carbonylation and the level of thiol groups) in patients with nephrolithiasis (before and after URSL). The following parameters of hemostasis were measured: blood platelet count, the level of fibrinogen and D-dimer, and coagulation times (the activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) of plasma).

Results: Different levels of plasma lipid peroxidation were observed in patients with nephrolithiasis before URSL and after URSL. However, no such difference in the level of oxidative damage to plasma proteins was observed. In addition, the tested hemostasis parameters were not influenced by the presence of nephrolithiasis, nor by treatment with URSL.

Conclusion: We suggest URSL does not induce the oxidative modifications of plasma proteins and does not change hemostatic parameters in patients with nephrolithiasis.

Keywords: hemostasis, nephrolithiasis, oxidative stress, ureteroscopic lithotripsy, extracorporeal shock wave lithotripsy

OPEN ACCESS

Edited by:
Ballamkonda K. Kishore, University of Utah Health Care, United States

Reviewed by:
Laith Farah Al-rabadi, University of Utah Hospital, United States
Alan Rao, Stanford University, United States

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Specialty section:
This article was submitted to Renal and Epithelial Physiology, a section of the journal Frontiers in Physiology

Received: *27 February 2019*
Accepted: *06 June 2019*
Published: *21 June 2019*

Citation:
Woźniak P, Kontek B, Skalski B, Król A, Różański W and Olas B (2019) Oxidative Stress and Hemostatic Parameters in Patients With Nephrolithiasis Before and After Ureteroscopic Lithotripsy. Front. Physiol. 10:799. doi: 10.3389/fphys.2019.00799

INTRODUCTION

Oxidative stress causes damage to different biomolecules – lipids, proteins and DNA. Moreover, oxidative stress may induce changes elements of hemostasis (including coagulation process and blood platelet activation) in various diseases. In recent years, various studies demonstrated that oxidative stress exists in patients with nephrolithiasis, however, the mechanism(s) of this process is not well known. It has been previously shown that especially lipid peroxidation [measured by different biomarkers: 8-isoprostaglandin F_2 (8-isoPGF$_{2\alpha}$) and thiobarbituric acid reactive substances (TBARS)] exists in these patients. Increase of lipid peroxidation was demonstrated in different biological materials (serum, plasma, erythrocytes, and urine) obtained from patients with nephrolithiasis (Khan, 2005, 2012, 2013a,b, 2014; Boonla et al., 2007; Ma et al., 2014; Ceban et al., 2016). In addition, various invasive methods [including extracorporeal shock wave lithotripsy (ESWL)] for treatment of nephrolithiasis may also induce the oxidative stress (Gecit et al., 2012; Garca et al., 2014; Wozniak et al., 2017, 2018) and modulate hemostasis (Wozniak et al., 2017). Modulation of hemostasis was observed 30–240 min after shock wave lithotripsy (SWL) (Hughes et al., 2015) and the day after ESWL (Wozniak et al., 2017).

Ureteroscopic lithotripsy (URSL) is one of the most common operations in urology. Moreover, it is and important that is an effective and safe method for managing ureteral stones (Seitz et al., 2007; Tao et al., 2015). Complications occur in less than 0.1% of cases (Nuttall et al., 2010). However, the effect of URSL on parameters of oxidative stress and hemostasis in patients with nephrolithiasis has not been studied yet. Thus, the main objective of our experiments was to examine the level of selected typical two biomarkers of oxidative modification of lipids (such as the production of TBARS and isoprostane concentration) and two biomarkers of oxidative damages of proteins (carbonylation and the level of thiol groups) in patients with nephrolithiasis (before and after URSL). Moreover, the aim of the present study was also determine selected parameters of hemostasis (blood platelet count, the level of fibrinogen and D-dimer, and coagulation times (the activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) of plasma) in these patients.

MATERIALS AND METHODS

Patients and Samples

The blood samples were collected from 56 patients (30 men and 26 women) whom had been referred to the 2nd Department of Urology, Medical University of Łódź, Poland, for URSL. The exclusion criteria were as follows: the presence of urinary tract infection, other inflammatory or malignant disease, diabetes mellitus, history of heart disease, or chronic kidney disease. Before URSL anatomy of the kidney and excretory path for all patients were investigated by intravenous urography, and no anomalies were found.

A group of 49 healthy individuals (27 men and 22 women) were collected from the hospital from routine controls of health and used as control. They were non-related men and women, that have never been diagnosed with nephrolithiasis or chronic disease and were randomly selected and frequency matched to the cases on age.

The blood and urine samples were collected from the patients before URSL, and 1 day after URSL. The blood samples and the urine samples were taken from patients and healthy participants keeping a balanced diet (meat and vegetables), with similar socio-economic background, using no antioxidant supplementation and any medications (f.e. anti-platelet drugs or anti-inflammatory agents).

Erythrocytes were separated from plasma by centrifugation (2800 × g for 10 min). Concentration of hemoglobin was determined by the cyanohemoglobin method using Drabkin's reagent. Participants provided also first morning void urine samples (50–100 ml), which was kept on ice and processed within 4 h. Plasma samples obtained from the participants were stored at −80°C within 2 h of removal.

The protocol was passed by the Committee for Research on Human Subjects of the Medical University of Łódź RNN/101/13/KE. The protocol was passed by the Committee for Research on Human Subjects of the Medical University of Łódź RNN/101/13/KE. The first, participants provided verbal consent to the researchers, and later participants provided written the documents. Authors had access to identifying participant information.

Parameters of Oxidative Stress

Measurement of Lipid Peroxidation – The Level of 8-isoPGF$_{2\alpha}$

The level of 8-isoPGF$_{2\alpha}$ was estimated in urine samples from control subjects and from patients using an immunoassay kit (Cayman Chemical) according to the manufacturer's instructions.

Measurement of Lipid Peroxidation – The Level of TBARS

Samples of plasma or erythrocytes were transferred to an equal volume of cold 20% (v/v) trichloroacetic acid in 0.6 M HCl and centrifuged at 1200 × g for 15 min. One volume of clear supernatant was mixed with 0.2 volume of 0.12 M thiobarbituric acid in 0.26 M Tris (pH 7.0) immersed in a boiling water bath for 15 min. and then absorbance was measured at 535 nm (the SPECTROstar Nano Microplate Reader – BMG LABTECH Germany) (Wachowicz, 1984; Bartosz, 2008). The TBARS concentration was calculated using the molar extinction coefficient (ε = 156,000 $M^{-1}cm^{-1}$).

Measurement of Protein Carbonylation

The detection of carbonyl groups in proteins was carried out according to Levine et al. (1990) and Bartosz (2008). The carbonyl group concentration was calculated using a molar extinction coefficient (ε = 22,000 $M^{-1}cm^{-1}$), and the level of carbonyl groups was expressed as nmol carbonyl

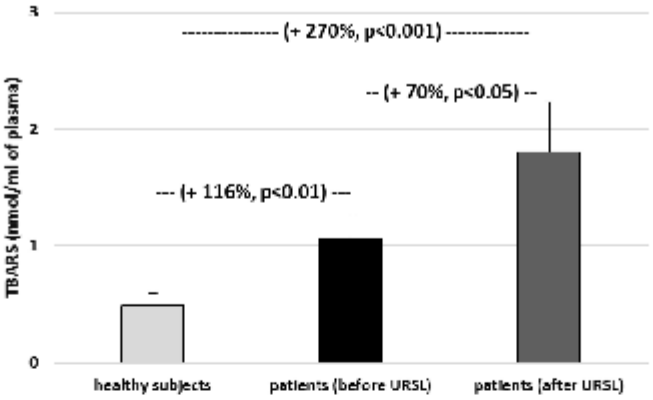

FIGURE 1 | The level of TBARS in plasma in patients with nephrolithiasis (before and after URSL), and in control plasma obtained from healthy volunteers. Results are means ± SD. The statistically analysis was done by Mann–Whitney test (for means ± SD).

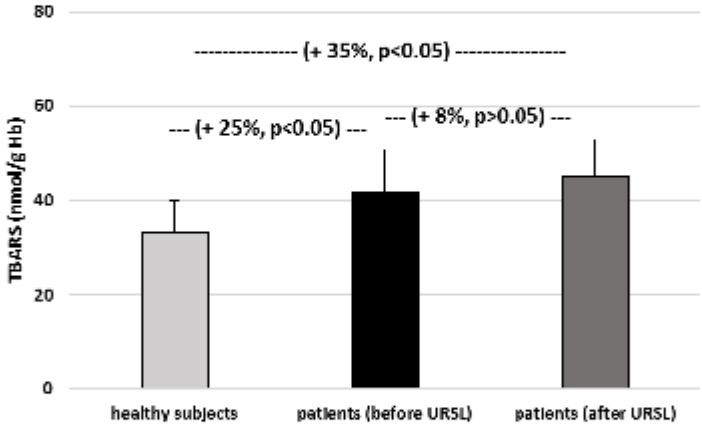

FIGURE 2 | The level of TBARS in erythrocytes in patients with nephrolithiasis (before and after URSL), and in control erythrocytes obtained from healthy volunteers. Results are means ± SD. The statistically analysis was done by Mann–Whitney test (for means ± SD).

groups/mg of protein. Carbonyl content was determined by taking the SPECTROstar Nano Microplate Reader – BMG LABTECH Germany.

Measurement of the Level of Thiol Groups

The thiol group content was measured spectrophotometrically (the SPECTROstar Nano Microplate Reader – BMG LABTECH Germany) by absorbance at 412 nm with Ellman's reagent: 5,5′-dithio-bis-(2-nitrobenzoic acid). The thiol group concentration was calculated using a molar extinction coefficient (ε = 13,600 $M^{-1}cm^{-1}$) (Ando and Steiner, 1973a,b; Bartosz, 2008). The level of thiol groups was expressed as nmol thiol groups/mg of plasma protein.

Parameters of Hemostasis

The Measurement of Prothrombin Time

The PT (seconds) was determined coagulometrically (BCS XP Healthcare Diagnostics Siemens, Germany) in citrated samples.

The Measurement of Thrombin Time

The TT (seconds) was determined coagulometrically (BCS XP Healthcare Diagnostics Siemens, Germany) in citrated samples.

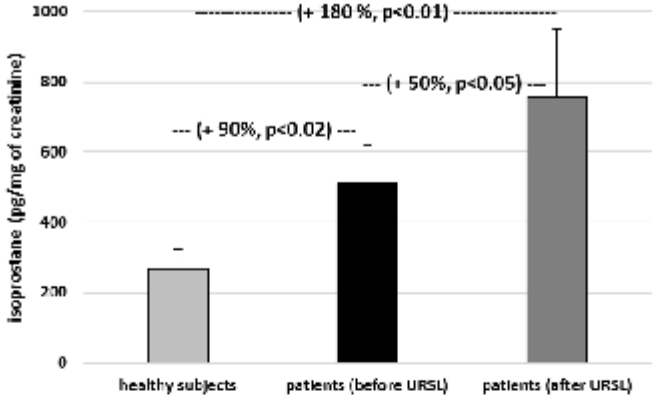

FIGURE 3 | The level of 8-isoPGF$_{2\alpha}$ in urine in patients with nephrolithiasis (before and after URSL), and in control urine obtained from healthy volunteers. Results are means ± SD. The statistically analysis was done by Mann–Whitney test (for means ± SD).

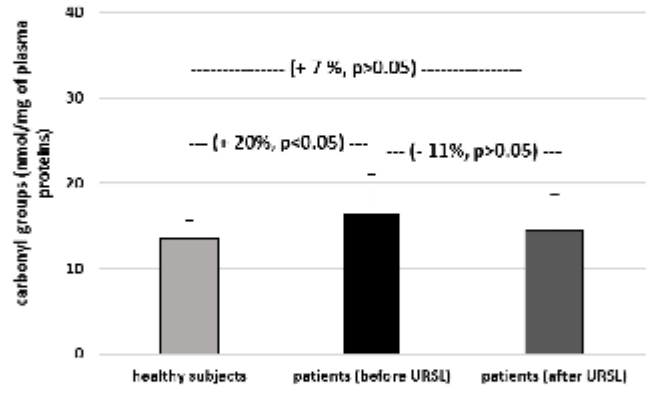

FIGURE 4 | The level of carbonyl groups in plasma proteins in patients with nephrolithiasis (before and after URSL), and in control plasma proteins obtained from healthy volunteers. Results are means ± SD. The statistically analysis was done by Mann–Whitney test (for means ± SD).

The Measurement of APTT

The APTT (seconds) was determined coagulometrically (BCS XP Healthcare Diagnostics Siemens, Germany) in citrated samples.

The Measurement of Blood Platelet Concentration

Blood platelet count was performed using an automated cell counter (Sysmex XN-2000, Sysmex, Japan) in citrated samples. The platelets were measured in units $\times 10^9$/l.

The Measurement of Fibrinogen

Fibrinogen (g/l) concentration (in citrated samples) was measured using an analyzer (BCS XP Healthcare Diagnostics Siemens, Germany).

The Measurement of D-Dimer

D-dimer (ng/ml) concentration was determined by an analyser (BCS XP Healthcare Diagnostics Siemens, Germany) in citrated samples.

Statistical Analysis

The statistical analysis was done by several tests. All the values in this study were expressed as mean ± SD. In order to eliminate uncertain data, Q-Dixon test was performed. Since levels of biomarkers of oxidative stress in studied material did not show normal distribution (Kolmogorov–Smirnov test) the non-parametrical statistical test (Mann-Whitney *U* test) was applied. Reported

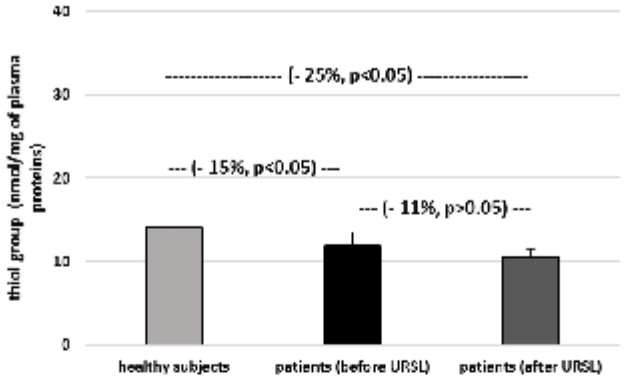

FIGURE 5 | The level of thiol groups in plasma proteins in patients with nephrolithiasis (before and after URSL), and in control plasma proteins obtained from healthy volunteers. Results are means ± SD. The statistically analysis was done by Mann–Whitney test (for means ± SD).

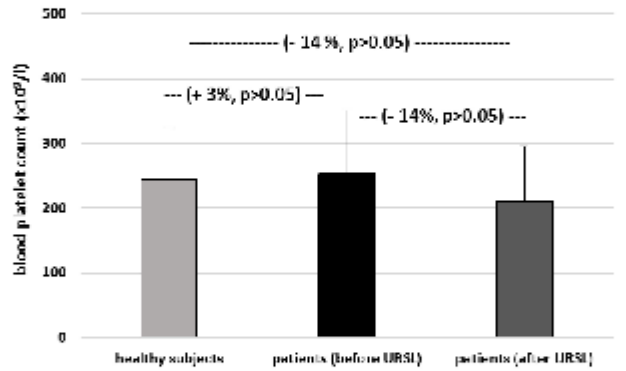

FIGURE 6 | The level of blood platelets in patients with nephrolithiasis (before and after URSL), and in from healthy volunteers. Results are means ± SD. The statistically analysis was done by Mann–Whitney test (for means ± SD).

p-values were two-sided. All analyses were completed using STATISTICA 12.

RESULTS

As demonstrated on **Figure 1**, the TBARS level in plasma was significantly increase in patients with nephrolithiasis (before URSL) than in control. In plasma samples from patients with nephrolithiasis (before URSL), the level of TBARS was increase by about 116% compare to control group (**Figure 1**). The concentration of TBARS in plasma from patients with nephrolithiasis (after URSL) was also higher than in patients with nephrolithiasis (before URSL) and higher than in healthy volunteers (**Figure 1**). In addition, the concentration of TBARS in erythrocytes in patients with nephrolithiasis (before URSL and after URSL) differed markedly from healthy volunteers (**Figure 2**). The TBARS content in erythrocytes in patients with nephrolithiasis (before URSL and after URSL) was significantly higher than in controls [and increase – about 25% (before URSL), and about 35% (after URSL)] (**Figure 2**). The level of TBARS in erythrocytes from patients after URSL was also changed, compared with patients before URSL, however, it was not statistically significant (**Figure 2**).

Other experiments showed that the concentration of isoprostane in urine from patients (before and after URSL) was found to be higher than the concentration of isoprostane in urine obtained from healthy volunteers (**Figure 3**). The concentration of isoprostane in urine from patients (after URSL) was also higher than in patients (before URSL) (**Figure 3**).

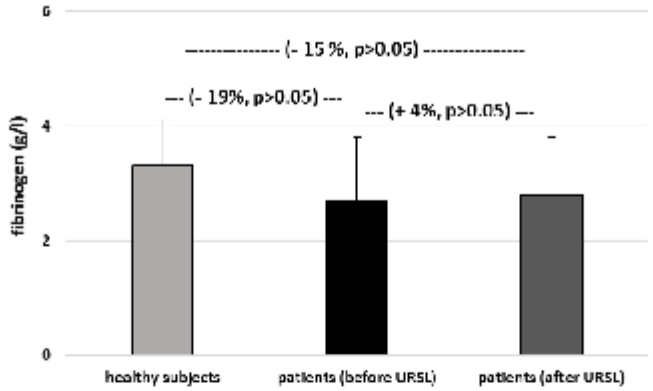

FIGURE 7 | The level of fibrinogen in plasma from patients with nephrolithiasis (before and after URSL), and in control plasma obtained from healthy volunteers. Results are means ± SD. The statistically analysis was done by Mann–Whitney test (for means ± SD).

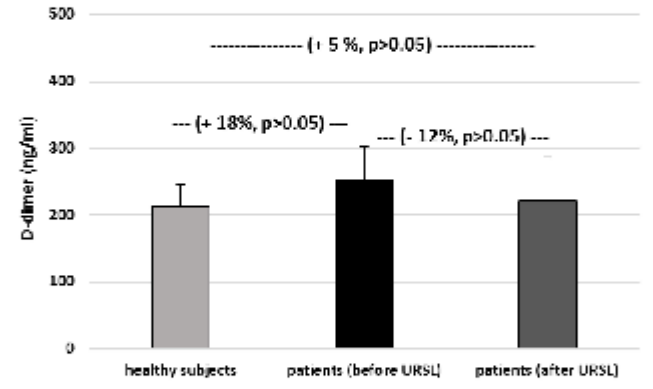

FIGURE 8 | The level of D-dimer in plasma in patients with nephrolithiasis (before and after URSL), and in control plasma obtained from healthy volunteers. Results are means ± SD. The statistically analysis was done by Mann–Whitney test (for means ± SD).

The level of carbonyl groups in plasma proteins from patients with nephrolithiasis (before and after URSL) was found to be higher than the level of carbonyl groups in plasma proteins obtained from healthy volunteers (**Figure 4**). On the other hand, the level of carbonyl groups in proteins from patients (after URSL) was lower than in proteins from patients (before URSL) (**Figure 4**). However, this change (in the concentration of carbonyl groups) was not statistically significant (**Figure 4**). **Figure 5** demonstrates that the level of thiol groups in proteins from patients (after URSL) was slightly lower than in proteins from patients (before URSL), but not statistically significant.

Figures 6–9 demonstrate parameters of hemostasis in patients with nephrolithiasis before and after URSL. For example, blood platelet counts were (about 14%) lower in patients after URSL than in healthy subjects or in patients before URSL, but these changes were not statistically significant (**Figure 6**). Neither, fibrinogen, D-dimer and coagulation times (APTT, PT, and TT) were influenced by the presence of nephrolithiasis, nor by treatment with URSL (**Figures 7–9**).

Moreover, we did not observe the difference in concentrations of biomarkers of oxidative stress between the group of men and the group of women.

DISCUSSION

Lipids and proteins are major targets for oxidants. Oxidative stress itself is known to play a role in nephrolithiasis through

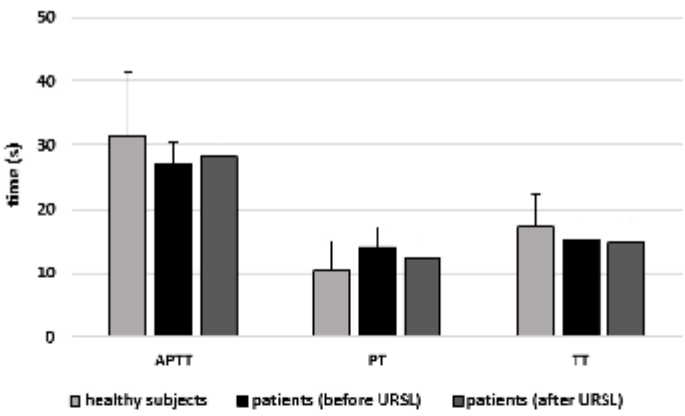

FIGURE 9 | The activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombin time (TT) of plasma in patients with nephrolithiasis (before and after URSL), and in control plasma obtained from healthy volunteers. Results are given as means ± SD. The statistical analysis was performed using the Mann–Whitney test (for all times: $p > 0.05$ – patients [(before/after URSL] *versus* healthy subjects; $p > 0.05$ patients [after ESWL] *versus* patients [(before URSL]).

the action of free radicals, which are believed to initiate the inflammation process and induce renal cellular injury (Khan, 2005, 2012, 2013a,b, 2014; Boonla et al., 2007; Ma et al., 2014; Ceban et al., 2016). In addition, increased oxidative stress may also be correlated with kidney stone development (Gaspar et al., 2010). For example, oxidative stress has been reported in the erythrocytes of patients with calcium oxalate stones, resulting in renal tubular damage and increases in stone size (Ma et al., 2014). Other studies suggest that urinary levels of oxalate and citric oxide may be correlated with lipid peroxidation (Huang et al., 2003). The present study by using different biomarkers of oxidative stress – damages of lipids, including the change of TBARS (in plasma and erythrocytes) and the change of isoprostane level in urine provides evidence that in patients with nephrolithiasis, oxidative stress – lipid peroxidation occurs. It should be underlined that in our present experiments tested samples from patients were taken before and after URSL. It is very important, because various drugs, which are used during treatment (i.e., surgery) may induce oxidative stress in different tissues, blood cells and plasma (Wang et al., 2017). However, Ceban et al. (2016) studied oxidant and antioxidant status in the blood of patients with complicated urolithiasis pre- and post-surgery. Oxidant and antioxidant status was measured also by various parameters, including the level of thiol groups and activity of different antioxidant enzymes (i.e., glutathione dismutase and glutathione reductase). Experiments of these authors demonstrated that the surgical treatment of complicated urolithiasis leads a decrease of the oxidative stress and an increase in potential of antioxidant status. On the other hand, *in vitro* and *in vivo* experiments show that increased oxidative stress is associated with kidney stone development (Khan, 2005, 2012, 2013a,b, 2014; Boonla et al., 2007; Ma et al., 2014; Ceban et al., 2016). Our earlier results indicate that ESWL also induces the oxidative stress (measured by the level of carbonyl groups in plasma proteins in patients with nephrolithiasis) and modulates hemostasis in these patients (Wozniak et al., 2017). A key novel finding of this experiment is that patients with nephrolithiasis undergoing other method for treatment – URSL, experience an increase in lipid peroxidation (measured by two typical biomarkers: TBARS and isoprostanes), as compared to healthy volunteers. Moreover, changes in lipid peroxidation were found between patients before URSL and patients who had completed treatment.

Hemostasis is thought to be modulated by oxidative stress (Nowak et al., 2010). However, in the present study, no changes in oxidative damage to plasma proteins, indicated by the levels of thiol groups and carbonyl groups, were observed in the nephrolithiasis patients before URSL and after URSL. In addition, a significant novel finding is that URSL does not appear to induce changes in hemostasis, measured by various typical parameters, including blood platelet count, fibrinogen concentration and coagulation times in these patients. The only possible explanation for this lack of observed change was that no oxidative damage had occurred to the plasma proteins. Hughes et al. (2015) also report that treatment of solitary kidney stones by SWL does not appear to influence the biochemical parameters of hemostasis, measured by coagulation times (APTT and PT).

This present study is the first to examine the effect of URSL on parameters of oxidative stress and hemostasis in patients with nephrolithiasis. Our findings indicate that URSL does not induce any oxidative modification in plasma proteins nor does it change the hemostatic parameters in these patients. However, the differences in levels of oxidative stress biomarkers are small, but they are statistically significant. Moreover, it is not

clear whether these statistical changes are clinically significant. Therefore, further experiments based on larger groups of patients are needed to more precisely determine its influence on oxidative stress and hemostasis.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The protocol was passed by the Committee for Research on Human Subjects of the Medical University of Łódź RNN/101/13/KE. The first, participants provided verbal consent to the researchers, and later participants provided written the documents. Authors had access to identifying participant information.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was supported by grant B1711000000044.01 from the University of Łódź.

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Woźniak, P., Kontek, B., Różański, W., and Olas, B. (2018). The lipid peroxidation in patients with nephrolithiasis before and after extracorporeal shock wave lithotripsy. *Future Med. Chem.* 10, 2685–2693. doi: 10.4155/fmc-2018-0149

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Podsumowanie

Materiał dysertacji stanowią trzy publikacje o łącznym **IF=9,584 (300 punktów MNiSzW)**

Praca nr 1

IF=2,766 (100 punktów MNiSzW)

Paweł Woźniak, Bogdan Kontek, Waldemar Różański, Beata Olas.

Evaluation of hemostasis parameters and the role of the oxidative damage to plasma proteins in the modulation of hemostasis in patients with nephrolithiasis before and after extracorporeal shock wave lithotripsy.

PLoS One 10:e0185157. doi: 10.1371/journal.pone.0185157 (2017)

W artykule oceniono wpływ metody ESWL na parametry hemostazy i wybrane markery stresu oksydacyjnego: poziom karbonylacji białek osocza i poziom grup tiolowych w białkach osocza. Nie zaobserwowano różnic pomiędzy pacjentami z kamicą nerkową przed i po ESWL oraz zdrowymi osobami z grupy kontrolnej w stosunku do czasów krzepnięcia: PT, TT i APTT. Natomiast stężenie fibrynogenu i liczba płytek krwi było niższe u pacjentów po zabiegu ESWL niż przed zabiegiem. Pacjenci z kamicą nerkową poddani zabiegowi mieli wyższe stężenie D-dimerów. Oceniany poziom markerów stresu oksydacyjnego okazał się wyższy u chorych z kamicą nerkową niż w grupie kontrolnej, jednak nie uległ istotnym zmianom po zabiegu ESWL. Stres oksydacyjny może indukować zmiany hemostazy u pacjentów z kamicą moczową zarówno przed, jak i po zabiegu ESWL. Zmiany parametrów hemostazy takie jak fibrynogen, liczba płytek krwi i D-dimery u pacjentów po ESWL mogą sugerować wpływ tego zabiegu na proces hemostazy.

Praca nr 2

IF=3,617 (100 punktów MNiSzW)

Paweł Woźniak, Bogdan Kontek, Waldemar Różański, Beata Olas.

The lipid peroxidation in patients with nephrolithiasis before and after extracorporeal shock wave lithotripsy.

Future Med. Chem. 10, 2685–2693. doi: 10.4155/fmc-2018-0149 (2018)

W przeprowadzonym badaniu oceniano poziom peroksydacji lipidów przed i po zabiegu ESWL u pacjentów z kamicą nerkową. Analizie poddano stężenie izoprostanów (8-isoPGF$_{2\alpha}$) oznaczanych w moczu i stężenie produktów peroksydacji lipidów z kwasem tiobarbiturowym (TBARS) w surowicy krwi i erytrocytach. Dodatkowo, badano stężenie kwasu moczowego, glukozy i kreatyniny w surowicy krwi. U chorych z kamicą nerkową zarówno przed, jak i po zabiegu ESWL obserwowano znacząco wyższy poziom dwóch różnych markerów peroksydacji lipidów i stężenia kwasu moczowego w porównaniu z grupą kontrolną. Nie stwierdzono różnicy w poziomie peroksydacji lipidów u chorych z kamicą nerkową przed zabiegiem ESWL w porównaniu z poziomem oznaczanym dzień po zabiegu. Podwyższony poziom kwasu moczowego może być związany z peroksydacją lipidów u chorych z kamicą nerkową.

Praca nr 3

IF=3,201 (100 punktów MNiSzW)

Paweł Woźniak, Bogdan Kontek, Bartosz Skalski, Anna Król, Waldemar Różański, Beata Olas.

Oxidative stress and hemostatic parameters in patients with nefhrolithiasis before and after ureteroscopic lithotripsy

Front. Physiol. 10:799. doi: 10.3389/fphys.2019.00799 (2019)

W publikacji poddano analizie zmiany oksydacyjne zachodzące w lipidach i białkach oraz parametry hemostazy chorych z kamicą moczową poddanych zabiegowi endoskopowego kruszenia złogów laserem holmowym (URSL). Podobnie jak w wyżej omówionych pracach peroksydacja lipidów określana była w moczu przy pomocy izoprostanów F_2 (8-iso$PGF_{2\alpha}$) i stężenia produktów peroksydacji lipidów z kwasem tiobarbiturowym (TBARS) w surowicy krwi i erytrocytach. Natomiast zmiany oksydacyjne w białkach na podstawie określenia ilości grup karbonylowych i tiolowych. Analizie poddano wybrane parametry hemostazy, takie jak: liczba płytek krwi, fibrynogen, D-dimery i czasy krzepnięcia: APTT, PT i TT.

Liczba płytek krwi była o 14% niższa u pacjentów po URSL w porównaniu do grupy kontrolnej i u pacjentów przed URSL, ale nie była to różnica istotna statystycznie. Żaden z pozostałych parametrów hemostazy i czasów krzepnięcia nie był zależny od kamicy nerkowej czy zabiegu URSL. Poziom TBARS w osoczu był znacząco wyższy u pacjentów z kamicą niż w grupie kontrolnej. U pacjentów przed URSL był o 116% wyższy w porównaniu do zdrowych ochotników, a po zabiegu wzrósł o kolejne 70%. Również poziom TBARS w erytrocytach u chorych na kamicę moczową różnił się znacząco od grupy kontrolnej, odpowiednio wyższy o 25% przed URSL i 35% po URSL. Badanie stężenia izoprostanów w moczu wykazało wyższe wartości u chorych w porównaniu do zdrowych ochotników i wyższe stężenie u pacjentów po URSL niż przed.

Poziom grup karbonylowych w białkach osocza pacjentów z kamicą moczową przed i po URSL był wyższy niż w grupie kontrolnej. Jednakże u pacjentów po URSL był niższy od pacjentów przed URSL, różnica ta nie była istotna statystycznie. Stężenie grup tiolowych w białkach osocza było niższe u chorych po URSL niż u chorych przed URSL, ale statystycznie nieistotne.

Wnioski

Lipidy i białka są głównym celem oksydantów. Stres oksydacyjny za pośrednictwem wolnych rodników wpływa na rozwój kamicy nerkowej w mechanizmie aktywacji procesu zapalnego i uszkodzenia komórek nerkowych[21,22,23,24]. Jak donoszą inne badania, poziom stresu oksydacyjnego mierzonego w erytrocytach chorych na kamicę szczawianowo-wapniową koreluje ze stopniem uszkodzenia kanalików nerkowych i wielkością kamienia[25].

Zaprezentowane w rozprawie doktorskiej badania oparte o dwa niezależne markery stresu oksydacyjnego lipidów - izoprostany w moczu i TBARS (w surowicy i erytrocytach) wykazały, że u pacjentów z kamicą nerkową poziom stresu oksydacyjnego jest znacząco wyższy zarówno w grupie chorych poddanych ESWL, jaki i URSL.

Poziom ocenianych markerów stresu oksydacyjnego lipidów nie uległ istotnym zmianom po zabiegu ESWL, natomiast znacząco wzrósł po zabiegu URSL. Stężenie izoprostanów w moczu okazało się wyższe u chorych w porównaniu do zdrowych ochotników i wyższe u pacjentów po URSL niż przed zabiegiem.

Wybrane w badaniu do oceny oksydacji białek grupy tiolowe i karbonylowe zarówno u chorych leczonych na drodze ESWL, jak i URSL nie wykazały istotnych statystycznie zmian.

Stres oksydacyjny może indukować zmiany hemostazy u pacjentów z kamicą moczową zarówno przed, jak i po zabiegu ESWL. Zmiany parametrów hemostazy takich jak spadek stężenia fibrynogenu i liczby płytek krwi oraz wzrost wartości D-dimerów u pacjentów po ESWL mogą sugerować wpływ tego zabiegu na hemostazę. Wyniki te nasuwają zachowanie szczególnej ostrożności w leczeniu kamicy na drodze ESWL u pacjentów z chorobą niedokrwienną serca, po zatorowości płucnej czy zakrzepicy kończyn dolnych, zwłaszcza przyjmujących leki przeciwpłytkowe. W świetle przeprowadzonych badań ryzyko powikłań krwotocznych wydaje się być większe u chorych poddanych ESWL, a litotrypsja za pomocą endoskopowej ureterorenoskopii bardziej odpowiednia.

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