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ROZPRAWA DOKTORSKA

Znaczenie aktywności selenoproteinaz i stężenia renalazy we krwi w patogenezie nadciśnienia tętniczego i obturacyjnego bezdechu sennego

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Podziękowania

Pragnę złożyć szczególne podziękowania
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Dziękuję ***Rodzinie i Przyjaciołom*** *za cierpliwość, motywację i nieustanną wiarę w moje możliwości.*

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WYKAZ SKRÓTÓW UŻYTYCH W PRACY

ABPM (*ang. ambulatory blood pressure monitoring*)
24-godzinne ambulatoryjne monitorowanie ciśnienia tętniczego

AHI (*ang. apnea/hypopnea index*) wskaźnik bezdechu/spłycenia oddechu

BMI (*ang. body mass index*) wskaźnik masy ciała

FAD (*ang. flavin adenine dinucleotide*) dinukleotyd flawinoadeninowy

HTN (*ang. hypertension, HTN*) nadciśnienie tętnicze

LVEF% (*ang. left ventricle ejection fraction*) frakcja wyrzutowa lewej komory

LVH (*ang. left ventricular hypertrophy*) przerost lewej komory

LVM (*ang. left ventricular mass)* masa lewej komory

LVMI (*ang. left ventricular mass index*) wskaźnik masy lewej komory

MAO-A (*ang. monoamine oxidase-A*) monoaminooksydaza A

MAO-B (*ang. monoamine oxidase-*B) monoaminooksydaza B

mBP (*ang. mean blood pressure*) średnie ciśnienie tętnicze

OSA (*ang. obstructive sleep apnea*) obturacyjny bezdech senny

PP (*ang. pulse pressure)* ciśnienie tętna

RWT (*ang. relative wall thickness*) względna grubość ściany lewej komory

SNP (*ang. single nucleotide plymorphism*) polimorfizm pojedynczego nukleotydu

TAS (*ang. Total Antioxidant Status*) całkowity status antyoksydacyjny

1,2-dihydroNAD(P) (*ang. 1,2-dihydro nicotinamide adenine dinucleotide [phosphate]*), 1,2-dihydro fosforan dinukleotydu nikotynoamidoadeninowego

1,4-dihydroNAD(P) (*ang. 1,4-dihydro nicotinamide adenine dinucleotide [phosphate]***)**, 1,4-dihydro fosforan dinukleotydu nikotynoamidoadeninowego

1,6-dihydroNAD(P) (*ang. 1,6-dihydro nicotinamide adenine dinucleotide [phosphate]*), 1,6-dihydro fosforan dinukleotydu nikotynoamidoadeninowego

WPROWADZENIE

Nadciśnienie tętnicze (ang. *hypertension*, HTN) pozostaje najważniejszym czynnikiem ryzyka chorób układu krążenia, a według Światowej Organizacji Zdrowia jest wciąż pierwszą przyczyną przedwczesnych zgonów na świecie [1]. Stale podwyższone wartości ciśnienia tętniczego prowadzą do uszkodzenia struktur układu krwionośnego, rozwoju subklinicznych powikłań narządowych, a w dalszej kolejności do rozwoju chorób sercowo-naczyniowych, takich jak niewydolność serca, zawał serca, czy udar mózgu [2]. Podstawową metodą stosowaną w diagnostyce nadciśnienia tętniczego są pomiary ciśnienia tętniczego wykonane w gabinecie lekarskim. Pomiar gabinetowy to metoda względnie tania i szerokodostępna, jednak wiąże się z ryzykiem nierozpoznania nadciśnienia tętniczego maskowanego lub rozpoznania nadciśnienia, które wynika wyłącznie z efektu białego fartucha [3]. Badaniem, które pozwala na bardziej wiarygodną ocenę jest 24-godzinne ambulatoryjne monitorowanie ciśnienia tętniczego (ang. ABPM – *ambulatory blood pressure monitoring*). Metoda ta polega na wielokrotnym odczycie wartości ciśnienia przy użyciu walidowanego sprzętu w naturalnym środowisku pacjenta [4].

Kluczowym elementem w ograniczeniu śmiertelności z przyczyn kardiologicznych jest wczesne wykrycie nadciśnienia tętniczego i wdrożenie odpowiedniego leczenia. Preferowana terapia ustalana jest w oparciu o stwierdzane wartości ciśnienia oraz ocenę całkowitego ryzyka sercowo-naczyniowego. Stratyfikacja ryzyka obejmuje czynniki genetyczne, czynniki związane ze stylem życia i środowiskiem zewnętrznym, a także ocenę subklinicznych uszkodzeń narządowych m.in. podwyższonego ciśnienia tętna (ang. PP – *pulse pressure)* i przerostu lewej komory (ang. LVH – *left ventricular hypertrophy*) [3]. Kalkulacji wartości PP można dokonać na podstawie pomiaru gabinetowego, aczkolwiek bardziej obiektywne wartości wynikają z ABPM. Badania naukowe wskazują, że ciśnienie tętnicze oraz PP mierzone za pomocą ABPM korelują z zachorowalnością i śmiertelnością z powodu chorób sercowo-naczyniowych i mózgowo-naczyniowych [5]. PP jest również uznawane za niezależny czynnik ryzyka przebudowy oraz przerostu lewej komory [6].

Dokładna ocena LVH w warunkach podstawowej opieki zdrowotnej może być trudna. Istnieją liczne kryteria możliwe do zastosowania w badaniu elektrokardiograficznym, jednak różnią się one dokładnością i mają dość niską czułość, często poniżej 50% [7]. Preferowaną metodą oceny geometrii oraz funkcji lewej komory jest echokardiografia [8]. Wśród pacjentów z HTN służy jako miarodajny test przesiewowy w kierunku specyficznych powikłań sercowo-naczyniowych, takich jak LVH, dysfunkcja rozkurczowa lewej komory oraz powiększenie lewego przedsionka. Wyżej wymienione zmiany w morfologii i funkcji serca sprzyjają

zachorowalności i śmiertelności z przyczyn sercowo-naczyniowych [9]. Należy podkreślić, że przerost koncentryczny wiąże się z największą śmiertelnością w porównaniu do innych typów geometrii lewej komory [10]. Ten typ przerostu charakteryzuje się podwyższoną względną grubością ściany (ang. *relative wall thickness, RWT*) i zwiększoną zindeksowaną masą lewej komory (ang. *left ventricular mass index, LVMI*) w badaniu echokardiograficznym.

Obturacyjny bezdech senny (ang. *obstructive sleep apnea,* OSA) to choroba, w której podczas snu dochodzi do powtarzających się epizodów bezdechu i/lub spłyconego oddechu [11]. Nasilenie OSA określa wskaźnik bezdechów i spłyconych oddechów (ang. *apnea-hypopnea index*, AHI), który podsumowuje liczbę zdarzeń oddechowych występujących w ciągu godziny. Rozpoznanie OSA stawiane jest, gdy AHI wynosi pięć lub więcej. Pacjenci z OSA są grupą szczególnego ryzyka zarówno pod względem zachorowania, jak i śmiertelności z powodu chorób układu krążenia [12]. Zaburzona fizjologia snu i powtarzające się epizody bezdechu skutkują nadmierną aktywacją współczulną oraz nasilonym stanem zapalnym, co stanowi znamienną przyczynę wzrostu ryzyka sercowo-naczyniowego w tej grupie pacjentów [13].

Zaburzenia równowagi redoks są uznawane za jeden z istotnych elementów w patogenezie chorób sercowo-naczyniowych. Obniżenie potencjału antyoksydacyjnego stwierdzano w przebiegu takich schorzeń jak nadciśnienie tętnicze, czy choroba naczyń obwodowych [14, 15]. W związku z powyższym, czynniki o charakterze przeciwutleniającym są często proponowane jako możliwe do zastosowania w profilaktyce, diagnostyce lub terapii chorób układu krążenia [16-19].

Selenoproteiny to grupa białek zawierających przynajmniej jedną resztę selenocysteiny. Do grupy tej, wraz z peroksydazą glutationową, reduktazą tioredoksyny i dejodynazą jodotyroniny, należy selenoproteina P [20]. Jest to białko, które w przeciwieństwie do innych selenoprotein zawiera 10 reszt selenocysteiny. Jedna N- końcowa reszta selenocysteiny znajduje się w domenie katalitycznej odpowiedzialnej za aktywność enzymatyczną, podczas gdy dziewięć C-końcowych reszt funkcjonuje jako magazyn selenu dla tkanek pozawątrobowych [21,22]. Istnieją doniesienia na temat potencjału antyoksydacyjnego selenoproteiny P, jednak dokładny mechanizm jej działania w utrzymaniu równowagi redoks pozostaje nieznany [23,24]. Brakuje również doniesień na temat jej udziału w patogenezie chorób układu krążenia.

Renalaza jest białkiem klasyfikowanym jako oksydaza zależna od dinukleotydu flawinoadeninowego (*ang. flavin adenine dinucleotide*, FAD) [25]. Za jej funkcję początkowo uznawano degradację krążących we krwi katecholamin [25,26], jednak założenie to okazało się błędne. W kolejnych latach ustalono, że substratami dla renalazy są izomery formy natywnej

1,4-dihydro fosforanu dinukleotydu nikotynoamidoadeninowego (1,4-dihydroNAD(P)). Renalaza katalizuje reakcję ich utlenienia i powrotu do formy natywnej poprzez formowanie β-NAD(P)+ [26]. W badaniach *in vitro* ustalono, że wspomniane izomery powstają przez spontaniczną nie-enzymatyczną redukcję lub tautomeryzację formy natywnej i są potencjalnie szkodliwe dla funkcjonowania komórki [27].

Peroksyredoksyny to rodzina peroksydaz zaangażowanych w utrzymanie równowagi tiolowej [28]. Istnieją doniesienia sugerujące ich korzystny wpływ na stan układu krążenia i zmniejszenie ryzyka sercowo-naczyniowego [29-32]. Większość badań koncentruje się jednak na peroksyredoksynie-1. Peroksyredoksyna-5 jest najpóźniej odkrytym przedstawicielem rodziny peroksyredoksyn, a jej wpływ na układ krążenia wciąż nie został dokładnie poznany.

W utrzymanie równowagi redoks zaangażowane są zarówno antyoksydanty endogenne, jak i egzogenne. Do określenia całkowitej aktywności antyoksydacyjnej stosowane są odpowiednie testy, w tym test TAS (ang. *Total Antioxidant Status*). Zastosowanie testu TAS jest użyteczną miarą informacji biologicznej, pozwala na określenie połączonego działania wszystkich przeciwutleniaczy obecnych w badanym materiale biologicznym [33].

ZAŁOŻENIA I CELE PRACY

Cel główny rozprawy stanowi weryfikacja założenia, że stężenia selenoproteiny P, renalazy i peroksyredoksyny-5 we krwi mają znaczenie w patogenezie nadciśnienia tętniczego i obturacyjnego bezdechu sennego.

Ponadto celem rozprawy była weryfikacja poniższych założeń szczegółowych:

1. Istnieje zależność pomiędzy stężeniem selenoproteiny P, peroksyredoksyny-5 a stężeniem renalazy.
2. Istnieje zależność pomiędzy stężeniem selenoproteiny P a całkowitym potencjałem antyoksydacyjnym (TAS).
3. Istnieje zależność pomiędzy stężeniem renalazy a całkowitym potencjałem antyoksydacyjnym (TAS).
4. Istnieje zależność pomiędzy stężeniem peroksyredoksyny-5 a całkowitym potencjałem antyoksydacyjnym (TAS).
5. Istnieje zależność pomiędzy stężeniem selenoproteiny P a występowaniem subklinicznych powikłań narządowych nadciśnienia tętniczego.
6. Istnieje zależność pomiędzy stężeniem peroksyredoksyny-5 a występowaniem subklinicznych powikłań narządowych nadciśnienia tętniczego.
7. Istnieje zależność pomiędzy stężeniem renalazy a występowaniem subklinicznych powikłań narządowych nadciśnienia tętniczego.

Powyższe cele znalazły odzwierciedlenie w celach poszczególnych prac cyklu:

Cel pracy nr 1: przegląd aktualnych doniesień na temat renalazy, a w szczególności jej funkcji enzymatycznej oraz nie-enzymatycznej.

Cel pracy nr 2: poznanie związku pomiędzy stężeniem selenoproteiny P, peroksyredoksyny-5 oraz renalazy we krwi, a całkowitym statusem antyoksydacyjnym, średnim ciśnieniem tętniczym oraz wskaźnikiem bezdechów i spłyconych oddechów.

Cel pracy nr 3: ocena związku pomiędzy stężeniem selenoproteiny P, peroksyredoksyny-5 i renalazy a wybranymi konsekwencjami sercowo-naczyniowymi ocenianymi w 24-godzinnym ambulatoryjnym monitorowaniu ciśnienia tętniczego oraz w echokardiografii.

GRUPA BADANA I METODY

Publikacja nr 1 – praca przeglądowa

Przegląd literatury przeprowadzono w bazie PubMed, Scopus oraz GoogleScholar. Wyszukiwanie ograniczono do artykułów opublikowanych w latach 2005-2020. Użyto następujących słów kluczowych: renalaza, oksydaza monoaminowa, enzym, polimorfizm, cytokina. Spośród wszystkich znalezionych artykułów tylko 43 skupiały się na temacie według tytułu i streszczenia. Te dokumenty przeanalizowano i podzielono na trzy obszary: funkcja enzymatyczna renalazy (16 rekordów), polimorfizm renalazy (16 rekordów), nie-enzymatyczna aktywność renalazy (11 rekordów).

Publikacja nr 2 – praca oryginalna

Grupa badana i metody

Grupę badanych stanowiło 112 pacjentów hospitalizowanych celem weryfikacji diagnozy obturacyjnego bezdechu sennego w Katedrze i Klinice Chorób Wewnętrznych, Zawodowych, Nadciśnienia Tętniczego i Onkologii Klinicznej we Wrocławiu.

Do wykonanych procedur zaliczono: badanie polisomnograficzne, pomiar ciśnienia tętniczego metodą Korotkowa oraz badania laboratoryjne - pomiar stężenia selenoproteiny P, renalazy i peroksyredoksyny-5, a także ocenę całkowitego statusu antyoksydacyjnego (TAS). Całonocne badanie polisomnograficzne wykonano w certyfikowanym Laboratorium snu Katedry i Kliniki Chorób Wewnętrznych, Zawodowych, Nadciśnienia Tętniczego i Onkologii Klinicznej Uniwersytetu Medycznego we Wrocławiu. Zakres badania obejmował elektroencefalogram, elektrokardiogram, elektrookulogram, elektromiogram oraz pomiar saturacji krwi. Uzyskane polisomnogramy oceniano w oparciu o wytyczne Amerykańskiej Akademii Medycyny Snu (ang. *American Academy of Sleep Medicine, AASM 2013*). AHI definiowano jako średnią liczbę epizodów bezdechu i spłycenia oddechu na godzinę całkowitego czasu snu. Bezdech stwierdzano w sytuacji zmniejszenia przepływu powietrza do mniej niż 10% wartości wyjściowej przez co najmniej 10 s. Epizod spłyconego oddechu definiowano jako spadek ciśnienia powietrza w jamie nosowej o co najmniej 30% w stosunku do wartości wyjściowej przez co najmniej 10 s, z redukcją saturacji o co najmniej 3% w stosunku do wartości wyjściowej przed zdarzeniem.

Badania laboratoryjne wykonano w laboratorium naukowym Zakładu Zdrowia

Środowiskowego i Medycyny Pracy Uniwersytetu Medycznego we Wrocławiu w oparciu o dostępne komercyjnie standaryzowane testy immunoenzymatyczne. Materiałem biologicznym użytym do badania była surowica krwi. Wykorzystano zestaw ELISA E1809h dla pomiaru stężenia selenoproetiny P, zestaw ELISA E3109Hu dla pomiaru stężenia renalazy, zestaw ELISA E0703h dla pomiaru stężenia peroksyredoksyny-5 oraz Antioxidant Assay Kit numer 709001 dla pomiaru TAS.

Publikacja nr 3 – praca oryginalna

Grupa badana i metody

Grupę badanych stanowiło 101 pacjentów hospitalizowanych celem weryfikacji diagnozy obturacyjnego bezdechu sennego w Katedrze i Klinice Chorób Wewnętrznych, Zawodowych, Nadciśnienia Tętniczego i Onkologii Klinicznej we Wrocławiu.

Do wykonanych procedur zaliczono: badanie polisomnograficzne, badanie echokardiograficzne, 24-godzinne ambulatoryjne monitorowanie ciśnienia tętniczego oraz badania laboratoryjne - pomiar stężenia selenoproteiny P, renalazy i peroksyredoksyny-5. Całonocne badanie polisomnograficzne wykonano w certyfikowanym Laboratorium snu Katedry i Kliniki Chorób Wewnętrznych, Zawodowych, Nadciśnienia Tętniczego i Onkologii Klinicznej Uniwersytetu Medycznego we Wrocławiu. Zakres badania obejmował elektroencefalogram, elektrokardiogram, elektrookulogram, elektromiogram oraz pomiar saturacji krwi. Uzyskane polisomnogramy oceniano w oparciu o wytyczne Amerykańskiej Akademii Medycyny Snu (ang. *American Academy of Sleep Medicine, AASM 2013*). AHI definiowano jako średnią liczbę epizodów bezdechu i spłycenia oddechu na godzinę całkowitego czasu snu. Bezdech stwierdzano w sytuacji zmniejszenia przepływu powietrza do mniej niż 10% wartości wyjściowej przez co najmniej 10 s. Epizod spłyconego oddechu definiowano jako spadek ciśnienia powietrza w jamie nosowej o co najmniej 30% w stosunku do wartości wyjściowej przez co najmniej 10 s, z redukcją saturacji o co najmniej 3% w stosunku do wartości wyjściowej przed zdarzeniem.

Echokardiografię przezklatkową wykonano aparatem ALOKA ProSound SSD-5500 SV, wyposażonym w głowicę 3,5/2,7 MHz (Aloka Inc, Tokio, Japonia). Wyniki oceniono stosując kryteria Kardiologicznych Towarzystw Naukowych. Pomiarów lewej komory dokonano w prezentacji M-mode z zastosowaniem konwencji pomiarowej Penn.

Frakcję wyrzutową lewej komory oceniono z zastosowaniem dwupłaszczyznowej metody Simpsona. Masę lewej komory (ang. *left ventricular mass*, LVM) obliczono wykorzystując formułę Devereux: LVM (g) = 0,8{1,04[(LVEDD + IVSd + PWd)^3 - LVEDD^3]} + 0,6. Indeks masy lewej komory (LVMI) uzyskano dzieląc LVM przez powierzchnię ciała obliczoną ze wzoru Du Bois. 24-godzinne ambulatoryjne monitorowanie ciśnienia tętniczego wykonano za pomocą systemu Welch Allyn ABPM 6100.

Badania laboratoryjne wykonano w laboratorium naukowym Zakładu Zdrowia Środowiskowego i Medycyny Pracy Uniwersytetu Medycznego we Wrocławiu w oparciu o dostępne komercyjnie standaryzowane testy immunoenzymatyczne. Materiałem biologicznym użytym do badania była surowica krwi. Wykorzystano zestaw ELISA E1809h dla pomiaru stężenia selenoproetiny P, zestaw ELISA E3109Hu dla pomiaru stężenia renalazy, zestaw ELISA E0703h dla pomiaru stężenia peroksyredoksyny-5 oraz Antioxidant Assay Kit numer 709001 dla pomiaru TAS.

METODY STATYSTYCZNE

Analizy statystyczne przeprowadzono przy użyciu oprogramowania Dell Statistica 13 (Dell Inc., Round Rock, TX, USA). Zmienne ilościowe wyrażono jako średnie i odchylenia standardowe. Zmienne jakościowe wyrażono w procentach. Ocenę zgodności rozkładu zmiennej z rozkładem normalnym przeprowadzono przy użyciu testu Shapiro-Wilka. W przypadku zmiennych ilościowych o rozkładzie normalnym przeprowadzono dalszą analizę statystyczną za pomocą testu t Studenta (dla porównań 2 grup) lub jednoczynnikowej parametrycznej analizy wariancji ANOVA (dla porównania >2 grup). Dla zmiennych ilościowych o rozkładzie innym niż normalny zastosowano test U Manna–Whitney'a (dla porównań 2 grup) lub nieparametryczny odpowiednik analizy wariancji ANOVA Kruskala-Wallisa (dla porównania >2 grup). W przypadku zmiennych jakościowych zastosowano test zgodności chi-kwadrat.

W celu określenia związku pomiędzy badanymi zmiennymi przeprowadzono analizy korelacji i regresji. W przypadku rozkładu normalnego wyznaczono współczynnik r korelacji Pearsona, natomiast w przypadku rozkładu innego niż normalny zastosowano współczynniki r Spearmana. Do dalszej analizy wykorzystano metodę wieloczynnikowej regresji krokowej wstecznej. Celem analizy było zidentyfikowanie możliwych niezależnych czynników predykcyjnych dla wyznaczonych zmiennych zależnych. Wyniki z $p < 0,05$ uznawano za istotne statystycznie.

WYKAZ PUBLIKACJI WCHODZĄCYCH W SKŁAD CYKLU

1. **Renalase—a new understanding of its enzymatic and non-enzymatic activity and its implications for future research**
   Czerwińska K, Poręba R, Gać P.
   Clinical and Experimental Pharmacology and Physiology
   2022, Vol. 49, no. 1, pages:3-9. doi: 10.1111/1440-1681.13594
   IF: **2,963**
   Punkty MEiN: **100,00**
2. **Selenoprotein P, peroxiredoxin-5, renalase and total antioxidant status in patients with suspected obstructive sleep apnea**
   Czerwińska K, Januszewska L, Markiewicz-Górka I, Jaremków A, Martynowicz H, Pawlas K, Mazur G, Poręba R, Gać P.
   Sleep and Breathing
   2023, doi.org/10.1007/s11325-023-02880-7

   IF: **2,500**
   Pkt. MEiN: **70,00**
3. **Selenoprotein P, peroxiredoxin-5, renalase and selected cardiovascular consequences tested in ambulatory blood pressure monitoring and echocardiography**
   Czerwińska K, Januszewska L, Markiewicz-Górka I, Jaremków A, Martynowicz H, Pawlas K, Mazur G, Poręba R, Gać P.
   Antioxidants 2023, Vol. 12, no. 6. doi:10.3390/antiox12061187
   IF: **7,675**
   Pkt. MEiN: **140,00**

Sumaryczny IF: 13,138
Punkty MEiN: 310,00

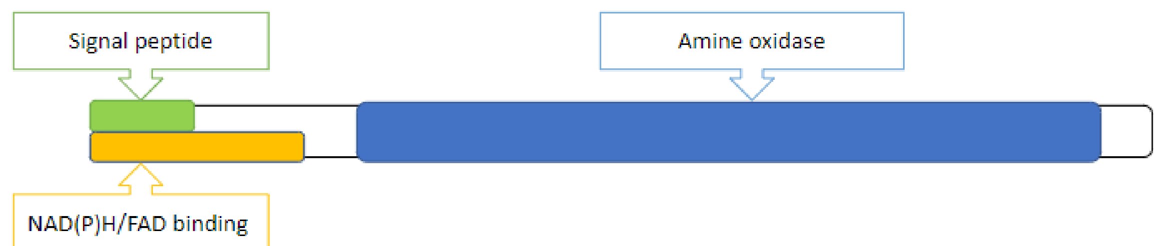
OMÓWIENIE POSZCZEGÓLNYCH PUBLIKACJI

1. Renalase—A new understanding of its enzymatic and non-enzymatic activity and its implications for future research

Jest to artykuł przeglądowy podsumowujący aktualny stan wiedzy na temat renalazy. W pracy opisano historię odkrycia renalazy, a także zmieniające się poglądy na temat jej domniemanej funkcji, zarówno enzymatycznej, jak i nie-enzymatycznej.

Renalaza jest białkiem klasyfikowanym jako oksydaza zależna od dinukleotydu flawinoadeninowego (FAD). Za jej odkrycie odpowiada Gary V. Desir, który poszukiwał nowych białek zaangażowanych w regulację czynności nerek.

Analiza strukturalna renalazy doprowadziła do identyfikacji trzech domen funkcjonalnych: peptydu sygnałowego, miejsca wiążącego FAD oraz domeny katalitycznej charakterystycznej dla enzymów z grupy oksydaz (Rycina 1). Do znanych enzymów zawierających zarówno domenę wiążącą FAD, jak i domenę oksydazową należą Monoaminooksydazy A oraz B (MAO-A, MAO-B). Enzymy te katalizują wewnątrzkomórkową oksydację katecholamin. Stwierdzenie podobieństwa pomiędzy renalazą a monoaminooksydazami doprowadziło do sugestii, że renalaza również katalizuje degradację katecholamin, ale w odróżnieniu od MAO-A i MAO-B jest wydzielana poza komórkę i degraduje katecholaminy obecne we krwi.

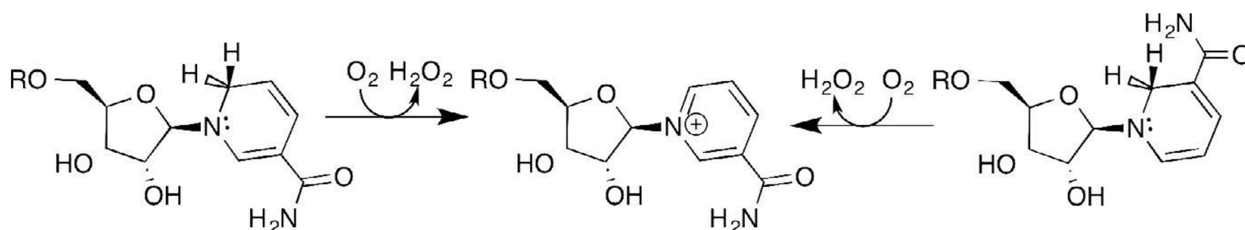


Rycina 1. Domeny funkcjonalne renalazy.

W celu weryfikacji postawionej hipotezy wykonano szereg analiz laboratoryjnych. Badania oparto o detekcję nadtlenku wodoru (H_2O_2), który jest produktem ubocznym powstającym w trakcie reakcji oksydacji. Hipotezę zweryfikowano na modelu zwierzęcym. Rekombinowaną renalazę podano dożylnie grupie szczurów, po podaniu odnotowano obniżenie tętna oraz kurczliwości mięśnia sercowego, a także spadek ciśnienia tętniczego. Artykuł podsumowujący odkrycie renalazy, a także opis jej funkcji enzymatycznej opublikowano

w 2005 roku, od tamtej pory z renalazą wiązano wiele nadziei odnośnie jej zastosowania w diagnostyce i terapii schorzeń układu krążenia. W 2007 r. pojawiły się pierwsze wątpliwości co do poprawności postawionej hipotezy oraz sugerowanej zdolności renalazy do degradacji katecholamin. Zasugerowano, że ilości H_2O_2 generowane w trakcie wcześniejszych analiz biochemicznych były zbyt niskie, aby przypisać je do działania enzymatycznego renalazy. Stwierdzenie argumentowano faktem, że w obecności tlenu katecholaminy ulegają reakcji naturalnej dekompozycji, w trakcie której produktem ubocznym jest H_2O_2. Te doniesienia sprawiły, że badania naukowe mające na celu poznanie prawdziwej funkcji enzymatycznej renalazy rozpoczęły się ponownie.

Poszukiwania zakończyły się w 2015 r., gdy ustalono, że substratami dla renalazy są 1,2-dihydroNAD(P) oraz 1,6-dihydroNAD(P). Związki te są izomerami formy natywnej 1,4-dihydroNAD(P). Renalaza katalizuje reakcję ich utlenienia i powrotu do formy natywnej poprzez formowanie β-NAD(P)+ (Rycina 2). W badaniach *in vitro* ustalono, że wyżej wymienione izomery powstają przez spontaniczną nie-enzymatyczną redukcję lub tautomeryzację formy natywnej i są potencjalnie szkodliwe dla funkcjonowania komórki.



Rycina 2. Konwersja 1,6-dihydro-NAD(P) (strona lewa) lub 1,2-dihydro-NAD(P) (strona prawa) do β-NAD(P)+.

Forma natywna 1,4-dihydroNAD(P) jest kofaktorem wielu enzymów, a formy izomeryczne mogą wiązać się z nimi kompetycyjnie i blokować ich działanie. Prace nad ustaleniem, które enzymy są podatne na hamowanie przez formy izomeryczne NAD wciąż trwają. Renalaza wydaje się więc swego rodzaju enzymem zmiatającym (ang. *scavanger enzyme*) chroniącym komórkę przed akumulacją cząsteczek, które mogą hamować inne szlaki enzymatyczne.

W powyższym artykule podsumowano najnowsze doniesienia na temat renalazy, podkreślając, że brak pełnego zrozumienia mechanizmu jej działania w komórkach ludzkich nie oznacza braku istotności klinicznej. Szczególną uwagę zwrócono na badania na temat znaczenia polimorfizmu pojedynczego nukleotydu (ang. *SNP – single nucleotide plymorphism*) w obrębie genu ranalazy, a także na nowe doniesienia na temat jej funkcji nie-enzymatycznej.

Trwają badania na temat związku polimorfizmów rs2296545, rs2576178 oraz rs10887800

ze wzrostem ryzyka wielu chorób, w tym: choroby nadciśnieniowej, przerostu mięśnia sercowego, choroby naczyń wieńcowych, rzucawki oraz stanu przedrzucawkowego u ciężarnych, a także bezpłodności. Co więcej, w ciągu ostatnich lat stwierdzono, że renalaza wydzielana poza komórkę traci domenę wiążącą FAD co skutkuje utratą funkcji enzymatycznej. Receptorem dla krążącej renalazy jest przezbłonowa ATP-aza wapniowa 4b-PMCA4b (ang. *Plasma membrane calcium-dependent ATPase 4b*). Łącząc się ze swoim receptorem pozakomórkowa renalaza funkcjonuje jako cytokina zaangażowana w transdukcję sygnałów do wnętrza komórki.

Powyższe doniesienia wskazują na plejotropizm działania renalazy i jej zaangażowanie nie tylko w regulację pracy szlaków enzymatycznych, ale również sygnałowych.

2. Selenoprotein P, peroxiredoxin-5, renalase and total antioxidants status in patients with suspected obstructive sleep apnea

Celem badań opisanych w tej publikacji było poznanie związku pomiędzy stężeniem selenoproteiny P, peroksyredoksyny-5 oraz renalazy, a całkowitym statusem antyoksydacyjnym (TAS), średnim ciśnieniem tętniczym (ang. *mean blood pressure*, mBP) oraz wskaźnikiem bezdechów i spłyconych oddechów (AHI).

Grupę badanych opisano we wcześniejszych rozdziałach dysertacji. Średnie ciśnienie krwi (mBP) obliczano stosując wzór: średnie ciśnienie krwi = rozkurczowe ciśnienie krwi + 1/3*(ciśnienie skurczowe - ciśnienie rozkurczowe). Nadciśnienie tętnicze rozpoznawano, gdy średnia z dwóch pomiarów wynosiła co najmniej 140 mmHg dla ciśnienia skurczowego lub 90 mmHg dla ciśnienia rozkurczowego.

W badaniu wyższe stężenie selenoproteiny P, peroksyredoksyny-5 oraz renalazy korelowało dodatnio z wyższym TAS, co potwierdza właściwości przeciwutleniające badanych substancji. Z wartością TAS najsilniej korelowało stężenie selenoproetiny P ($r = 0,57$; $p < 0,05$), następnie peroksyredoksyny-5 ($r = 0,48$; $p < 0,05$) i renalazy ($r = 0,25$; $p < 0,05$). Obniżony poziom badanych substancji był również niezależnym czynnikiem ryzyka obniżonego TAS w analizie regresji. Według naszej wiedzy jest to pierwsze badanie opisujące bezpośredni związek pomiędzy poziomem renalazy a TAS.

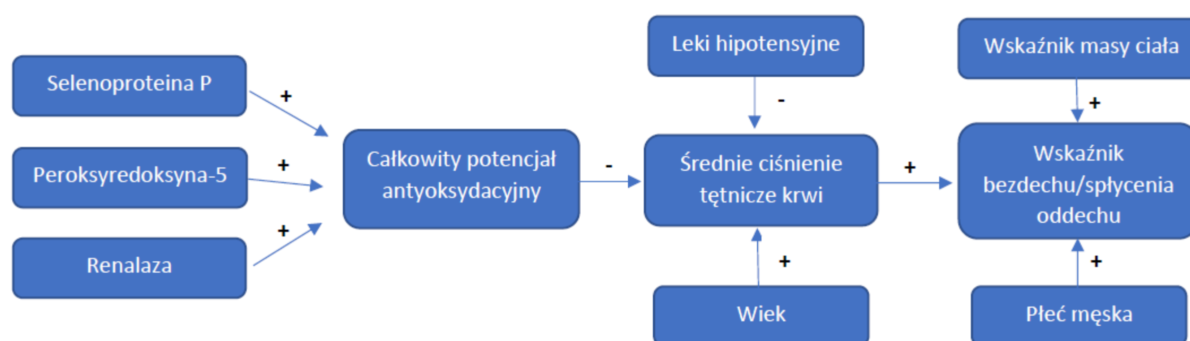
W celu szczegółowej analizy zgromadzonych danych pacjentów podzielono na podgrupy. Początkowo wyróżniono cztery podgrupy na podstawie diagnozy nadciśnienia tętniczego (HTN) i obturacyjnego bezdechu sennego (OSA). Podgrupa A składała się z pacjentów z rozpoznaniem zarówno HTN, jak i OSA (HTN+, OSA+), podgrupa B z pacjentów z HTN, ale bez OSA (HTN+, OSA−), podgrupa C z pacjentów z rozpoznaniem OSA, ale bez HTN (HTN−, OSA+), podgrupa D z pacjentów bez HTN i OSA (HTN−, OSA−). Kolejny podział uwzględniał mediany wartości mBP (Me = 105 mmHg) i AHI (Me = 10,95). Wyróżniono następujące podgrupy: E — pacjenci z wysokim mBP i wysokim AHI ($mBP \geq Me$, $AHI \geq Me$), F — pacjenci z wysokim mBP, ale niskim AHI ($mBP \geq Me$, $AHI < Me$), G — pacjenci z niskim mBP i wysokim AHI ($mBP < Me$, $AHI \geq Me$) oraz H — pacjenci z niskim mBP i niskim AHI ($mBP < Me$, $AHI < Me$).

Selenoproteina P była substancją, której stężenie najistotniej różniło się pomiędzy badanymi podgrupami, zwłaszcza w kontekście wartości ciśnienia tętniczego krwi. Jej poziom był statystycznie istotnie wyższy w podgrupie D (HTN−, OSA−) niż w podgrupie A (HTN+, OSA+). Był również istotnie wyższy w podgrupie H (niskie mBP, niskie AHI) niż w podgrupie E (wysokie mBP, wysokie AHI). Różnice były również istotne statystycznie przy podziale

pacjentów wyłącznie na podstawie diagnozy nadciśnienia tętniczego lub wartości mBP. Poziom renalazy i peroksyredoksyny-5 korelował z TAS, ale nie różnił się istotnie pomiędzy pacjentami ze zdiagnozowanym nadciśnieniem tętniczym lub bez. W związku z powyższym, wysunięto hipotezę, że wśród badanych substancji zaburzenia w stężeniu selenoproteiny P są najsilniej zaangażowane w obniżenie TAS w tej grupie pacjentów.

Dzieląc pacjentów względem mediany wskaźnika AHI nie stwierdzono istotnych statystycznie różnic w stężeniu badanych substancji. Podział względem diagnozy OSA wykazał istotnie statystycznie obniżony poziom TAS wśród pacjentów z rozpoznanym bezdechem sennym.

W analizie regresji wieloczynnikowej wstecznej uwzględniono trzy zmienne zależne: TAS, mBP oraz AHI. Na podstawie uzyskanych modeli stwierdzono istnienie pośredniej zależności pomiędzy parametrami laboratoryjnymi a mBP i AHI. Wyższe stężenia selenoproteiny P, peroksyredoksyny-5 i renalazy były związane z wyższym TAS. Wyższy TAS był związany z niższymi wartościami mBP, a niższe wartości mBP były związane z niższym wskaźnikiem AHI. Wyniki te wskazują na pośredni związek między selenoproteiną P, peroksyredoksyną-5 i renalazą a wartościami mBP i AHI. Powyższą zależność podsumowano na Rycinie nr 3.



Rycina nr 3. Graficzne podsumowanie analizy regresji.

3. Selenoprotein P, Peroxiredoxin-5, Renalase and Selected Cardiovascular Consequences Tested in Ambulatory Blood Pressure Monitoring and Echocardiography

Celem pracy była ocena związku pomiędzy wybranymi antyoksydantami: selenoproteiną P, peroksyredoksyną-5 i renalazą a wybranymi konsekwencjami sercowo-naczyniowymi ocenianymi w ABPM i echokardiografii. W pracy konsekwencje sercowo-naczyniowe rozumiano jako: podwyższone średnie ciśnienie krwi (mBP) i ciśnienie tętna (PP) w badaniu ABPM, a także powiększenie lewego przedsionka, przerost lewej komory (LVH) i obniżenie frakcji wyrzutowej lewej komory (LVEF%) w badaniu echokardiograficznym.

Grupę badanych opisano we wcześniejszych rozdziałach dysertacji.

W celu szczegółowej analizy pacjentów dzielono na podgrupy. W sumie wyróżniono 20 podgrup oznaczonych skrótem literowym od A do U. Podziału dokonano na podstawie: diagnozy nadciśnienia tętniczego (podgrupy A i B), diagnozy obturacyjnego bezdechu sennego (podgrupy C i D), mediany stężenia selenoproteiny P (podgrupa E i F), mediany stężenia peroksyredoksyny-5 (podgrupa G i H), mediany stężenia renalazy (podgrupa I i J), mediany mBP (podgrupa K i L), mediany PP (podgrupa M i N), mediany wymiaru lewego przedsionka (podgrupa O i P), mediany LVEF% (podgrupa R i S) oraz diagnozy LVH (podgrupa T i U).

W badanej grupie pacjenci z rozpoznanym HTN (podgrupa A) mieli istotnie statystycznie niższy poziom selenoproteiny P niż pacjenci bez HTN (podgrupa B). Poziom peroksyredoksyny-5 i renalazy nie różnił się znacząco pomiędzy wspomnianymi podgrupami. Pod względem parametrów mierzonych w badaniu ABPM oraz w echokardiografii pacjenci z HTN mieli istotnie wyższe mBP, PP, a także większe ryzyko powiększenia lewego przedsionka oraz LVH. Podczas podziału pacjentów według rozpoznania OSA (podgrupy C i D) nie stwierdzono istotnych statystycznie różnic w stężeniu selenoproteiny P, peroksyredoksyny-5 i renalazy, jak również w wartościach mBP, wymiaru lewego przedsionka i wartości LVEF%. Aczkolwiek pacjenci z OSA (podgrupa C) mieli istotnie wyższe PP i zwiększone ryzyko wystąpienia LVH.

W badanej grupie pacjenci z poziomem selenoproteiny P wyższym niż mediana mieli istotnie niższe mBP i PP. Co więcej, stwierdzono ujemną korelację liniową pomiędzy stężeniem selenoproteiny P a średnim ciśnieniem skurczowym ($r = -0,33$; $p < 0,05$), średnim ciśnieniem rozkurczowym ($r = -0,21$; $p < 0.05$), mBP ($r = -0,28$; $p < 0,05$) i PP ($r = -0,32$; $p < 0.05$). W analizie regresji obniżony poziom selenoproteiny P był niezależnym czynnikiem ryzyka wyższego PP z wartością $p < 0,01$. Pacjenci ze stężeniem selenoproteiny P wyższym niż mediana mieli istotnie mniejszy lewy przedsionek i niższe prawdopodobieństwo wystąpienia LVH ($p < 0,05$). Na podstawie uzyskanych informacji wskazano na możliwość wykorzystania

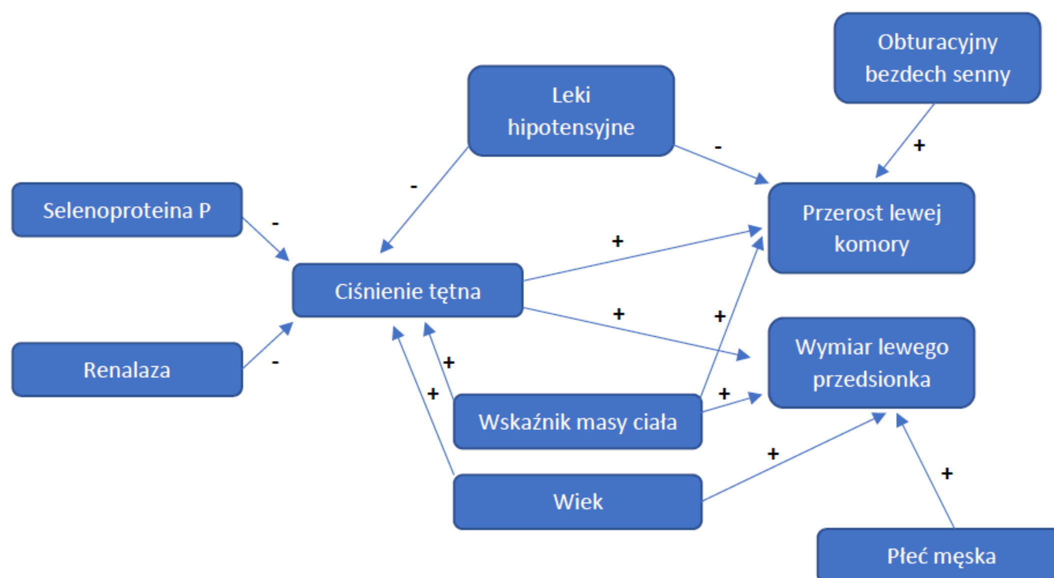
pomiaru stężenia selenoproteiny P w selekcji pacjentów ze zwiększonym ryzykiem LVH, którzy powinni być skierowani do dalszej diagnostyki w badaniu echokardiograficznym.

Pacjenci ze stężeniem renalazy powyżej mediany mieli istotnie niższe wartości PP i wymiaru lewego przedsionka niż pacjenci z niższym stężeniem renalazy. Ponadto, stwierdzono ujemną korelację liniową pomiędzy renalazą a PP ($r = -0{,}22$; $p <0{,}05$). Stężenie renalazy nie różniło się istotnie pomiędzy pacjentami z podwyższonym i prawidłowym ciśnieniem tętniczym. W związku z powyższym, wysunięto hipotezę, że renalaza może być zaangażowana w utrzymanie równowagi między skurczowym i rozkurczowym ciśnieniem tętniczym bardziej niż w regulowanie surowych wartości ciśnienia.

W badaniu stwierdzono istnienie ujemnej korelacji liniowej pomiędzy stężeniem selenoproteiny P i renalazy a wybranymi parametrami ocenianymi w ABPM i w echokardiografii. Nie stwierdzono korelacji pomiędzy poziomem peroksyredoksyny-5 a żadnym z badanych parametrów.

Wykonaną w badaniu analizę wieloczynnikowej regresji krokowej wstecznej oparto o trzy zmienne zależne: PP, wymiar lewego przedsionka oraz LVH. W uzyskanym modelu niezależnymi czynnikami ryzyka LVH były wyższe PP w ABPM, wyższy wskaźnik masy ciała (ang. *body mass index*, BMI), brak leczenia hipotensyjnego oraz rozpoznanie obturacyjnego bezdechu sennego.

Diagram podsumowujący uzyskane wyniki analizy regresji przedstawiono na Rycinie nr 4.



Rycina nr 4. Graficzne podsumowanie analizy regresji.

DYSKUSJA

Badania będące podstawą niniejszej rozprawy pozwoliły na weryfikację postawionych założeń. Ustalono, że stężenia selenoproteiny P, renalazy i peroksyredoksyny-5 korelowały w sposób liniowy dodatni z całkowitym statusem antyoksydacyjnym (TAS). Co więcej, ich obniżony poziom był niezależnym czynnikiem ryzyka obniżonego TAS w analizie regresji. Uzyskane wyniki potwierdzają zaangażowanie badanych substancji w utrzymanie równowagi redoks. Według naszej wiedzy jest to pierwsze badanie opisujące bezpośredni związek pomiędzy poziomem renalazy a TAS. Potwierdzenie wpływu renalazy na potencjał antyoksydacyjny pozostaje w zgodzie z jej sugerowaną funkcją enzymatyczną, jednak konieczne są dalsze badania, aby ustalić dokładny mechanizm jej działania antyoksydacyjnego i stwierdzić czy działanie antyoksydacyjne związane jest z jej funkcją enzymatyczną, czy też sygnałową.

W trakcie analiz będących podstawą rozprawy zauważono, że pomimo korelacji z TAS, stężenia badanych substancji nie korelowały pomiędzy sobą w sposób istotny statystycznie. Co więcej, wyniki uzyskiwane dla każdej z nich w trakcie analizy pomiędzy podgrupami pacjentów, a także w analizie korelacji i regresji istotnie się różniły. Najwięcej istotnych statystycznie zależności stwierdzono dla stężenia selenoproteiny P. W trakcie badań przedstawionych w pracy numer 2 stężenie selenoproteiny P różniło się istotnie pomiędzy utworzonymi w badaniu podgrupami pacjentów - pacjenci z nadciśnieniem tętniczym lub mBP powyżej mediany mieli istotnie niższe stężenie selenoproteiny P. W pracy numer 3 niższy poziom selenoproteiny P był związany z niekorzystnym konsekwencjami sercowo-naczyniowymi stwierdzanymi zarówno w badaniu ABPM, jak i w echokardiografii. W badaniu ABPM pacjenci ze stężeniem selenoproteiny P wyższym niż mediana mieli istotnie niższe mBP i PP. Co więcej, stężenie selenoproteiny P korelowało w sposób liniowy ujemny ze średnim skurczowym ciśnieniem krwi, średnim rozkurczowym ciśnieniem krwi, mBP oraz PP. Ponadto analiza regresji wykazała, że niższy poziom selenoproteiny P był niezależnym czynnikiem ryzyka wyższego PP z wartością p poniżej 0,01. W badaniu echokardiograficznym pacjenci ze stężeniem selenoproteiny P wyższym niż mediana mieli istotnie mniejszy wymiar lewego przedsionka i niższe prawdopodobieństwo rozwoju LVH. Stężenie selenoproteiny P korelowało również negatywnie z LVMI oraz RWT. Podsumowując, niższe stężenie selenoproteiny P było związane z czynnikami, które w sposób istotny zwiększają ryzyko zachorowania oraz zgonu z przyczyn sercowo-naczyniowych. Stwierdzenie różnic w stężeniu selenoproteiny P pomiędzy pacjentami z podwyższonym i prawidłowym ciśnieniem tętniczym wskazuje na jej możliwe zaangażowanie w patogenezę nadciśnienia tętniczego.

W związku z powyższym, wskazujemy na możliwość zastosowania oznaczania poziomu selenoproteiny P we wstępnej selekcji pacjentów z grupy wysokiego ryzyka sercowo-naczyniowego, zwłaszcza w przypadku ograniczonego dostępu do bardziej zaawansowanych badań - ABPM lub echokardiografii. Ponadto sugerujemy pomiar selenoproteiny P jako możliwy wskaźnik pacjentów ze zwiększonym ryzykiem przerostu lewej komory, którzy powinni być skierowani do dalszej diagnostyki. Uzyskane przez nas wyniki pozostają w zgodzie ze szwedzkim prospektywnym badaniem kohortowym, które obejmowało 4366 pacjentów. W badaniu stwierdzono istotnie wyższe ryzyko zachorowania i zgonu z przyczyn sercowo-naczyniowych wśród 20% badanych z najniższym stężeniem selenoproteiny P [34]. Warto zauważyć, że średnie stężenie selenoproteiny P w naszej grupie badanych było dość niskie, zbieżne z poziomem uważanym za najniższy kwantyl we wspomnianym badaniu kohortowym. Aktualnie zastosowanie pomiaru stężenia selenoproteiny P w procesie diagnostycznym i w selekcji pacjentów wysokiego ryzyka wydaje się bardziej osiągalne niż zastosowanie suplementacji selenu w celu zwiększenia stężenia selenoproteiny P we krwi. Doniesienia na temat skuteczności suplementacji selenu w prewencji chorób sercowo-naczyniowych są niejednoznaczne, istnieją badania wskazujące na jej pozytywne jak i negatywne skutki [35-37]. Wydaje się, że suplementacja wśród pacjentów z wyjściowo niskim poziomem selenu może być korzystna, jednak w przypadku wyjściowo wysokiego poziomu selenu może wiązać się z brakiem efektu lub działaniami niepożądanymi. W związku z powyższym, konieczne są dalsze badania kliniczne, aby ocenić ewentualne wskazania oraz zakres bezpieczeństwa dla suplementacji selenu.

W przeprowadzonym badaniu stężenie selenoproteiny P nie różniło się znacząco pomiędzy pacjentami z obturacyjnym bezdechem sennym i bez, sugerując, że nie jest ona bezpośrednio zaangażowana w patogenezę OSA. Warto podkreślić, że dzieląc pacjentów według rozpoznania obturacyjnego bezdechu sennego, nie stwierdzono istotnych różnic w wartościach mBP, jednak pacjenci z OSA mieli istotnie wyższe PP. W związku z powyższym wskazujemy na istotność kalkulacji wartości PP u każdego pacjenta z OSA, nawet jeśli ciśnienie tętnicze pozostaje w granicach normy.

W przypadku renalazy na podstawie wyników badań przedstawionych w pracy nr 2 stwierdzono, że pacjenci ze stężeniem renalazy powyżej mediany mieli istotnie niższe wartości PP i wymiaru lewego przedsionka niż pacjenci z niższym stężeniem renalazy. Stężenie renalazy nie różniło się istotnie pomiędzy pacjentami z podwyższonym i prawidłowym ciśnieniem tętniczym. W związku z tym wskazujemy na możliwy wpływ renalazy na utrzymanie równowagi pomiędzy skurczowym i rozkurczowym ciśnieniem krwi. W badaniu nie znaleziono istotnych statystycznie różnic w stężeniu renalazy dzieląc pacjentów względem AHI lub

diagnozy obturacyjnego bezdechu sennego. Aczkolwiek, stwierdzono związek pomiędzy wartościami TAS a mBP, które z kolei było związane z AHI. Wskazujemy na możliwe pośrednie połączenie pomiędzy renalazą i AHI poprzez TAS i mBP.

Poznanie klinicznego znaczenia aktywności enzymatycznej renalazy, jak również jej wpływu na międzykomórkowe szlaki sygnałowe wciąż wymaga dalszych badań. Istotne jest ustalenie, które enzymy są podatne na hamowanie przez izomeryczne formy β-NAD (P)H oraz jakie są konsekwencje ich inhibicji *in vivo*. Biorąc pod uwagę fakt, że istnieje wiele enzymów potencjalnie podatnych na hamowanie przez izomeryczne formy β-NAD(P)H, nie można stwierdzić, że niezdolność do degradacji katecholamin wyklucza jej istotność kliniczną.

W trakcie badań będących podstawą powyższej rozprawy stężenie peroksyredoksyny-5 w sposób istotny statystycznie korelowało w sposób liniowy dodatni z TAS. Należy jednak podkreślić, że w trakcie dalszych analiz stężenie peroksyredoksyny-5 nie korelowało istotnie z żadnym z badanych parametrów klinicznych. W pracy nr 1 nie różniło się istotnie pomiędzy grupami pacjentów z nadciśnieniem tętniczym lub bez, ani w grupach pacjentów z obturacyjnym bezdechem sennym lub bez. W pracy nr 3 nie stwierdzono istotnych statystycznie zależności pomiędzy stężeniem peroksyredoksyny-5 a żadnym z parametrów ocenianych w badaniu ABPM lub w echokardiografii. Obniżone stężenie peroksyreodksyny-5 nie było związane ze zwiększonym ryzykiem ocenianych konsekwencji sercowo-naczyniowych, w tym podwyższonego mBP i PP w badaniu ABPM, a także powiększenia lewego przedsionka, LVH i LVEF% w badaniu echokardiograficznym. W związku z powyższym wskazujemy, że nie wszystkie substancje o charakterze przeciwutleniającym są jednakowo istotne w patogenezie nadciśnienia tętniczego i jego powikłań.

WNIOSKI

1. W przeprowadzonym badaniu nie obserwowano istotnych statystycznie korelacji pomiędzy stężeniami selenoproteiny P, renalazy i peroksyredoksyny-5 we krwi.
2. Wyższe stężenie selenoproteiny P, renalazy i peroksyredoksyny-5 było związane z wyższym całkowitym statusem antyoksydacyjnym. Najsilniejszą dodatnią korelację liniową stwierdzono pomiędzy całkowitym statusem antyoksydacyjnym a stężeniem selenoproteiny P.
3. Niższe wartości stężenia selenoproteiny P oraz renalazy były niezależnym czynnikiem ryzyka podwyższonego ciśnienia tętna.
4. W przeprowadzonym badaniu wyższe ciśnienie tętna było niezależnym czynnikiem ryzyka przerostu lewej komory oraz powiększenia lewego przedsionka.
5. Istnieje zależność pomiędzy stężeniem selenoproteiny P we krwi a wartościami średniego ciśnienia tętniczego, średniego ciśnienia skurczowego, średniego ciśnienia rozkurczowego oraz ciśnienia tętna mierzonymi w ABPM, a także wartościami wskaźnika masy lewej komory oraz względną grubością jej ściany w badaniu echokardiograficznym.
6. Oznaczenie stężenia selenoproteiny P może mieć zastosowanie we wstępnej selekcji pacjentów z grupy wysokiego ryzyka sercowo-naczyniowego, zwłaszcza jeśli dostęp do bardziej zaawansowanych badań jest ograniczony.
7. Pomiar stężenia selenoproteiny P może być potencjalnym wskaźnikiem pacjentów ze zwiększonym ryzykiem przerostu lewej komory, którzy powinni być skierowani do dalszej diagnostyki i mogą odnieść korzyści z badania echokardiograficznego.
8. Wśród pacjentów z obturacyjnym bezdechem sennym podwyższenie wartości ciśnienia tętna może poprzedzać rozwój nadciśnienia tętniczego. Wskazuje się, aby u każdego pacjenta z obturacyjnym bezdechem sennym standardowo wykonywać zarówno pomiary ciśnienia tętniczego, jak i kalkulację ciśnienia tętna.

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STRESZCZENIE

Nadciśnienie tętnicze pozostaje najważniejszym czynnikiem ryzyka chorób układu krążenia i główną przyczyną przedwczesnych zgonów na świecie. Stale podwyższone wartości ciśnienia tętniczego prowadzą do stopniowego uszkodzenia struktur układu krwionośnego, rozwoju subklinicznych powikłań narządowych, a w dalszej kolejności do rozwoju chorób sercowo-naczyniowych. Grupą pacjentów szczególnie narażoną na rozwój nadciśnienia tętniczego i jego konsekwencji są pacjenci z obturacyjnym bezdechem sennym (*ang. obstructive sleep apnea,* OSA*).* Jest to choroba, w której podczas snu dochodzi do powtarzających się epizodów bezdechu i/lub spłyconego oddechu. Nasilenie OSA określa wskaźnik bezdechów i spłyconych oddechów (ang. *apnea-hypopnea index*, AHI), który podsumowuje liczbę zaburzeń oddechowych występujących w ciągu godziny. Zaburzona fizjologia snu i powtarzające się epizody bezdechu skutkują nadmierną aktywacją współczulną oraz nasilonym stresem oksydacyjnym. Zaburzenia równowagi redoks są uznawane za jeden z istotnych elementów w patogenezie chorób sercowo-naczyniowych. W związku z powyższym czynniki o charakterze przeciwutleniającym są często proponowane jako możliwe do zastosowania w profilaktyce, diagnostyce lub terapii chorób układu krążenia.

Celem pracy była ocena istotności stężenia selenoproteiny P, renalazy oraz peroksyredoksyny-5 we krwi w patogenezie nadciśnienia tętniczego i obturacyjnego bezdechu sennego. W trakcie badań oceniano wpływ badanych substancji na całkowity status antyoksydacyjny (ang. *total antioxidant status*, TAS), wskaźnik AHI, a także ich korelację z wybranymi konsekwencjami sercowo-naczyniowymi ocenianymi w 24-godzinnym ambulatoryjnym monitorowaniu ciśnienia tętniczego i w echokardiografii. W pracy konsekwencje sercowo-naczyniowe rozumiano jako: podwyższone średnie ciśnienie tętnicze i ciśnienie tętna w badaniu ABPM, a także powiększenie lewego przedsionka, przerost lewej komory i obniżenie frakcji wyrzutowej lewej komory w badaniu echokardiograficznym.

Grupę badanych stanowiło 112 pacjentów hospitalizowanych celem weryfikacji diagnozy obturacyjnego bezdechu sennego w Katedrze i Klinice Chorób Wewnętrznych, Zawodowych, Nadciśnienia Tętniczego i Onkologii Klinicznej we Wrocławiu. Do wykonanych procedur zaliczono: pełne badanie polisomnograficzne, pomiar ciśnienia tętniczego metodą Korotkowa, badanie echokardiograficzne, 24-godzinne ambulatoryjne monitorowanie ciśnienia tętniczego oraz badania laboratoryjne - pomiar stężenia selenoproteiny P, renalazy i peroksyredoksyny-5, a także ocenę TAS.

Wyniki przeprowadzonych badań przedstawiono w cyklu trzech prac opublikowanych w trzech różnych czasopismach o łącznej punktacji Ministerstwa Edukacji i Nauki: 310,00

i łącznym współczynniku Impact Factor = 13,138.

Stężenia badanych substancji we krwi korelowały w sposób liniowy dodatni z całkowitym statusem antyoksydacyjnym, co potwierdziło ich zaangażowanie w utrzymanie równowagi redoks. W przypadku parametrów klinicznych najwięcej istotnych statystycznie zależności stwierdzono dla stężenia selenoproteiny P. Niższy poziom selenoproteiny P był związany z niekorzystnymi konsekwencjami sercowo-naczyniowymi stwierdzanymi zarówno w badaniu ABPM, jak i w echokardiografii. W badaniu ABPM pacjenci z niższym stężeniem selenoproteiny P mieli wyższe średnie ciśnienie tętnicze oraz ciśnienie tętna. W badaniu echokardiograficznym pacjenci z obniżonym stężeniem selenoproteiny P mieli istotnie większy wymiar lewego przedsionka i większe prawdopodobieństwo rozwoju przerostu lewej komory. Pacjenci ze stężeniem renalazy powyżej mediany mieli istotnie niższe wartości ciśnienia tętna w badaniu ABPM i wymiaru lewego przedsionka w badaniu echokardiograficznym w porównaniu do pacjentów z niższym stężeniem renalazy. Stężenie renalazy nie różniło się istotnie pomiędzy pacjentami z podwyższonym i prawidłowym ciśnieniem tętniczym. W przypadku peroksyredoksyny-5 pomimo stwierdzenia wpływu na TAS, nie znaleziono żadnej istotnej statystycznie zależności pomiędzy jej stężeniem a badanymi parametrami klinicznymi.

Wyniki przeprowadzonych badań dostarczają cennych informacji na temat związku badanych substancji z całkowitym statusem antyoksydacyjnym, wskaźnikiem bezdechów/spłyconych oddechów oraz wybranymi konsekwencjami sercowo-naczyniowymi. Wśród badanych substancji najwięcej istotnych statystycznie zależności stwierdzono dla selenoproteiny P, w związku z czym wskazano na możliwe zastosowanie oznaczania jej poziomu we wstępnej selekcji pacjentów z grupy wysokiego ryzyka sercowo-naczyniowego, zwłaszcza jeśli dostęp do bardziej zaawansowanych badań jest ograniczony. Pomiar stężenia selenoproteiny P może być również potencjalnym wskaźnikiem pacjentów ze zwiększonym ryzykiem przerostu lewej komory, którzy powinni być skierowani do dalszej diagnostyki i mogą odnieść korzyści z badania echokardiograficznego. Niższe stężenie badanych substancji nie było niezależnym czynnikiem ryzyka wyższego AHI. Aczkolwiek w badaniu stwierdzono związek pomiędzy wartościami TAS, a średnim ciśnieniem tętniczym, które z kolei było związane z AHI. Wskazujemy na możliwe pośrednie połączenie pomiędzy badanymi substancjami i AHI poprzez TAS i średnie ciśnienie tętnicze.

SUMMARY

High blood pressure is the main risk factor for cardiovascular disease and the leading cause of premature death globally. Consistently elevated blood pressure causes damage to the structures of the circulatory system. That can result in subclinical organ complications and cardiovascular diseases. Patients suffering from obstructive sleep apnea (OSA) are at a higher risk for developing hypertension and its associated effects. OSA is characterised by repeated episodes of apnea or shallow breathing during sleep. The severity of OSA is determined by the apnea-hypopnea index (AHI), which expresses the number of respiratory disturbances occurring per hour. The disrupted sleep patterns and repeated apnea episodes cause excessive sympathetic activation and increased oxidative stress. Redox imbalances are considered a significant factor in the development of cardiovascular diseases. As a result, antioxidant agents are often proposed as a potential preventative, diagnostic, or therapeutic treatment for cardiovascular diseases.

The aim of this study was to investigate the role of selenoprotein P, renalase, and peroxiredoxin-5 in the development of hypertension and obstructive sleep apnea. The study examined how these substances affected the total antioxidant status (TAS) and the AHI index. Additionally, the study explored the correlation between these substances and various cardiovascular outcomes, such as elevated mean blood pressure and pulse pressure in ambulatory blood pressure monitoring, as well as left atrial enlargement, left ventricular hypertrophy, and reduced left ventricular ejection fraction in echocardiography.

The research group consisted of 112 consecutive patients admitted to the Department and Clinic of Internal and Occupational Diseases, Hypertension and Clinical Oncology in Wroclaw to verify the diagnosis of OSA. The procedures performed included: polysomnography, echocardiography, ambulatory blood pressure monitoring, blood pressure measurement using the Korotkov method and laboratory tests - measurement of selenoprotein P, renalase and peroxiredoxin-5 blood concentrations, as well as assessment of TAS.

This dissertation consisted of three papers, published in three different journals, with a total Ministry of Education and Science score of 310.00 and total Impact Factor of 13.138.

The levels of selenoprotein P, renalase and peroxiredoxin-5 correlated in a positive linear manner with the total antioxidant status, which confirmed their involvement in maintaining the redox balance. In terms of clinical parameters, the strongest statistically significant relationships were observed for the concentration of selenoprotein P. Lower selenoprotein P levels were associated with adverse cardiovascular consequences found in both ABPM and echocardiography. In ABPM, patients with lower levels of selenoprotein P had

higher mean blood pressure and pulse pressure. In echocardiography, patients with reduced selenoprotein P concentration had a significantly larger left atrial diameter and a higher probability of developing left ventricular hypertrophy. Patients with selenoprotein P levels higher than the median had lower mean blood pressure, pulse pressure and left atrium diameter values and were less likely to develop left ventricular hypertrophy than patients with selenoprotein P levels lower than the median. Renalase levels did not differ significantly between hypertensive and normotensive patients. Although peroxiredoxin-5 influenced TAS, no statistically significant relationship was found between its concentration and the examined clinical parameters.

The study results offer valuable insights into the link between the tested substances, total antioxidant status, apnea/hypopnea index, and selected cardiovascular consequences. Among all the substances tested, selenoprotein P showed the greatest difference in concentration between the subgroups, and it was found to have the strongest correlation with different clinical parameters. That suggests that measuring its blood level could be useful in identifying patients from the high cardiovascular-risk group, particularly if more advanced studies are unavailable. Additionally, measuring selenoprotein P could indicate patients at risk of left ventricular hypertrophy who should be referred for further evaluation and could benefit from echocardiography. Reduced concentration of the tested substances did not independently increase the risk of higher AHI. However, the study found a relationship between TAS values and mean blood pressure, which in turn affected AHI. That suggests a possible indirect connection between the tested substances and AHI through TAS and mean blood pressure.

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REVIEW ARTICLE

Renalase—A new understanding of its enzymatic and non- enzymatic activity and its implications for future research

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Abstract

Renalase was first described in 2005 and since then it became an object of scien- tific interest because of its proposed ability to catalyse circulating neurotransmitters and its promising antihypertensive effects. However, further research on the enzy- matic activity of renalase did not confirm these initial findings and yielded that re- nalase serves to oxidize isomeric forms of β-NAD(P)H and recycle them by forming β-NAD(P)+. Moreover, in contrast to initial assumptions, it is indicated that renalase's enzymatic activity is confined to the cell and that extracellular renalase loses its enzymatic properties. These new reports led scientists to question as to whether renalase, as an enzyme, still has the potential to influence various systemic physiological responses (e.g. blood pressure). It was also put into question whether many physiological discoveries published based on the notion that renalase is secreted into the blood and acts by oxidation of catecholamines can still be considered valid. In this article, we attempt to review the literature to confront these doubts and find further possible directions of research on the importance of renalase. Our aim was to evaluate recent reports of non-enzymatic activity for renalase.

KEYWORDS
cytokine, enzyme, monoamine, neurotransmitters, renalase

1 | INTRODUC TION

Renalase was first described in 2005 by the research team of Gary V. Desir who aimed to find additional proteins that may be involved in endocrine function of the kidneys.[1] To do so, the scientists searched the Mammalian Gene Collection (MCG) for genes that: encode pro- teins, have <20% sequence similarity to known proteins, contain a signal peptide sequence and do not contain transmembrane do- mains. Their initial search identified 114 candidate genes encoding novel secretory proteins; however, in further Northern blot anal- ysis only one of them was preferably expressed in human kidney. The cDNA of this gene was then applied to the Human Genome Project database, identified to residue on chromosome 10 at q23.33 and named renalase.[1] Further analysis of the gene showed that it encompasses ~309,469 base pairs (bp), has 10 exons and at least four alternatively spliced isoforms with renalase1 being the most highly expressed.[2] The protein encoded by renalase1 is the most abundant one in the human body and can be detected in plasma, kidney, heart, skeletal muscle and liver.[2] The fact that it incorpo- rates three main functional domains (i.e., a signal peptide, a flavin adenine dinucleotide (FAD)-binding region and an amine oxidase domain) (Figure 1) became the basis for its classification as a novel flavin adenine dinucleotide-dependent amine oxidase.[1,2] The main representatives of this group are monoamine oxidases A and B (MAO-A and MAO-B), both known to catalyse the intracellular ox- idation of monoamine neurotransmitters, such as dopamine, sero- tonin, epinephrine and norepinephrine.[3] This prompted scientists to hypothesize that renalase is a sister enzyme to MAO-A and MAO-B and is also engaged into the oxidation of neurotransmitters, but unlike aforementioned enzymes it is secreted into plasma and acts

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extracellularly.[1] To check their hypothesis, Xu et al carried out an experiment with various amines as substrates. They used an assay based on the detection of H_2O_2, which was related to the fact that H_2O_2 is a by-product of the oxidation reaction. In more detail, flavoprotein oxidases catalyse substrate oxidation via two half-reactions; in the first step the flavin cofactor is reduced when it accepts a hydride equivalent from the substrate, whereas in the second step, the reduced flavin is re-oxidised by molecular oxygen and the H_2O_2 is formed.[3] The results of the assay led to the conclusion that renalase specifically metabolizes catecholamines, with dopamine being the preferred substrate, followed by epinephrine and norepinephrine.[1] Moreover, the scientists decided to intravenously administer re- combinant renalase to rats to test its impact on hemodynamic pa- rameters. It was described that renalase infusion caused a decrease in cardiac contractility, heart rate, blood pressure and prevented a compensatory increase in peripheral vascular tone.[1] These results became a starting point for further research and aroused great ex- pectations for renalase as a potential therapeutic agent. However, in 2007 the first doubts appeared and the ability of renalase to metabolize catecholamines was questioned.[4] These doubts were based on the finding that the rate of H_2O_2 generation reported by Xu et al[1] was far too low to be ascribed to enzymatic conversion of catecholamines by renalase, for catecholamines emanate H_2O_2 in the presence of O_2 in a natural decomposition reaction.[4] This report began the pursuit for the true substrates for renalase.[5-8] Finally, in 2015 Beaupre et al[9] published an article in which they recognised 1,2-dihydroNAD(P) and 1,6-dihydroNAD(P), which are the isomeric forms of 1,4-dihydroNAD(P)H (β-nicotinamide adenine dinucleotide [phosphate]), to be the substrates for renalase. These isoforms are created either by non-enzymatic reduction of 1,4-dihydroNAD(P)+ or by tautomerization of 1,4-dihydroNAD(P)H, which is the native form of β-NAD(P)H in cells. Renalase was described to oxidize these isomers and recycle them by forming β-NAD(P)+ (Figure 2). These results changed the view on renalase and introduced uncertainty about its potential effect on blood pressure.[10] Previously considered substrates (neurotransmitters) were well known and had a documented role in blood pressure regulation, whereas the isomeric forms of β-NAD(P)H have not been considered in this context so far. Moreover, these isomeric forms had not been detected in vivo to date.[10] They were detected in β-NAD(P)H solutions and the method of their artificial formation in vitro is known, nevertheless, further

FIGURE 1 Functional domains of renalase 1. Own elaboration based on Moran and Hoag10

experimentation is needed to confirm their formation within the cell.[9-11] Our aim was to review novel findings on enzymatic and non-enzymatic activity of renalase. We also attempted to find further possible directions of research on the importance of renalase.

2 | METHODS

The literature review was conducted in PubMed, Scopus and Google Scholar electronic databases. The search was limited to studies published between 2005 and 2020. The following keywords were used: renalase, monoamine oxidase, enzyme, renalase polymorphism, cytokine and intra-cellular signalling. Among all potentially applicable records only 43 focused on the subject by title and abstract. These papers were screened and divided into three areas: renalase enzymatic activity (16 records),[1,2,4,5,6,7,8,9,10,11,12,13,14,15,16,17] renalase polymorphisms (16 records)[18-33] and renalase non-enzymatic activity (11 records).[34-44]

2.1 | *Renalase new understanding of its enzymatic activity*

The current understanding of the importance of β-NAD(P)H iso- meric forms for the cell is that they are formed in rare non-enzymatic redox events and can be potentially harmful. This notion is based on the fact that these isoforms were found to competitively bind to other β-NAD(P)H (1,4-dihydroNAD[P]) dependent enzymes, namely some dehydrogenases, and inhibit their activity.[9,12] From this stand point, renalase seems to be a type of a scavenger enzyme that pro- tects cells against the accumulation of substances that may nega- tively affect other enzymatic reactions. Several dehydrogenases have been tested for their susceptibility to inhibition by isomeric forms of β-NAD(P)H, for instance, porcine heart lactate dehydroge- nase (LDH), Escherichia coli malate dehydrogenase (MDH) and rab- bit muscle lipoamide dehydrogenase (DLD).[9] The research team of Graham R. Moran demonstrated that both MDH and LDH are sub- ject to inhibition by both 1,2- and 1,6-dihydroNAD, whereas DLD is not.[9] This indicates that some enzymes are able to differentiate between β-NAD(P)H isomeric forms and bind selectively to the na- tive form, whereas others have no such capacity. With that in mind, it seems acceptable to hypothesise that some human enzymes that use β-NAD(P)H as a cofactor may be possibly inhibited by β-NAD(P) H isoforms. In such case, recent discovery of renalase's enzymatic function is only a starting point in the pursuit for its metabolic/ biochemical importance within the cell. Even a cursory look at the Braunschweig enzyme database (BRENDA) shows that there are

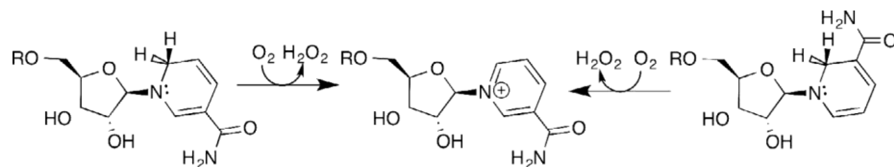


FIGURE 2 The chemistry catalysed by renalase. Conversion of 1,6-dihydro-NAD(P)H (left) or 1,2-dihydro-NAD(P)H (right) to β-NAD(P)+

many threads and paths to explore. A search limited to *Homo sapiens* enzymes using β-NADPH or β-NADH as a cofactor yields 117 and 85 results, respectively. Many of them are vital for maintaining proper cell metabolism.[13] For example, LDH (EC 1.1.1.27) catalyses the in- terconversion of pyruvate and lactate with concomitant interconver- sion of NADH and NAD+. This is the reaction that permits glycolysis under anaerobic conditions when NAD+ regeneration cannot take place by means of the electron transport chain within the mitochon- dria. It is then possible to hypothesise that lower renalase availability can cause an increase in 1,2- and 1,6-dihydroNAD and subsequently greater inhibition of LDH. This suspected indirect connection be- tween renalase and cellular respiration pathways may possibly ex- plain recent reports on renalase's involvement in reducing ischemic cell damage.[14-17] Li et al[14] carried out a study on the effects of renal- ase on Sprague-Dawley (SD) rats subjected to myocardial ischemia reperfusion injury (MIRI). They used lentivirus-mediated RNA inter- ference (RNAi) to inhibit the renalase gene expression in the heart tissue. The results from the study were as follows, the (RNAi) group rats exhibited lower renalase levels than did the controls and also exhibited more serious necrosis (7.12% ± 0.56% vs 3.32% ± 0.93%,
$P < .05$, $n = 6$) and apoptosis (151.8% ± 8.2% vs 66.8% ± 6.5%, $P < .05$, $n = 6$); however, pre-treatment with the recombinant re- nalase significantly reduced myocardial cell necrosis (1.51% ± 0.12% vs 4.13% ± 0.02%, $P < .05$, $n = 6$) and apoptosis (21.3% ± 5.0% vs 52.6% ± 10.4%, $P < .05$, $n = 6$) relative to the control rats.[14] In an- other study, Tokinoya et al[15] examined renalase concentration in the blood and renalase expression levels in different organs after moderate-intensity exercise (MEX) in rats. In conclusions, they re- ported that MEX for 60 minutes increased both renalase concentra- tion in the blood and its expression in skeletal muscle. Therefore, it can be suggested that renalase's expression/concentration increases in cells subjected to different stress conditions and is responsible for supporting their proper function. However, despite these compel- ling results, whether renalase has the ability to protect human cells against ischemic damage still requires confirmation. Moreover, even if it is possible to confirm such an effect, it is still unknown whether it results from the above-mentioned enzymatic function of renalase. Other enzymes that might possibly be inhibited by isomeric forms of β-NAD(P)H are nitric-oxide synthases (NOSs, EC 1.14.13.39); a fam- ily of enzymes catalysing the production of nitric oxide (NO) from L-arginine. In general, NOSs require five cofactors and one of them is β-NADPH. The presumption that there may be a link between re- nalase and NO production is one of the pathways that may unravel the mystery of its reported cardiovascular effects, for NO is well known to induce vasodilation, inhibit platelet aggregation, prevent neutrophil/platelet adhesion to endothelial cells and inhibit smooth muscle cell proliferation and migration.[45]

2.2 | *Renalase - Polymorphysms*

Understanding how mutations influence renalase's structure and function may be pivotal in our perception of its importance. According to the National Center for Biotechnology Information (NCBI) single nucleotide polymorphism (SNP) database, more than 95,700 renal- ase's SNPs were identified (available at https://www.ncbi.nlm.nih.gov/variation/view/; last accessed April 1, 2021). To date, several of the polymorphisms have been included in scientific research, namely rs2296545, rs2576178, rs10887800. Single nucleotide poly- morphism rs2296545 results in a conserved amino acid substitution (glutamic acid to aspartic acid at codon 37, Glu37Asp). Compared with Glu37, Asp37 has lower affinity for NADH that may possibly alter renalase's enzymatic activity.[19] Most studies on this polymor- phism concern its possible relationship with arterial hypertension (HT), but the results are contradictory. In 2015, Shi and Wang[20] conducted a meta-analysis on the association of rs2296545 and risk of hypertension and indicated that such an association does not exist.[20] This conclusion was based on the data extracted from four studies including 2493 cases and 2205 controls. Interestingly, an- other meta-analysis published only a year later showed diametrically opposite results. Lv et al[21] concluded that renalase gene rs2296545 polymorphism is significantly associated with increased risk of HT. In detail, a significant association between SNP rs2296545 and risk of HT in all genetic models was found. This meta-analysis included six studies with 2871 cases and 2279 controls; all four studies from the previously-mentioned meta-analysis were also encompassed. The authors acknowledged contradictory results indicated in previous studies, but also pointed out many strengths of their own findings that included supplementary analyses, such as subgroup analyses and sensitivity analyses. The association between rs2296545 and increased risk of HT would indicate that even without the ability to oxidise neurotransmitters renalase still plays a role in regulating blood pressure. Nevertheless, it is difficult to draw firm conclusions from similar, but conflicting reports.

Interestingly, Orlowska-Baranowska et al[22] suggested that re- nalase might play a role in hypertrophic response to pressure over- load in the heart muscle. They carried out a study on 657 patients with aortic stenosis referred for aortic valve replacement and de- scribed that in females the SNP rs2296545 gene polymorphism was associated with higher left ventricular mass, intraventricular septal thickness and posterior wall thickness all indexed to body surface area, as well as relative wall thickness. No significant associations were found among male patients. This possible correlation was also reported in previous studies,[23] however, the exact molecular mecha- nism in which renalase may influence cardiac hypertrophy in gender-related manner is still not known. Detailed studies on animal models of pressure overload are required to elucidate this matter.

Another direction of research on the importance of rs2296545 gene polymorphism is its influence on blood pressure during pregnancy. In a recent case-control study, which consisted of 185 patients with hypertensive disorders of pregnancy (HDP) and 380 normotensive pregnant women from the north-eastern Chinese Han population, SNP rs2296545 was significantly associated with HDP and pre-eclampsia (PE) risk.[24] Applied logistic regression analy- sis adjusted by maternal age and body mass index (BMI) showed that SNP rs2296545 was significantly associated with HDP susceptibility

TABLE 1 Research on possible correlations between renalase SNPs and selected health conditions

Renalase SNPs	Health condition	Reference
rs2296545	Arterial hypertension	20,21
	Cardiac hypertrophy	22,23
	Hypertensive disorders of pregnancy	24,25
rs2576178	Arterial hypertension	21
	Coronary artery disease	27,28
	Infertility	29
	Hypertensive disorders of pregnancy	25,30,31,32,33
rs10887800	Coronary artery disease	27,28
	Infertility	29
	Hypertensive disorders of pregnancy	25,30,31,32,33
	Gestational diabetes mellitus	34

in the dominant model with 1.91-fold higher risk and with a 1.80-fold higher PE risk.[24] In contrast, Chinese cohort study failed to demon- strate such an association.[25]

Other renalase gene polymorphisms included in scientific research are SNP rs2576178 and SNP rs10887800. In rs2576178, polymorphism adenine is replaced by guanine in the 5′-flanking region of the gene, which may possibly affect the binding of transcription factors. Whereas in rs10887800 guanine is replacing adenine in intron 6 of the gene.[26] Single nucleotide polymorphism rs2576178 was also considered in terms of its possible correlation with arterial hypertension. However, the results from the aforementioned meta- analysis[21] indicate that this particular polymorphism may not be as- sociated with the susceptibility to HT. Interestingly, both rs2576178 and rs10887800 were suggested to be involved in the pathogen- esis of coronary artery disease (CAD).[27,28] Hu et al[27] carried out a case-control study that included a total of 449 CAD patients and 507 healthy controls. Their results revealed that renalase rs2576178 GG (homozygous mutant) was significantly associated with in- creased risk of CAD (GG compared with AA – wild type, OR = 1.60, 95% CI = 1.07-2.39, P = .022). Moreover, subgroup analyses showed that the increased risk of CAD mostly applied to females, smokers and alcoholics.[27] Nonetheless, these results are in conflict with the results obtained in a Polish case-control study on a total of 309 pa- tiens treated with hemodialysis (107 with and 202 without CAD). In this study, the rs2576178 polymorphism did not influence the risk of CAD.[28] Such erratic research results highlight the need to carry out studies with similar study designs, unified and vast selection of study groups, as well as unified diagnostic criteria of CAD that often differ between studies. Aforementioned Polish case-control study also in- cluded SNP rs10887800 and showed that GG genotype was associ- ated with an increased risk of CAD under the codominant model and under the recessive model.[28] However, reliable conclusions cannot be drawn without more data.

Renalase, although initially detected in the kidney, is found in many other organs and tissues.[2,32] Among others it can be detected within female and male gonads, which led scientists to suggest that it may be involved in maturation of germ cells and steroid hormone regulation. Moreover, it initiated a series of studies on its potential effects on fertility,[29] PE[25,30,31,32,33] or gestational diabetes.[34] In their recent study, Fatima et al[29] indicated that both renalase polymorphism rs2576178 and rs10887800 are associated with infertility in women. In this cross-sectional study, a total of 672 women were recruited, of whom 508 were fertile and 164 were infertile. The causes of infertility were marked as endometriosis (n = 15), poly- cystic ovary syndrome (PCOS, n = 76) or unexplained (n = 73). The results showed that the variant AG/GG of rs2576178 was signifi- cantly associated with overall infertility (OR = 2.266; P < .001), with a strong G allele association with unexplained infertility OR = 2.796 (P = .002). Similarly, rs10887800 AG/GG and G allele showed signif- icant association with both infertility because of polycystic ovarian syndrome and unexplained infertility.[29] Recent studies also indicate that renalase SNP polymorphisms rs2576178 and rs10887800 have the potential to be new genetic markers of hypertensive disorders of pregnancy. It seems that mutant GG/GA genotypes are associated with lower blood renalase levels and increased risk of PE.[25,30,31,32,33] What is more, this association seems meaningful, for it was recently reported that rs10887800 and rs2576178 GG/GG combined genotypes were associated with 8.4- and 16.7-fold higher risk of PE and severe PE, respectively (OR = 8.4; 95% CI = 1-71.1; P = .048 and OR = 16.7; 95% CI = 1.6-167; P = .018).[30] Considering the above, renalase seems to be an interesting and multidirectional research object. Potential correlations between its gene polymorphisms and health conditions indicate that even without the ability to catalyse neurotransmitters it still may have an impact on physiology. Possible correlations between its gene polymorphisms and different disorders/diseases are summarized in Table 1.

2.3 | *Renalase as a cytokine*

It was initially claimed that extracellular renalase has enzymatic properties; however, recent reports seem to contradict it.[35] Fedchenko et al[35] reported that extracellular renalase lacks its N-terminal pep- tide that prevents the FAD cofactor from binding and translates into the loss of amine oxidase activity. They further suggested that cleav- age of this peptide occurs on secretion of intracellular renalase from

the cell. This concept was also supported by their recent observation that renalase secreted with urine lacks its N-terminal peptide and formation of this truncated renalase cannot be attributed to urinary/ blood proteases.[36] Nevertheless, these studies do not provide suf- ficient proof and it is yet to be clarified whether the cleavage occurs on secretion or otherwise (e.g. in blood circulation). Taken together, extracellular renalase cannot accommodate FAD and loses its enzy- matic activity.

Many scientific studies imply that extracellular renalase acts as a cytokine and exerts cytoprotective properties.[37] Among others, renalase cytoprotective properties were described in terms of ischemic acute kidney injury (AKI),[17,38] cisplatin-induced toxic injury,[39] acute pancreatitis,[40] renal fibrosis,[41] cardiac remodelling,[38] as well as fatty liver ischemia/reperfusion injury.[42] For example, renalase knock-out (KO) mice were found to have significantly increased renal injury indicated by higher plasma creatinine levels compared with renalase wild-type (WT) mice after both moderate and severe renal ischaemia reperfusion (IR) injury. Moreover, administration of human recombinant renalase ameliorated ischemic AKI by targeting all three pathways (necrosis, apoptosis and inflammation) of renal cell injury.[17] In another study, subtotally (5/6) nephrectomised rats (STNx) showed higher expression levels of pro-inflammatory cytokines in the remnant kidney, including tumour necrosis factor (TNF)-α, interleukin (IL)-6, monocyte chemotactic protein (MCP)-1 and NADPH oxidase components when compared with sham op- erated rats. At the same time, supplementary adenovirus-renalase treatment decreased the expression of pro-inflammatory cytokines and NADPH oxidase components, attenuated hypertension, cardio- myocytes hypertrophy and cardiac interstitial fibrosis.[38]

Numerous reports indicate the involvement of renalase in inflammatory response. Scientists hypothesize that reduced plasma renalase levels could be a marker of coronavirus disease 2019 (COVID-19) disease severity.[43] In the study including 51 hospitalized COVID-19 patients and 15 uninfected non-hospitalized controls, Wang et al reported that patients with COVID-19 disease had lower renalase levels than controls. Moreover, plasma renalase levels were negatively correlated with inflammatory markers, including IL-1β, IL-6 and TNF-α. Low renalase levels were also correlated with higher risk of mechanical ventilation (84.6% vs 50.0%, $P = .048$), as well as worse overall survival (HR = 4.54; 95% CI = 1.06-19.43; $P = .005$). Conclusively, it was suggested that low plasma renalase may be a cause and/or marker for the systemic biologic perturbations that lead to a poor outcome in COVID-19.[43]

The exact mechanism of renalase's extracellular action is still not fully understood. Recent research shows that the plasma membrane calcium ATPase isoform (PMCA4b) serves as a receptor for extracellular renalase.[39] This membrane ATPase is one of four known PMCA isoforms. It is involved in maintaining optimal Ca^{2+} homeostasis by ejecting Ca^{2+} from the cytosol of a eukaryotic cell to the external environment using one molecule of ATP in transporting one molecule of Ca^{2+}. This exchange supports the maintenance of a low resting intracellular Ca^{2+} concentration that is crucial for preserving proper cell physiology. What is more,

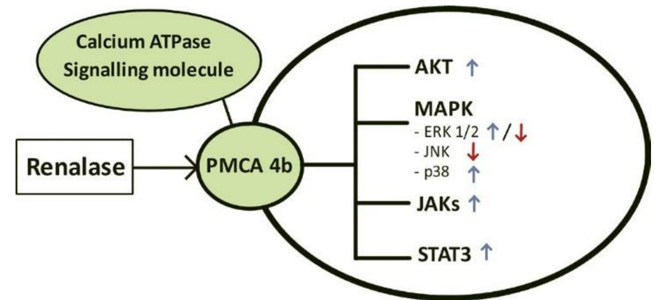


FIGURE 3 Model of interaction between renalase and its receptor-plasma membrane calcium ATPase isoform 4b (PMCA 4b). Renalase binds to PMCA 4b protein and interacts with many signalling molecules, including protein kinase B (AKT), mitogen activated protein kinases (MAPK), Janus kinases (JAKs), as well as signal transducer and activator of transcription proteins (STATs). ERK 1/2: extracellular-regulated kinase, JNK: C-Jun N-terminal kinase, p38: p38 mitogen-activated kinase

PMCA4b serves as a signalling molecule via interactions with pro- tein partners. Renalase is currently thought to bind to PMCA4b protein and interact with many signalling molecules, including pro- tein kinase B (AKT), Janus kinases (JAKs), signal transducer and activator of transcription proteins (STATs) and mitogen activated protein kinases (MAPK).[37] A schematic model of this interaction is shown in Figure 3. In general, these molecules and pathways enable the cell to respond to external stimuli by regulating the activity of various proteins, enzymes and transcription factors. MAPK pathway consists of 14 MAPK members divided into 7 sub- groups, including four conventional MAPK subgroups, such as extracellular-regulated kinase (ERK1/2), C-Jun N-terminal kinase (JNK), p38 MAPK, and ERK5 and three atypical MAPK subgroups, such as ERK3/4, ERK7/8 and nemo-like kinase (NLK). In general, these kinases can either get activated or inactivated by their phos- phorylation or dephosphorylation. JNK and p38 MAPK are acti- vated most notably following cell exposure to stress evoked by a variety of physical, chemical and biological stress stimuli and lead to cell death. ERK1/2 cascades tend to promote cell survival and mostly process cell growth factor-stimulated signalling. Renalase was first described to activate AKT, ERK 1/2, p38 kinase, B cell lymphoma 2 and inhibit JNK.[44] Nevertheless, there are inconsis- tent reports as to whether renalase activates or inhibits these mol- ecules and the effects of such action. In their recent study, Wang et al[39] indicated that the early activation of the stress kinase p38 pathway, and not ERK pathway, is crucial in mediating the protec- tion of renalase against cisplatin cytotoxicity. Within cells treated with cisplatin and renalase peptide, chemical inhibition of p38 com- pletely eliminated the peptide's protective effect.[39] These results stand in contrast with the common notion that activation of p38 pathway leads to cell death. As an explanation, the authors sug- gest that the effects of p38 pathway activation are complex and may vary depending on the presence of other factors.[39] In another study on STNx rats, renalase was found to protect against renal injury and cardiac remodelling via inhibition of ERK 1/2. Renalase

supplementation by systemic delivery of adenovirus-renalase significantly decreased the activation of ERK-1/2 compared with sham-treated rats and had anti-fibrotic effects.[38] Renoprotective properties of renalase dependent on ERK 1/2 pathway inhibition were also confirmed in a study on rats with complete unilateral ureteral obstruction (UUO) and on human proximal renal tubular epithelial (HK-2) cells.[41] On the contrary, a study aimed at examining the role of renalase in promoting the growth of melanoma cells showed that renalase activates ERK pathway through its binding to PMCA4b. This action was described as highly unfavourable because of enhanced cancer cell survival. It was also proposed that abnormal upregulation of renalase signalling promotes tumour growth and inhibition of these pathways may provide a novel therapeutic option.[45] The exact mechanism behind renalase's extracellular activity and signalling properties remain unclear. Some authors suggest that inconsistencies may arise because of renalase's biphasic activity expressed as direct and indirect effects on signalling pathways. It was suggested that changes caused by renalase treatment (e.g. blood pressure decline and sympathetic hyperactivity) may indirectly influence the inactivation of ERK 1/2 pathway.[38] However, whether these assumptions are valid and whether there are other mechanisms involved remains to be further explored. It is worth mentioning that signalling and cytoprotective properties of renalase were described for both intact renalase and renalase peptide devoid of its enzymatic activity.[44]

3 | CONCLUSIONS

Many important discoveries regarding renalase have been made in recent years. It is currently acknowledged that it has both intracellular and extracellular activity. The former associated with enzymatic oxidation of isomeric forms of β-NAD(P)H and the latter non-enzymatic related to intercellular signalling and regulation of various cellular responses. Initial high expectations for the physiological role of renalase led to multiple studies with conflicting results. It seems inaccurate to rule out the results of clinical studies that were based on the notion that renalase catalyses neurotransmitters, neverthe- less, credence should not be applied to all claims equally. We be- lieve that novel research based on the finding that renalase serves to oxidase isomeric forms of β-NAD(P)H are another introduction to the pursuit for understanding renalase's importance and impact on human physiology. The importance of renalase's enzymatic activity, as well as renalase's involvement in intercellular signalling pathways still remain poorly understood. It is yet to be discovered which en- zymes are prone to inhibition by isomeric forms of β-NAD(P)H and what consequences this may have for the functioning of the cell and the whole organism. Given that the list of potential enzymes is long, it cannot be concluded that the inability to catalyse neurotransmitters excludes renalase's influence on physiological responses. Clarification of the existing doubts and questions may provide a response to whether renalase or its inhibitors may be considered potential therapeutic agents, especially in terms of cardiology, nephrology, gynaecology and oncology.

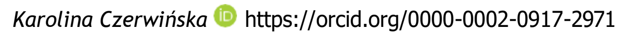
CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data available on request from authors.

ORCID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Selenoprotein P, peroxiredoxin-5, renalase, and total antioxidant status in patients with suspected obstructive sleep apnea

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Abstract

Purpose The aim of this study was to investigate the relationship between selenoprotein P, peroxiredoxin-5, renalase, total antioxidant status (TAS), mean blood pressure (mBP), and apnea-hypopnea index (AHI).

Methods The study group consisted of 112 patients hospitalized to verify the diagnosis of obstructive sleep apnea (OSA). The inclusion criteria were consent to participate in the study and age ≥ 18 years. Patients with active proliferative disease, severe systemic diseases, or mental diseases were excluded from the study. Each patient underwent full polysomnography and had blood pressure measured. Blood samples were collected and laboratory test was performed.

Results Among 112 patients enrolled, there was a statistically significant negative linear correlation between blood pressure values (sBP, dBP, mBP) and selenoprotein P, renalase, and TAS levels. Similarly, there was a negative linear correlation between AHI and selenoprotein P, renalase, and TAS levels, but none between AHI and peroxiredoxin-5. Based on the obtained regression models, higher selenoprotein P, peroxiredoxin-5, and renalase levels were independently associated with higher TAS. Lower mBP values were independently associated with the use of antihypertensive drugs, higher TAS, and younger age. Male gender, higher BMI, and higher mBP were independently associated with higher AHI.

Conclusions Higher concentrations of selenoprotein P, peroxiredoxin-5, and renalase were associated with higher TAS, which confirms their antioxidant properties. There was an indirect connection between tested antioxidants and blood pressure values.

Keywords Antioxidants · Hypertension · Obstructive sleep apnea · Peroxiredoxin-5 · Renalase · Selenoprotein P

Introduction

Redox imbalance plays a role in the pathogenesis of cardiovascular diseases (CVDs). Overwhelmed cellular antioxidant capacity is described in the course of such diseases as hypertension, atherosclerosis, or ischemic heart disease [1, 2]. These diseases remain the leading cause of death worldwide, and any factors that could be used in their prevention, diagnosis, or therapy are in-demand [3]. There is potential in using antioxidants as a supplement for treating or preventing cardiovascular diseases. While many substances with antioxidant properties have been researched, none has been recommended for this purpose thus far [4]. It is related to a large number of conflicting reports [5, 6]. It seems that such supplementation has potential; however, the difficulty lies in choosing the dose and substance suit- able for patients with a specific disease or risk factor [7]. Therefore, the search for appropriate antioxidants continues. In our study, we focused on peroxiredoxin-5, which is a known antioxidant, as well as on selenoprotein P and renalase, which are suspected to exert antioxidant properties.

SelP is a unique protein that contains multiple selenocysteine (Sec) residues [8]. It is primarily produced in the liver and released into the bloodstream after the removal of its signal peptide. The abbreviation "P" in SelP signifies its presence in the plasma. SelP consists of two domains: the N-terminal and C-terminal domains. The N-terminal Sec residue acts as the enzyme's active site, reducing phospholipid

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hydroperoxide. Meanwhile, the nine C-terminal Sec residue serves as the Se transporter [8]. In vitro studies have shown that SelP acts as a phospholipid hydroperoxide glutathione peroxidase and a peroxynitrite reductase [9]. Steinbrenner et al. reported that SelP derived from human plasma safeguards low-density lipoproteins (LDL) from oxidation [9]. Moreover, it is suggested that cells pretreated with SelP are protected from oxidative damage caused by tert-butyl hydroperoxide due to an increase in the production of intracellular selenoenzymes [10]. It appears that SelP plays a role in promoting cardiovascular health by working together with endothelial cells. Plasma SelP not only supplies selenium to these cells, but also acts as an external antioxidant. This helps safeguard plasma proteins against damage from peroxynitrite-induced oxidation and nitration [10]. In the case of selenium deficiency, the concentration of plasma SelP decreases, making it a possible indicator of selenium's nutritional status [9, 10]. However, more research is needed to fully understand SelP's role in maintaining redox balance and cardiovascular health.

Renalase was first described in 2005 and since then it became an object of scientific interest due to its proposed ability to catalyze circulating neurotransmitters and its promising antihypertensive effects [11]. Based on preliminary animal model reports, it was found that administering renalase intravenously can lower blood pressure, heart rate, and cardiac contractility [11]. This effect was entitled to renalase breaking down catecholamines in the bloodstream. However, subsequent research on its enzymatic activity did not support these initial findings. Instead, it was discovered that renalase helps to oxidize isomeric forms of β-NAD(P)H and recycle them by forming β-NAD(P)+ [12]. These isomers were found to inhibit some β-NAD(P)H-dependent enzymes in vitro, namely, porcine heart lactate dehydrogenase (LDH) and *Escherichia coli* malate dehydrogenase (MDH) [12]. There is speculation that some human enzymes which use β-NAD(P)H as a cofactor might be inhibited by β-NAD(P)H isoforms too. From this standpoint, renalase seems to act as a scavenger enzyme that protects cells against the accumulation of substances that may negatively affect other enzymatic reactions. The data on renalase's involvement in redox balance in humans is scarce. We have discussed recent findings on renalase's enzymatic and non-enzymatic activity in our previous work [13].

Peroxiredoxins (Prdxs) are a family of peroxidases that participate in maintaining thiol balance by reducing organic hydroperoxides, H_2O_2, and peroxynitrite [14]. According to scientific research, Prdxs have the potential to be used in the diagnosis and treatment of cardiovascular disease (CVD) [15–18]. Developing derivatives or mimicking the catalytic activity of Prdxs could be a promising approach for antioxidant therapy in treating CVD [18]. It is important to note that the majority of research focuses on Prdx-1. Prdx-5 is the most recently discovered member of the peroxiredoxins family, and its impact on CVD requires further investigation.

Fighting ROS and keeping the redox balance require the cooperation of many compounds with antioxidant properties. The total antioxidant capacity of the body is composed of both endogenous and food-derived antioxidants. The collaboration among different antioxidants offers superior protection against reactive oxygen or nitrogen species. Therefore, the total antioxidant status is a more useful measure of biological information than the measurement of individual components, as it considers the combined effect of all antioxidants present in the plasma.

Obstructive sleep apnea (OSA) is a respiratory sleep disorder caused by repeated obstruction of the upper airway during sleep, leading to interruptions in oxygen flow, arousal, and fragmented sleep [19, 20]. The severity of OSA is measured by the apnea/hypopnea index (AHI), which counts the number of respiratory events per hour. A diagnosis of OSA is made when the AHI is five or more. Mild OSA is indicated by an AHI from 5 up to 15; moderate OSA is indicated by an AHI from 15 up to 30, and severe OSA requires an AHI of 30 or more [19]. It is important to note that OSA increases the risk of cardiovascular disease [21]. Repeated episodes of apnea cause sympathetic overactivation and acute inflammation, leading to an increase in cardiovascular morbidity and mortality [22].

The aim of this study was to investigate the relationship between selenoprotein P, peroxiredoxin-5, renalase, total antioxidant status (TAS), mean blood pressure (mBP), and apnea-hypopnea index (AHI).

Materials and methods

The study group included patients who were admitted consecutively to an internal medicine clinic for the purpose of verifying the diagnosis of obstructive sleep apnea. Group size was determined using a sample size calculator. The selection conditions were as follows: population size 2.9 million (population size of the macroregion from which patients are referred to our study center), fraction size 0.5, maximum error 10%, confidence level 95%. The required minimum number of subjects was 96. To be eligible for the study, participants had to be 18 years or older and provide their consent. Patients with severe systemic diseases, severe mental illness/disorders, or active proliferative disease were excluded from the study.

A single-night recording of PSG was conducted at the Sleep Laboratory of the Department of Internal Medicine, Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Poland. The Nox-A1 machine from Nox Medical, Iceland, was used according to the standard diagnostic criteria for nocturnal recording.

The assessment of the polysomnograms was carried out in 30-s epochs based on the American Academy of Sleep Medicine (AASM) 2013 criteria for sleep scoring, by a qualified physician (H.M.) from the Sleep Laboratory. For a thorough explanation of PSG methodology, please refer to our recent work [23].

Blood was collected from the patient's ulna vein in the morning after polysomnography. The blood samples were stored at a constant temperature until renalase determinations were performed simultaneously on all samples. The serum renalase levels were checked using the E3109Hu kit ELISA from the Bioassay Technology Laboratory in Shanghai, China, as per the manufacturer's instructions. The renalase concentration was measured in ng/ml, with a reference range of 1–400 ng/ml. The ELISA test used had a sensitivity of 0.52 ng/ml, and the coefficient of intra- and inter-assay variation was less than 8% and 10%, respectively.

We utilized the E1809h ELISA Kit for Human SeP from ElAab in Wuhan, China, to measure serum selenoprotein P. Following the manufacturer's instructions diligently, we expressed the selenoprotein P concentration in ng/ml. The coefficient of intra-assay variation was less than 4.9% and inter-assay variation was less than 7.1%.

We used the E0703h ELISA Kit for Human Peroxiredoxin-5, mitochondrial (ElAab) from Wuhan, China, to measure serum peroxiredoxin-5. The test was conducted following the manufacturer's instructions, and the results were presented as nanograms/milliliter (ng/ml). The reference range of the assay was 0.78–50 ng/ml.

Because the effects of different antioxidants are additive, the total antioxidant status (TAS) was used to measure the overall antioxidant status of the body. The USA Antioxidant Assay Kit No709001 was used to measure TAS in our study group. This protocol does not differentiate between aqueous- and lipid-soluble antioxidants. Instead, it evaluates the overall antioxidant activity of all constituents such as vitamins, proteins, lipids, glutathione, and uric acid. This assay measures the ability of antioxidants in the sample to inhibit the oxidation of ABTS® (2,2′-Azino-di-[3-ethylbenzthiazoline sulfonate]) to ABTS®$^{\bullet+}$ by metmyoglobin. The capacity of the antioxidants in the sample to prevent ABTS® oxidation is compared with that of Trolox, a water-soluble tocopherol analog, and is quantified as millimolar Trolox equivalents.

The auscultatory method using mercury sphygmomanometer was used to measure blood pressure. During the measurement, the patients were relaxed and seated comfortably with their feet resting on the ground. The arm was relaxed, uncovered, and supported at the level of the heart. Blood pressure was measured twice by the Korotkoff method. Mean blood pressure (mBP) was calculated using the formula: mean blood pressure = diastolic blood pressure + 1/3*(systolic blood pressure − diastolic blood pressure). Arterial hypertension was diagnosed when the mean of two measurements was at least 140 mmHg for systolic pressure or 90 mmHg for diastolic pressure. In a situation where the patient declared taking any antihypertensive drugs, arterial hypertension was diagnosed, regardless of the measured blood pressure values.

We used Dell Statistica 13 software (Dell Inc., USA) to conduct statistical analyses. Mean and standard deviation were used to express quantitative variables, while percentages were used for qualitative variables. We tested the distribution of variables using the W-Shapiro-Wilk test. For normally distributed quantitative variables, we used the *T*-test for further statistical analysis. To analyze non-normally distributed quantitative variables, we used the Mann-Whitney *U* test. For qualitative variables, we used the chi-square test of maximum likelihood. Additionally, correlation and regression analyses were conducted to establish the relationship between the variables. The Pearson correlation *r* factor was used for normal distribution, while the Spearman *r* factor was used for non-normal distribution. We used multivariable stepwise backward regression to identify possible predictor variables for changes in mBP, number of AHI events, and TAS level. Variables were removed from the model based on *p* values.

Ethical approval for this study was obtained from the Bioethics Committee of Wroclaw Medical University. Before the study, all participants gave their informed consent.

ClinicalTrials.gov Identifier: NCT05040516.

Results

The study group comprised 112 participants, 52.7% men (n = 59) and 47.3% women (n = 53). The complete characteristics of the study group are presented in Table 1.

For detailed analysis, the patients were divided into subgroups. First, four subgroups were distinguished based on the diagnosis of HTN and OSA. Subgroup A consisted of patients diagnosed with both HTN and OSA (HTN+, OSA+), subgroup B of patients with HTN but without OSA (HTN+, OSA−), subgroup C of patients diagnosed with OSA but without HTN (HTN−, OSA+), and subgroup D of patients without HTN and OSA (HTN−, OSA−). The second division included median values of mBP (Me = 105 mmHg) and AHI (Me = 10.95). The following subgroups were distinguished: E—patients with high mBP and high AHI (mBP ≥ Me, AHI ≥ Me), F—patients with high mBP but low AHI (mBP ≥ Me, AHI<Me), G—patients with low mBP and high AHI (mBP < Me, AHI ≥ Me), and H—patients with low mBP and low AHI (mBP < Me, AHI < Me).

Statistical analysis of the selenoprotein P, peroxiredoxin-5, and renalase levels, as well as total antioxidant status in the study subgroups, are presented in Table 2. Patients in the subgroup designated as D (HTN− OSA−)

Table 1 Characteristics of the study group ($n = 112$)

	Mean	Standard deviation
Age (years)	49.8	14.7
BMI (kg/m^2)	28.7	5.3
sBP (mmHg)	139.5	20.7
dBP (mmHg)	89.6	12.8
mBP (mmHg)	106.2	14.7
AHI (events/h)	18.0	18.8
Average SpO2 (%)	93.2	2.5
Minimum SpO2 (%)	83.5	8.0
SpO2 <90% (%)	9.5	18.23
Selenoprotein P (ng/ml)	1.49	1.88
Peroxiredoxin-5 (ng/ml)	1.82	4.52
Renalase (ng/ml)	186.98	213.86
TAS (nM)	1.15	0.33
	Number	Percent
Men	59	53
Women	53	47
Overweight/obesity	55	49
HTN	43	38
Diuretics	19	17
β-Blockers	22	20
ACE inhibitors	20	18
Angiotensin receptor blockers	13	12
Calcium channel blockers	12	11
OSA	80	71
Mild OSA	32	29
Moderate OSA	21	189
Severe OSA	27	24
Type 2 diabetes	10	9
Coronary artery disease	8	7

AHI apnea-hypopnea index, *BMI* body mass index, *dBP* diastolic blood pressure, *HTN* arterial hypertension, *mBP* mean blood pressure, *OSA* obstructive sleep apnea, *sBP* systolic blood pressure, *SpO2* oxygen saturation; *TAS* total antioxidant status

had statistically significantly higher selenoprotein P and TAS levels when compared to subgroup A (HTN+ OSA+). There were no significant differences between the other subgroups in terms of these parameters. Peroxiredoxin-5 and renalase levels did not differ significantly between any of the subgroups A–D.

Dividing patients by diagnosis of arterial hypertension showed significant differences in selenoprotein P and TAS levels between HTN+ ($n = 43$) and HTN− ($n = 69$) subgroups, but no differences in peroxiredoxin-5 and renalase levels. Division by diagnosis of obstructive sleep apnea found statistical differences in TAS levels between OSA+ ($n = 80$) and OSA− ($n = 32$) patients, but not for the other parameters.

Statistically significant differences for selenoprotein P, renalase, and TAS levels, but no for peroxiredoxin level, were found when dividing patients by median values of mBP and AHI. Subjects in the E subgroup (high mBP, high AHI) had lower selenoprotein P, renalase, and TAS levels when compared to subjects in the H subgroup (low mBP, low AHI). Moreover, there was a statistically significant difference between TAS levels between the E subgroup (high mBP, high AHI) and the G subgroup (low mBP, high AHI).

Selenoprotein P, renalase, and TAS levels, but no peroxiredoxin level, were statistically significantly lower in patients with high mBP (≥Me, $n = 58$) when compared to patients with low mBP (<Me, $n = 54$). No such differences were found when comparing patients with high AHI (≥Me, $n = 56$) and low AHI (<Me, $n = 56$).

Statistically significant positive linear correlation was observed between TAS and selenoprotein P ($r = 0.57$), peroxiredoxin-5 ($r = 0.48$), and renalase ($r = 0.25$) levels. No significant linear relationship was found between selenoprotein P, peroxiredoxin-5, and renalase levels.

There was a statistically significant negative linear correlation between blood pressure values (sBP, dBP, mBP) and selenoprotein P, renalase, and TAS levels. No such correlation was found for the peroxiredoxin-5 level. Similarly, there was a negative linear correlation between AHI and selenoprotein P, renalase, and TAS levels, but none between AHI and peroxiredoxin-5. In addition, it has been shown that there are positive linear correlations between mean saturation and TAS, and minimum saturation and TAS. The results of the linear correlation analysis including the correlation coefficient are presented in Table 3.

A multivariable stepwise backward regression analysis was performed for three different dependent variables: mBP, AHI, and TAS level. A final model obtained for mBP as a dependent variable was as follows: mBP = 94.645 − 10.761 antihypertensive drugs − 6.486 TAS + 0.24 age. Based on the obtained regression model, use of antihypertensive drugs, higher TAS, and younger age were independently associated with lower mBP values. A final model obtained for AHI as a dependent variable was as follows: AHI = −54.449 + 11.540 male gender + 0.660 BMI + 0.621 mBP. Based on the obtained regression model, it was shown that male gender, higher BMI, and higher mBP values were independently associated with higher AHI values. A final model obtained for TAS level was as follows: TAS = 0.889 + 0.103 selenoprotein P + 0.040 peroxiredoxin-5 + 0.001 renalase. Based on the obtained regression model, higher selenoprotein P, peroxiredoxin-5, and renalase levels were independently associated with higher TAS. Detailed results of multivariable stepwise backward regression analysis in the study group are presented in Table 4. Figure 1 shows a diagram summarizing the results of regression analysis.

Table 2 Selenoprotein P, peroxiredoxin-5, renalase, and total antioxidant status in the study subgroups

Subgroup	Selenoprotein P (ng/ml)	Peroxiredoxin-5 (ng/ml)	Renalase (ng/ml)	TAS (nM)
A (*n* = 38)	1.04 ± 0.96	1.09 ± 0.99	132.28 ± 189.87	1.07 ± 0.26
B (*n* = 5)	0.52 ± 0.16	2.56 ± 3.09	308.10 ± 266.10	1.15 ± 0.10
C (*n* = 42)	1.78 ± 2.46	2.34 ± 5.81	203.34 ± 207.20	1.15 ± 0.41
D (*n* = 27)	1.96 ± 1.88	1.85 ± 5.38	216.69 ± 238.15	1.29 ± 0.31
p	A vs. D: <0.05	ns	ns	A vs. D: <0.05
HTN+ (*n* = 43)	0.98 ± 0.92	1.27 ± 1.43	152.72 ± 204.34	1.08 ± 0.25
HTN− (*n* = 69)	1.85 ± 2.25	2.16 ± 5.62	208.64 ± 218.36	1.20 ± 0.37
p	<0.05	ns	ns	<0.05
OSA+ (*n* = 80)	1.42 ± 1.92	1.76 ± 4.34	169.16 ± 200.98	1.11 ± 0.34
OSA− (*n* = 32)	1.71 ± 1.79	1.97 ± 5.03	230.97 ± 240.50	1.26 ± 0.29
p	ns	ns	ns	<0.05
E (*n* = 40)	1.02 ± 1.19	1.07 ± 1.03	136.67 ± 192.75	1.05 ± 0.25
F (*n* = 18)	1.47 ± 1.67	3.10 ± 6.41	187.87 ± 216.22	1.20 ± 0.35
G (*n* = 16)	1.79 ± 2.82	3.30 ± 9.13	216.91 ± 192.17	1.29 ± 0.51
H (*n* = 38)	1.94 ± 2.09	1.87 ± 1.63	225.59 ± 238.28	1.19 ± 0.29
p	E vs. H: <0.05	ns	E vs. H: <0.05	E vs. G: <0.05 E vs. H: <0.05
mBP ≥ Me (*n* = 58)	1.16 ± 1.36	1.72 ± 3.79	152.84 ± 199.95	1.10 ± 0.29
mBP < Me (*n* = 54)	1.89 ± 2.30	1.93 ± 5.23	223.02 ± 223.84	1.22 ± 0.37
p	<0.05	ns	<0.05	<0.05
AHI ≥ Me (*n* = 56)	1.23 ± 1.79	2.03 ± 5.15	160.01 ± 194.31	1.12 ± 0.36
AHI < Me (*n* = 56)	1.78 ± 1.96	1.62 ± 3.82	213.46 ± 230.13	1.19 ± 0.31
p	ns	ns	ns	ns

AHI apnea-hypopnea index, *HTN* arterial hypertension, *mBP* mean blood pressure, *Me* median value, *OSA* obstructive sleep apnea, *TAS* total antioxidant status

Discussion

The most important result of this study is the finding of a relationship between the tested laboratory parameters, mBP, and AHI. Higher concentrations of selenoprotein P, peroxiredoxin-5, and renalase were associated with higher TAS. Higher TAS was associated with lower mBP values, and lower mBP values were associated with lower AHI index. These results indicate an indirect connection between selenoprotein P, peroxiredoxin-5, and renalase levels and blood pressure values. It is an argument for the concept that a decrease in antioxidant levels, redox imbalance, and intensification of oxidative stress provoke an increase in blood pressure values. This causative role of oxidative stress in hypertension pathophysiology has also been proposed by other authors [24–26]. Increased intracellular ROS levels are described to promote oxidative modification of signaling proteins and cause altered vascular signaling. Moreover, ROS were found to increase vasoconstriction and reduce endothelium-dependent vasodilation by increasing the production of prostanoids [27]. The concept that overwhelmed cellular antioxidant capacity promotes hypertension is also supported by findings that blood pressure can be lowered by antioxidants, ROS scavengers, and Nox inhibitors [26, 28]. Nevertheless, this matter seems more complicated as there are studies with contrary results [29, 30].

It is worth noting that renalase has only recently been proposed as a scavenger enzyme [12]. Initially, it was thought to oxidize circulating catecholamines but that assumption was not proven in more detailed trials [11, 31]. Instead, Beaupre et al. recognized 2- and 6-dihydroNAD(P) molecules to be the real substrates for renalase [12]. These molecules are isomeric forms of native β-NAD(P)H (4-dihydroNAD(P)H) that arise either by nonspecific reduction of β-NAD(P)+ or by tautomerization of β-NAD(P)H. Renalase serves to oxidase these isomers and recycles them by forming β-NAD(P)+. This action seems highly favorable for the cell because β-NAD(P)+ isomeric forms were found to competitively bind to β-NAD(P)+ dependent enzymes and inhibit their activity [12]. In short, renalase is a scavenger enzyme that protects cells against the accumulation of substances that may negatively affect other enzymatic reactions. However, these assumptions are not based on human studies and it is yet to be established whether human enzymes are prone to inhibition by isomeric forms of β-NAD(P)H. We have discussed enzymatic and non-enzymatic activity of renalase

Table 3 The results of the correlation analysis in the study group

	selenoprotein P (ng/ml)	peroxiredoxin -5 (ng/ml)	renalase (ng/ml)	TAS (nM)
selenoprotein P (ng/ml)	x	ns	ns	0.57
peroxiredoxin-5 (ng/ml)	ns	x	ns	0.48
renalase (ng/ml)	ns	ns	x	0.25
TAS (nM)	0.57	0.48	0.25	x

ns – non significant (p>0.05); TAS – total antioxidant status

b) between antioxidants and blood pressure values, AHI, saturation

	sBP (mmHg)	dBP (mmHg)	mBP (mmHg)	AHI (events/h)	average SpO2 (%)	minimum SpO2 (%)	SpO2 <90% (%)
selenoprotein P (ng/ml)	-0.26	-0.24	-0.26	-0.19	0.23	ns	ns
peroxiredoxin-5 (ng/ml)	ns	ns	ns	ns	ns	ns	ns
renalase (ng/ml)	-0.20	-0.28	-0.26	-0.19	ns	ns	ns
TAS (nM)	-0.22	-0.20	-0.22	-0.22	0.25	0.20	ns

AHI apnea-hypopnea index, *dBP* diastolic blood pressure, *mBP* mean blood pressure, *ns* non-significant ($p > 0.05$), *sBP* systolic blood pressure, *SpO2* oxygen saturation, *TAS* total antioxidant status

in our previous work [13]. In this study, we have found a positive linear relationship between renalase blood concentration and TAS level which seems to confirm renalases' antioxidant properties. To our knowledge, this is the first study to describe a direct connection between renalase level and total antioxidant status. However, the strength of this correlation was weaker than for selenoprotein P or peroxiredoxin-5. The mechanism behind renalases' antioxidant activity remains unknown. It may be connected with the fact that many enzymes involved in redox balance, such as a family of nonphagocytic NADPH oxidases or nitric oxide synthase, are NAD(P)+ dependent. However, to date, no studies have addressed this issue. Another possible mechanism involves renalase's extracellular action and its impact on calcium balance, which is considered an important part of maintaining redox homeostasis [32]. Recently plasma membrane calcium ATPase isoform (PMCA4b) was found to serve as a receptor for extracellular renalase [33]. The interaction between renalase and PMCA4b receptor is involved in maintaining optimal Ca^{2+} homeostasis. Alterations in extracellular calcium levels influence intracellular calcium levels and possibly play an important role in the pathogenesis of essential hypertension [34]. Nevertheless, the clinical relevance of the interaction between renalase and PMCA4b receptor remains undiscovered.

In our study, we have found that during subgroup analyses selenoprotein P was the substance that differed the most between the study subgroups, especially in the context of blood pressure. It was statistically significantly higher in subgroup D (HTN− OSA−) than in subgroup A (HTN+ OSA+). It was also significantly higher in subgroup H (low mBP, low AHI) than in subgroup E (high mBP, high AHI). The differences were also significant when dividing patients by the diagnosis of hypertension or mBP only (Table 3). Therefore, we suggest that among studied substances, the disturbances in selenoprotein P concentration are the most involved in lowering TAS in this group of patients. We further suggest that increasing selenoprotein P concentration may be a promising therapeutic strategy to increase TAS and lower blood pressure. The possible involvement of selenoprotein P in the maintenance of cardiovascular health was also described by Schomburg et al. In their study, quintiles of selenoprotein P concentration were related to the risk of all-cause mortality, cardiovascular mortality, and a first cardiovascular event. The main conclusion was that 20% with the lowest selenoprotein P concentration had markedly increased risk of cardiovascular morbidity and mortality [35]. However, this was a population-based prospective cohort study. Clinical trials testing if cardiovascular morbidity and mortality can be reduced in subjects belonging to this low selenoprotein P group are still needed.

Table 4 Results of multivariable stepwise backward regression analysis in the study group

model for: mBP (mmHg)			
	Rc	SEM of Rc	p
intercept	94.645	11.713	< 0.001
age (years)	0.240	0.109	< 0.05
TAS (nM)	- 6.486	2.929	< 0.05
antihypertensive drugs	-10,761	3.612	< 0.01
R2 = 0.637, p < 0.001, standard error of estimation: 14.053			

b) estimation for the AHI as a dependent variable

model for: AHI (events/h)			
	Rc	SEM of Rc	p
intercept	- 54.449	15.623	< 0.001
male gender	11.540	3.429	< 0.01
BMI (kg/m^2)	0.660	0.251	< 0.05
mBP (mmHg)	0.621	0.124	< 0.001
R2 = 0.591, p < 0.001, standard error of estimation: 13.870			

c) estimation for the TAS as a dependent variable

model for: TAS (nM)			
	Rc	SEM of Rc	p
intercept	0.889	0.031	< 0.001
selenoprotein P (ng/ml)	0.103	0.011	< 0.001
peroxiredoxin-5 (ng/ml)	0.040	0.005	< 0.001
renalase (ng/ml)	0.001	0.000	< 0.05
R2 = 0.622, p < 0.001, standard error of estimation: 0.205			

AHI apnea-hypopnea index, *BMI* body mass index, *mBP* mean blood pressure, *Rc* regression coefficient, *SEM* standard error of mean, *TAS* total antioxidant status

It is worth noting that even though statistically significant positive linear correlation was observed between TAS and peroxiredoxin-5, we did not find any significant differences in its levels between chosen subgroups. It may suggest that not all antioxidants are of equal importance in this patient group. Results of multivariable stepwise backward regression analysis showed that AHI was directly influenced by BMI, male gender, and mBP but not TAS. We did not find significant differences between selenoprotein P, peroxiredoxin-5, renalase, and TAS levels when dividing patients by the diagnosis of OSA or the number of AHI. In short, AHI was not directly influenced by TAS or antioxidants. However, estimation for the mBP as a dependent variable found a relationship between mBP values and AHI, which may indicate an indirect connection between TAS and AHI through mBP. An increase in TAS may decrease mBP values and consequently decrease AHI. Our results stay consistent with the notion that weight reduction and proper blood

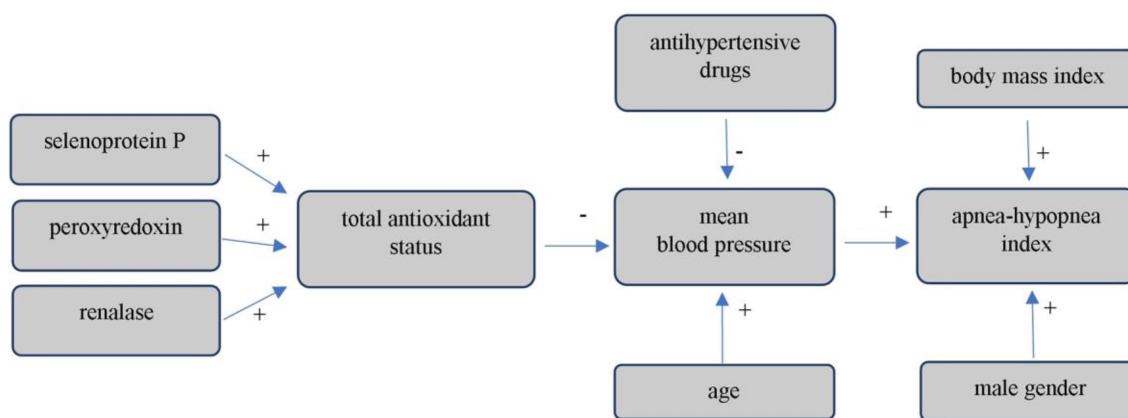

Fig. 1 Diagram summarizing the regression analysis

pressure control are one of the most effective methods to reduce the number of apnea/hypopnea episodes [36]. Our recent study showed an inversely proportional linear relationship between renalase concentration and AHI values in the entire study group, suggesting the association between OSA and renalase [20]. In this study, we further investigated this issue. We did not find significant differences between renalase levels when dividing patients by the diagnosis of OSA, or by the AHI value. However, renalase level was significantly lower in patients with mBP ≥ Me than in patients with mBP < Me. It seems that the relationship between renalase and OSA is indirect and that renalase impacts AHI through its influence on TAS and mBP.

The strengths of our research include the performance of a full polysomnographic examination in patients with clinical suspicion of obstructive sleep apnea, the inclusion of other polysomnographic examination parameters (saturation) in the analyses apart from the AHI, the performance of polysomnographic examinations in a recognized sleep laboratory, the analysis of all polysomnographic examinations by one qualified physician with extensive clinical and scientific experience, standardization of blood pressure measurement, determination of the total antioxidant status in addition to the determination of selected antioxidants, and the performance of multivariate analyses taking into account the impact of potential modifying factors on the examined relationships.

There are limitations to this study. The number of subjects involved in the study was rather small. In addition, the lack of randomization must also be considered a significant limitation. The group of patients consisted of successive patients admitted to the hospital. In the characteristics of the group, the relatively high value of the saturation index and the high percentage of patients using several antihypertensive drugs are noteworthy. The limitations of the research methodology include the lack of night-time blood pressure monitoring, as well as the subjective selection of antioxidants.

Conclusions

In our study, higher concentrations of selenoprotein P, peroxiredoxin-5, and renalase were associated with higher TAS, which confirms their antioxidant properties. The strongest correlation was found for selenoprotein P, which was also the substance that differed the most between the study subgroups, especially in the context of blood pressure. In contrast, peroxiredoxin-5 level correlated with TAS but did not differ significantly between study subgroups, which may suggest that not all antioxidants are of equal importance in this patient group.

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Data availability Data will be made available on reasonable request.

Declarations

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Bioethics Committee of Wroclaw Medical University.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no competing interests.

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Selenoprotein P, Peroxiredoxin-5, Renalase and Selected Cardiovascular Consequences Tested in Ambulatory Blood Pressure Monitoring and Echocardiography

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Abstract: This study aimed to assess the relationship between chosen antioxidants, namely seleno- protein P (SELENOP), peroxiredoxin-5 (Prdx-5), renalase and selected cardiovascular consequences tested in ambulatory blood pressure monitoring (ABPM) and echocardiography (ECHO). In our work, cardiovascular consequences refer to higher mean blood pressure (MBP) and pulse pressure (PP) on ABPM, as well as to left atrial enlargement (LAE), left ventricular hypertrophy (LVH) and lower left ventricular ejection fraction (LVEF%) on ECHO. The study group consisted of 101 consecutive patients admitted to the Department of Internal Medicine, Occupational Diseases and Hypertension to verify the diagnosis of Obstructive Sleep Apnoea (OSA). Each patient underwent full polysomnography, blood tests, ABPM and ECHO. Both selenoprotein-P and renalase levels correlated with different ABPM and ECHO parameters. We found no correlation between the peroxiredoxin-5 level and none of the tested parameters. We point to the possible application of SELENOP plasma-level testing in the initial selection of high cardiovascular-risk patients, especially if access to more advanced exami- nations is limited. We further suggest SELENOP measurement as a possible indicator of patients at increased left ventricular hypertrophy risk who should be of particular interest and may benefit from ECHO testing.

Keywords: selenoprotein P; renalase; peroxiredoxin-5; OSA; ABPM; ECHO

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1. Introduction

Increased oxidative stress is a major contributing factor in cardiovascular disease (CVD) pathogenesis. Disbalance between reactive oxygen species (ROS) and antioxidants leads to a decrease in the bioavailability of nitric oxide (NO), endothelial dysfunction, and damage to numerous cellular structures. Therefore, antioxidants are often proposed as promising agents in both the diagnosis and therapy of CVDs. Despite growing evidence supporting the role of antioxidants in CVD pathogenesis, the measurement of antioxidants is not officially recommended for initial CVD diagnosis or cardiovascular risk assessment. Selenoprotein P (SELENOP) belongs to a group of selenocysteine-containing seleno- proteins, along with glutathione peroxidases (GPx), thioredoxin reductases (TrxR) and iodothyronine deiodinases (DIO) [1]. It is mainly produced in the liver and subsequently excreted into the plasma. SELENOP, unlike other selenoproteins, contains 10 selenocysteine (Sec) residues, which constitute a substantial selenium store. One N-terminal Sec residue forms an active site of enzyme activity to reduce phospholipid hydroperoxide, while the nine C-terminal Sec residues function as Se transporters to extrahepatic tissues [2,3]. To date, three kinds of SELENOP receptors have been identified, namely, ApoER2 (LRP8), megalin (LRP2), and LRP1. 3 After entering the cell, SELENOP is degraded to amino acids

in the lysosome [3]. According to in vitro studies, SELENOP may act as a phospholipid hydroperoxide-GPx and as a peroxinitrite reductase [4]. Moreover, SELENOP-pretreated cells were protected from oxidative damage induced by tert-butyl hydroperoxide through an elevated biosynthesis of intracellular selenoenzymes [5]. Nevertheless, the exact mecha- nism of SELENOP involvement in redox balance remains unknown. Studies on SELENOP knockout (KO) mice underline the essential role of SELENOP in delivering Se to the brain and testes; however, the data on SELENOP significance in cardiovascular disease are scarce. Recent studies point to its possible involvement in the pathogenesis of Pulmonary Arterial Hypertension (PAH) [6]. It has been reported that serum levels of SeP were significantly higher in patients with PAH in comparison with control subjects. Moreover, patients with higher levels of SeP showed poorer prognoses in comparison with those with lower SeP levels. However, additional research is needed to understand the underlying mechanism behind the described correlation.

Renalase is a flavin adenine dinucleotide-dependent amine oxidase that serves as a scavenger enzyme. It oxidizes isomeric forms of β-NAD(P)H and recycles them by forming β-NAD(P)+. This action protects cells from the accumulation of β-NAD(P)H isomers that may inhibit other enzymatic reactions [7]. The importance of renalase's enzymatic activity, as well as renalase's involvement in intercellular signalling pathways, still remains poorly understood. It is yet to be discovered which enzymes are prone to inhibition by isomeric forms of β-NAD(P)H and what consequences this may have for the functioning of the cell. In our recent study, we found a positive linear relationship between the renalase blood concentration and the total antioxidant status (TAS) level, which seemed to confirm its antioxidant properties. Moreover, higher renalase levels were independently associated with TAS on regression analysis. We have summarised the latest reports on renalase's enzymatic and non-enzymatic activity in our previous work [8].

Peroxiredoxins (Prdxs) are a family of peroxidases that maintain thiol homeostasis by catalysing the reduction of organic hydroperoxides, H_2O_2, and peroxinitrite [9]. Various experimental studies have confirmed the potential of Prdxs as a therapeutic strategy against CVD [9–11]. Moreover, it was suggested that the development of derivatives or mimetics of the catalytic activity of Prdxs offers great promise for antioxidant therapy in CVD [10]. Twenty four-hour ambulatory blood pressure monitoring (ABPM) is a method to measure blood pressure continuously. According to the American and European Guide- lines on hypertension diagnosis and treatment, it is a preferable method to confirm office hypertension [12,13]. ABPM provides more reliable measurements and it helps to recog- nize masked and white coat hypertension. Moreover, increased blood pressure and pulse pressure on ABPM were found to correlate with the likelihood of cardiovascular and cere- brovascular disease and organ damage [14]. Dechen Liu et al. investigated whether PP was associated with all-cause and cause-specific mortality in a rural Chinese population. The study demonstrated that the risk of all-cause and other causes of mortality increased with increasing PP [15]. Pulse pressure was also described to predict left ventricular remodelling and left ventricular hypertrophy, particularly of the concentric type that is a significant independent risk factor for increased cardiovascular morbidity and mortality [16,17]. The use of ABPM is beneficial; however, it entails the availability of special equipment, knowl- edgeable personnel and time for interpretation, and thus, its accessibility may be limited in the outpatient care settings.

Echocardiography is an essential tool in the diagnosis, assessment and management of patients with clinical signs or symptoms of heart disease. Among hypertensive patients, it serves as a meaningful test in screening for specific signs of hypertensive heart disease, namely LV hypertrophy, LV diastolic dysfunction and LA enlargement [18]. These changes in heart morphology and function promote cardiovascular morbidity and mortality [19,20]. It is worth noting that concentric hypertrophy is related to the highest mortality and morbid- ity compared with other types of left ventricular geometry [21,22]. The concentric type of hypertrophy is characterised by elevated relative wall thickness (RWT) and increased mass indexed to body surface area (LVMI, g/m^2) both measured during ECHO. While ECHO

is highly useful, it is often not accessible in outpatient settings. In general, ECHO is recommended for hypertensive patients suspected of left ventricular hypertrophy. The office assessment of LVH may be difficult, and numerous ECG criteria may be applied; however, they differ in accuracy and have quite a low sensitivity of less than 50% [23]. For patients who have LVH but do not meet the ECG-LVH criteria, other predictive factors/clinical tests are being searched for [24].

Obstructive sleep apnoea (OSA) is a common respiratory sleep disorder characterized by recurrent partial (hypopnea) or complete (apnoea) obstruction of the upper airway dur- ing sleep, which results in intermittent hypoxia, arousals and sleep fragmentation [25,26]. Polysomnography (PSG) is the gold standard for OSA diagnosis. The PSG recording in- cludes electroencephalography, electromyography, electrooculography, pulse oximetry, electrocardiography and a microphone. The parameter that defines the severity of OSA is the apnoea/hypopnoea index (AHI), which indicates the number of episodes per hour. OSA is diagnosed if the AHI is $\geq$ 5 events per hour [25]. OSA is a recognized cause of secondary hypertension. It is independently associated with cardiovascular morbidity and mortality [27]. Disturbed sleep physiology with repeated episodes of apnoea results in sympathetic over-activation and severe inflammation, which is considered a significant cause of increased cardiovascular risk in this patient group [28].

The aim of this study was to assess the relationship between chosen antioxidants, namely selenoprotein P (SELENOP), peroxiredoxin-5 (Prdx5), renalase and selected cardio- vascular consequences tested using ambulatory blood pressure monitoring (ABPM) and echocardiography (ECHO). In our work, cardiovascular consequences refer to a higher mean blood pressure (MBP) and pulse pressure (PP) on ABPM, as well as to left atrial enlargement (LAE), left ventricular hypertrophy (LVH) and a lower left ventricular ejection fraction (LVEF%) on ECHO.

2. Materials and Methods

The research group consisted of 101 consecutive patients admitted to an internal medicine clinic to verify the diagnosis of OSA. The inclusion criteria for the study were as follows: consent to participate in the study and age $\geq$ 18 years. The exclusion criteria included the coexistence of severe systemic diseases, severe mental illness/mental disorders, and active proliferative disease. The characteristics of the study group with basic anthropometric measurements, and the diagnosis of OSA or hypertension (HTN), hypotensive treatment and mean blood selenoprotein P, renalase and peroxiredoxin-5 level are presented in Table 1.

For detailed analysis, the patients were divided into subgroups. In total, 20 subgroups (A–U) were distinguished. The division was made on the basis of the diagnosis of arterial hypertension (subgroups A and B), the diagnosis of obstructive sleep apnoea (subgroups C and D), the median level of selenoprotein P (subgroup E and F), the median level of peroxiredoxin-5 (subgroup G and H), the median level of renalase (subgroup I and J), the median of the mean blood pressure (subgroup K and L), the median pulse pressure (subgroup M and N), the median left atrium diameter (subgroup O and P), the median left ventricular ejection fraction (subgroup R and S), and the diagnosis of left ventricular hypertrophy (subgroup T and U). All selected subgroups are summarised in Table 2.

PSG was performed in the Sleep Laboratory of the Department of Internal Medicine, Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Poland, according to a diagnostic standard as a nocturnal, single-night recording, using the Nox-A1 machine (Nox Medical, Reykjavík, Iceland). Polysomnograms were assessed in 30 s epochs according to the American Academy of Sleep Medicine (AASM) 2013 standard criteria for sleep scoring. The analysis of the collected data was performed by a qualified physician (H.M.) from the Sleep Laboratory. The PSG recordings included electroencephalography, electromyography, electrooculography, pulse oximetry, electrocar- diography and a microphone. The airflow was measured using a nasal pressure transducer, and the respiratory effort of thoracoabdominal movement was measured using respiratory

inductance plethysmography. The AHI was defined as the average number of episodes of apnoea and hypopnea per hour of total sleep time (TST). Apnoea was attained with the reduction of airflow to less than 10% of the baseline for at least 10 s. A hypopnea episode was defined as a decrease in the nasal pressure signal by at least 30% from baseline for at least 10 s, with a reduction in O_2 saturation of at least 3% from the pre-event baseline or arousal.

Table 1. Characteristics of the study group (*n* = 101).

Variable	Prevalence in the Study Group
age (years) [a]	51.06 ± 13.93
height (cm) [a]	172.22 ± 10.16
body mass (kg) [a]	85.96 ± 15.36
BMI (kg/m^2) [a]	29.04 ± 5.05
BSA (m^2) [a]	1.99 ± 0.20
HTN [b]	40.6
sBP (mmHg) [a]	139.90 ± 20.83
dBP (mmHg) [a]	89.95 ± 12.44
diuretics [b]	17.8
β-blockers [b]	19.8
ACE inhibitors [b]	18.8
angiotensin receptor blockers [b]	10.9
calcium channel blockers [b]	8.9
OSA [b]	75.2
mild OSA [b]	30.7
moderate OSA [b]	20.8
severe OSA [b]	23.8
AHI (events/h) [a]	18.34 ± 18.46
type 2 diabetes [b]	8.9
coronary artery disease [b]	7.9
selenoprotein P (ng/mL) [a]	1.50 ± 1.91
peroxiredoxin-5 (ng/mL) [a]	1.48 ± 3.67
renalase (ng/mL) [a]	178.56 ± 208.00

[a]—values represent mean ± standard deviation, [b]—values represent percentages, AHI—apnoea-hypopnea index, BMI—body mass index, BSA—body surface area, dBP—diastolic blood pressure, HTN—arterial hypertension, OSA—obstructive sleep apnoea, sBP—systolic blood pressure.

Blood was collected in the morning after polysomnography, usually by puncturing the veins of the ulna. Until renalase determinations were performed simultaneously in all samples, the blood was stored at a constant temperature. Serum renalase determinations were performed using the E3109Hu kit ELISA (enzyme-linked immunosorbent assay) (Bioassay Technology Laboratory, Shanghai, China). The determinations were made strictly according to the test manufacturer's instructions. The renalase concentration was expressed as nanogram per millilitre (ng/mL). The reference range of the assay used was 1–400 ng/mL. According to the manufacturer, the sensitivity of the ELISA test used was 0.52 ng/mL. The coefficient of intra- and inter-assay variation was <8% and <10%, respectively.

Serum selenoprotein P determinations were performed using the E1809h ELISA Kit for Human SeP (ElAab, East Lake Hi-Tech Development Zone, Wuhan, China). The determinations were made strictly according to the test manufacturer's instructions. The selenoprotein P concentration was expressed as a nanogram per millilitre (ng/mL). The coefficient of intra- and inter-assay variation was <4.9% and <7.1%, respectively.

Table 2. Criteria for selecting subgroups.

	Subgroup	Classification Criterion	Subgroup Size
division by HTN diagnosis	A	diagnosed with HTN	60
	B	without HTN	41
division by OSA diagnosis	C	diagnosed with OSA	76
	D	without OSA	25
division by median of selenoprotein P	E	≥median of selenoproteins P (≥0.64 ng/mL)	51
	F	<median of selenoproteins P (<0.64 ng/mL)	50
division by median of peroxiredoxin	G	≥median of peroxiredoxin (≥0.77 ng/mL)	52
	H	<median of peroxiredoxin (<0.77 ng/mL)	49
division by median of renalase	I	≥median of renalase (≥60.43 ng/mL)	51
	J	<median of renalase (<60.43 ng/mL)	50
division by median of MBP	K	≥median of MBP (≥93.47 ng/mL)	51
	L	<median of MBP (<93.47 ng/mL)	50
division by median of PP	M	≥median of PP (≥51.00 mmHg)	53
	N	<median of PP (<51.00 mmHg)	48
division by median of LA	O	≥median of LA (≥43.00 mm)	53
	P	<median of LA (<43.00 mm)	48
division by median of LVEF	R	≥median of LVEF (≥66%)	54
	S	<median of LVEF (<66%)	57
division by diagnosis of LVH	T	diagnosed LVH	64
	U	without LVH	37

HTN—arterial hypertension, LA—left atrium diameter, LVEF—left ventricular ejection fraction, LVH—left ventricular hypertrophy, MBP—mean blood pressure, OSA—obstructive sleep apnoea, PP—pulse pressure.

Serum peroxiredoxin-5 determinations were performed using the E0703h ELISA Kit for Human Peroxiredoxin-5, mitochondrial (ElAab, East Lake Hi-Tech Development Zone, Wuhan, China). The determinations were made strictly according to the test manufacturer's instructions. The peroxiredoxin-5 concentration was expressed as a nanogram per millilitre (ng/mL). The reference range of the assay used was 0.78–50 ng/mL.

In every examined individual, 24 h ambulatory blood pressure monitoring was per- formed using the Welch Allyn ABPM 6100 system (Welch Allyn, UK, Aston Abbotts, Buckinghamshire, UK). The studied variables included the mean blood pressure (MBP), mean systolic blood pressure (MSBP), mean diastolic blood pressure (MDBP), variability of systolic blood pressure (VSBP), variability of diastolic blood pressure (VDBP) and pulse pressure (PP). Pulse pressure was calculated as the difference between MSBP and MDBP. Standard deviation (SD) from all measurements of systolic/diastolic blood pressure, taken at 30 min intervals, was accepted as a measure of VSBP and VDBP.

Transthoracic echocardiography was performed using the ALOKA ProSound SSD-5500 SV, equipped with a 3.5/2.7 MHz transducer (Aloka Inc., Tokyo, Japan). The results were evaluated using the criteria of the American Society of Echocardiography (ACC/AHA, 1990). Using an M-mode echocardiogram, following Penn convention, left ventricular end-diastolic diameter (LVEDd) and left ventricular end-systolic diameter (LVESd), interventricular septum diastolic diameter (IVSDd) and posterior wall diastolic diameter (PWDd) were measured. The mean of three measurements was recorded with an accuracy of 1 mm. The ejection fraction (EF) was determined from the api- cal four-chamber and two-chamber views, with the biplane Simpson's method. The left ventricular mass (LVM), expressed in grams, was calculated using the formula sug- gested by the American Society of Echocardiography (ASE), modified by Devereux et al.

(1986): LVM = $0.8 \times [1.04 \times (LVEDd + PWDd + IVSDd)^3 - LVEDd^3] + 0.6$ (LVEDd, PWDd, and IVSDd expressed in centimetres). The left ventricular mass index (LVMI) was calculated, by dividing the LVM value by the body surface area (BSA), expressed in square meters. The body surface area was calculated using the formula of Du Bois: BSA [m^2] = 0.007184
× (body weight [kg]) 0.425 × (body height [cm]) 0.725. The relative wall thickness (RWT) was calculated using the following formula: RWT = (IVSDd + PWDd)/LVEDd. The results of ABPM and ECHO in the study group are presented in Table 3.

Table 3. Selected parameters of 24-h ambulatory blood pressure monitoring and echocardiography in the study group (n = 101).

Measured Parameter	Results
MSBP (mmHg) [a]	131.57 ± 19.02
MDBP (mmHg) [a]	76.70 ± 10.63
MBP (mmHg) [a]	94.81 ± 12.68
VSBP (mmHg) [a]	13.78 ± 4.19
VDBP (mmHg) [a]	11.31 ± 3.78
PP (mmHg) [a]	54.87 ± 12.45
LVEDD (mm) [a]	51.24 ± 4.83
LVESD (mm) [a]	31.80 ± 4.28
IVSEDD (mm) [a]	12.81 ± 2.26
PWEDD (mm) [a]	11.21 ± 2.32
LA (mm) [a]	42.03 ± 4.66
Ao (mm) [a]	35.13 ± 3.95
LVEF (%) [a]	65.67 ± 4.63
LVMI (g/m^2) [a]	115.61 ± 41.78
RWT [a]	0.47 ± 0.09
LVH [b]	63.4

[a]—values represent mean ± standard deviation, [b]—values represent percentages, Ao—aortic bulb diameter, IVSEDD—interventricular septum end-diastolic diameter, LA—left atrium diameter, LVEDD—left ventricular end-diastolic diameter, LVEF—left ventricular ejection fraction, LVESD—left ventricular end-systolic diameter, LVH—left ventricular hypertrophy, LVMI—left ventricular mass index, MBP—mean blood pressure, MDBP— mean diastolic blood pressure, MSBP—mean systolic blood pressure, PP—pulse pressure, PWEDD—posterior wall diastolic diameter, RWT—relative wall thickness, VDBP—variability of diastolic blood pressure, VSBP—variability of systolic blood pressure.

Statistical analyses were conducted using the Dell Statistica 13 software (Dell Inc., Round Rock, TX, USA). The quantitative variables were expressed as means and standard deviations. The qualitative variables were expressed as percentages. The distribution of variables was tested with the W-Shapiro–Wilk test. In the case of the quantitative variables of normal distribution, a further statistical analysis was performed using the t-test. For non-normally distributed quantitative variables, the Mann–Whitney U test was used. For qualitative variables, the chi-square test of maximum likelihood was used. To determine the relationship between the examined variables, correlation and regression analyses were conducted. In the case of normal distribution, the Pearson correlation r factors were determined whereas, in the case of non-normal distribution, the Spearman r factors were applied. Multivariable stepwise backward regression was used to identify possible predictor variables for pulse pressure, left atrium diameter and left ventricular hypertrophy. Three separate analyses were performed with PP, LA and LVH as dependent variables. At each step, independent variables were removed from the model based on p-values. Results with $p < 0.05$ were considered statistically significant.

Ethical approval for this study was obtained from the Bioethics Committee of Wroclaw Medical University. Informed consent was obtained from all subjects before the study.

ClinicalTrials.gov Identifier: NCT05040516.

3. Results

In our study, patients with diagnosed hypertension (subgroup A) had statistically significantly lower selenoprotein P levels than patients without HT (subgroup B). Levels of peroxiredoxin-5 and renalase did not differ significantly between these groups. In terms of ABPM/ECHO parameters, patients with elevated blood pressure had significantly higher MBP, PP, and LA diameter and were more likely to have left ventricular hypertrophy. These results are shown in Table 4.

Table 4. Selenoprotein P, peroxiredoxin-5, renalase, selected parameters of the ABPM and echocardio- graphy in the study subgroups divided based on the criteria of arterial hypertension and obstructive sleep apnoea: A vs. B (diagnosed arterial hypertension vs. without arterial hypertension) and C vs. D (diagnosed obstructive sleep apnoea vs. without obstructive sleep apnoea).

Subgroup	SELENOP (ng/mL) [a]	Prdx-5 (ng/mL) [a]	Renalase (ng/mL) [a]	MBP (mmHg) [a]	PP (mmHg) [a]	LA (mm) [a]	LVEF (%) [a]	LVH [b]
A	0.95 ± 0.92	1.27 ± 1.43	159.16 ± 207.19	97.39 ± 11.81	56.98 ± 12.73	44.51 ± 4.39	65.44 ± 4.86	78.0
B	1.87 ± 2.29	1.62 ± 4.63	191.81 ± 209.24	91.78 ± 13.25	52.80 ± 12.37	39.70 ± 4.84	65.83 ± 4.50	53.0
p A-B	<0.05	ns	ns	<0.05	<0.05	<0.05	ns	<0.05
C	1.44 ± 1.95	1.61 ± 4.15	167.37 ± 197.29	95.09 ± 12.98	56.00 ± 12.42	42.46 ± 4.51	65.36 ± 4.88	71.0
D	1.69 ± 1.82	1.96 ± 1.50	212.56 ± 238.79	93.98 ± 11.93	51.44 ± 12.18	40.72 ± 4.93	66.64 ± 3.67	40.0
p C-D	ns	ns	ns	ns	<0.05	ns	ns	<0.05

[a]—values represent mean ± standard deviation, [b]—values represent percentages, LA—left atrium diameter, LVEF—left ventricular ejection fraction, LVH—left ventricular hypertrophy, MBP—mean blood pressure, PP— pulse pressure.

When dividing patients by the diagnosis of OSA (subgroup C and D), we did not find any significant differences in selenoprotein P, peroxiredoxin-5 and renalase levels, as well as in MBP values, LA diameter and the occurrence of LVH. However, patients with OSA (subgroup C) had significantly higher PP and the possibility of LVH. These results are shown in Table 4.

Comparison of results based on the criteria of median selenoprotein P, peroxiredoxin-5 and renalase levels showed that patients with selenoprotein P level ≥ median (subgroup E) had lower MBP, PP and LA diameter values and were less likely to develop LVH than patients with selenoprotein P level < median (subgroup F). None of the considered parameters differed significantly between patients with peroxiredoxin level ≥ median (subgroup G) and < median (subgroup H). Division by the median of renalase disclosed significantly lower PP and LA diameter values in patients with renalase level ≥ median (subgroup I) than in the subgroup with lower renalase levels (subgroup J); there were no differences in MBP, LVEF and LVH incidence between these subgroups. The analysis of selected ABPM/ECHO parameters based on the division by laboratory parameters is presented in Table 5.

No differences in laboratory parameters (SELENOP, peroxiredoxin-5 and renalase) levels were found when dividing patients by the median of MBP (subgroup K and L). However, both selenoprotein P and renalase levels were notably lower in patients with PP value ≥ the median (subgroup M) than in patients with lower PP values (subgroup N). Division by the median of MBP showed no differences between these subgroups in terms of LVH incidence. On the contrary, patients with higher PP (subgroup M) had a notably higher incidence of LVH than patients with PP lower than the median (subgroup N). These results are shown in Table 6.

Table 5. Selected parameters of the ABPM and echocardiography in the study subgroups divided based on the criteria of median selenoprotein P, peroxiredoxin-5 and renalase: E vs. F (≥median of selenoprotein P vs. <median of selenoprotein P), G vs. H (≥median of peroxiredoxin-5 vs. <median of peroxiredoxin-5) and I vs. J (≥median of renalase vs. <median of renalase).

Subgroup	MBP (mmHg) [a]	PP (mmHg) [a]	LA (mm) [a]	LVEF (%) [a]	LVH [b]
E	91.54 ± 11.07	50.72 ± 9.50	40.12 ± 4.49	66.52 ± 4.22	50.0
F	98.02 ± 13.42	58.94 ± 13.70	43.90 ± 4.05	64.84 ± 4.89	76.5
p E-F	<0.05	<0.05	<0.05	ns	<0.05
G	94.58 ± 12.35	53.82 ± 10.79	42.76 ± 4.85	65.24 ± 4.28	59.2
H	95.03 ± 13.10	55.87 ± 13.87	41.35 ± 4.41	66.08 ± 4.94	67.3
p G-H	ns	ns	ns	ns	ns
I	94.90 ± 12.78	52.51 ± 10.51	40.86 ± 4.85	65.57 ± 4.18	56.9
J	94.72 ± 12.70	57.28 ± 13.86	43.22 ± 4.17	65.78 ± 5.08	70.0
p I-J	ns	<0.05	<0.05	ns	ns

[a]—values represent mean ± standard deviation, [b]—values represent percentages, LA—left atrium diameter, LVEF—left ventricular ejection fraction, LVH—left ventricular hypertrophy, MBP—mean blood pressure, PP— pulse pressure.

Table 6. Selenoprotein P, peroxiredoxin-5, renalase and selected parameters of the echocardiography in the study subgroups divided based on the criteria of selected parameters of the ABPM: K vs. L (≥median of mean blood pressure vs. <median of mean blood pressure) and M vs. N (≥median of pulse pressure vs. <median of pulse pressure).

Subgroup	SELENOP (ng/mL) [a]	Prdx-5 (ng/mL) [a]	Renalase (ng/mL) [a]	LA (mm) [a]	LVEF (%) [a]	LVH [b]
K	1.28 ± 1.66	1.77 ± 5.01	184.68 ± 206.70	40.04 ± 4.34	65.47 ± 5.17	66.7
L	1.73 ± 2.13	1.17 ± 1.32	172.32 ± 211.23	44.02 ± 5.01	65.30 ± 3.66	60.0
p K-L	ns	ns	ns	<0.05	ns	ns
M	0.80 ± 1.04	1.68 ± 4.72	143.45 ± 176.30	43.64 ± 3.98	64.95 ± 5.09	77.6
N	2.45 ± 2.37	1.20 ± 1.31	225.92 ± 238.38	39.86 ± 4.66	66.65 ± 3.75	44.2
p M-N	<0.05	ns	<0.05	<0.05	ns	<0.05

[a]—values represent mean ± standard deviation, [b]—values represent percentages, LA—left atrium diameter, LVEF—left ventricular ejection fraction, LVH—left ventricular hypertrophy.

Analysis based on selected ECHO parameters showed that selenoprotein P and renalase levels were significantly lower in patients with LA diameter ≥ median (subgroup O) than in patients with LA diameter < Me, as well as in patients with diagnosed LVH (subgroup T) than in subjects without LVH (subgroup U). Patients with lower LVEF% (sub- group R) had markedly lower PP values than patients with LVEF% ≥ median (subgroup S). On the other hand, PP was significantly higher in patients with higher LA diameter (subgroup O) and in patients with LVH (subgroup T). None of the tested laboratory param- eters differed significantly between people with lower or higher LVEF% (groups R vs. S). Analysis based on selected ECHO parameters is presented in Table 7.

In this study, negative linear correlation was found between selenoprotein P and certain ABPM and ECHO parameters, namely: MSBP ($r = -0.33$, $p < 0.05$), MDBP ($r = -0.21$, $p < 0.05$), MBP ($r = -0.28$), $p < 0.05$), PP ($r = -0.32$, $p < 0.05$), IVSEDD ($r = -0.28$, $p < 0.05$), PWEDD ($r = -0.27$, $p < 0.5$), LA ($r = -0.3$, $p < 0.05$) and LVMI ($r = -0.33$, RWT ($r = -0.2$,$p < 0.05$). Renalase level correlated in a negative linear manner with PP ($r = -0.22$, $p < 0.05$), IVSEDD ($r = -0.2$, $p < 0.05$) and LA ($r = -0.22$, $p < 0.05$). The results of correlation analysis are presented in Table 8.

Table 7. Selenoprotein P, peroxiredoxin-5, renalase and selected parameters of the ABPM in the study subgroups divided based on the criteria of selected parameters of the echocardiography: O vs. P (≥median of left atrium diameter vs. <median of left atrium diameter), R vs. S (≥median of left ventricular ejection fraction vs. <median of left ventricular ejection fraction) and T vs. U (diagnosed left ventricular hypertrophy vs. without left ventricular hypertrophy).

Subgroup	SELENOP (ng/mL) [a]	Prdx-5 (ng/mL) [a]	Renalase (ng/mL) [a]	MBP (mmHg) [a]	PP (mmHg) [a]
O	0.85 ± 1.07	1.83 ± 4.89	123.55 ± 172.81	96.06 ± 13.06	57.81 ± 12.24
P	2.23 ± 2.34	1.08 ± 1.40	239.30 ± 227.66	93.43 ± 12.23	51.63 ± 11.99
p O-P	<0.05	ns	<0.05	ns	<0.05
R	1.35 ± 1.59	1.74 ± 5.12	177.66 ± 204.45	95.13 ± 13.71	57.45 ± 13.07
S	1.60 ± 2.08	1.25 ± 1.61	179.34 ± 212.94	94.92 ± 11.04	52.63 ± 11.55
p R-S	ns	ns	ns	ns	<0.05
T	1.02 ± 1.54	1.58 ± 4.47	131.18 ± 157.18	97.21 ± 13.53	57.06 ± 13.29
U	2.33 ± 2.21	1.29 ± 1.57	260.51 ± 257.00	90.67 ± 9.91	51.08 ± 10.37
p T-U	<0.05	ns	<0.05	<0.05	<0.05

[a]—values represent mean ± standard deviation, MBP—mean blood pressure, PP—pulse pressure.

Table 8. The results of the correlation analysis in the study group. The table shows statistically significant correlation coefficients.

Tested Parameter	Selenoprotein P (ng/mL)	Peroxiredoxin-5 (ng/mL)	Renalase (ng/mL)
MSBP (mmHg)	−0.33	ns	ns
MDBP (mmHg)	−0.21	ns	ns
MBP (mmHg)	−0.28	ns	ns
VSBP (mmHg)	ns	ns	ns
VDBP (mmHg)	ns	ns	ns
PP (mmHg)	−0.32	ns	−0.22
LVEDD (mm)	ns	ns	ns
LVESD (mm)	ns	ns	ns
IVSEDD (mm)	−0.28	ns	−0.20
PWEDD (mm)	−0.27	ns	ns
LA (mm)	−0.30	ns	−0.22
Ao (mm)	ns	ns	ns
LVEF (%)	ns	ns	ns
LVMI (g/m^2)	−0.33	ns	ns
RWT	−0.20	ns	ns

Ao—aortic bulb diameter, IVSEDD–interventricular septum end-diastolic diameter, LA—left atrium diameter, LVEDD–left ventricular end-diastolic diameter, LVEF—left ventricular ejection fraction, LVESD—left ventricular end-systolic diameter, LVMI—left ventricular mass index, MBP–mean blood pressure, MDBP—mean diastolic blood pressure, MSBP—mean systolic blood pressure, PP–pulse pressure, PWEDD—posterior wall diastolic diameter, RWT—relative wall thickness, VDBP–variability of diastolic blood pressure, VSBP–variability of systolic blood pressure.

A regression analysis was performed for three different dependent variables: PP, LA and LVH. A final model obtained for PP as a dependent variable was:

$$PP = 44.801 - 3.757\ \text{antihypertensive drugs} - 1.876\ \text{selenoprotein P} - 0.011\ \text{renalase} + 0.118\ \text{age} + 0.355\ \text{BMI}$$

The obtained model demonstrated that no antihypertensive drug therapy, low selenoprotein P level, low renalase level, more advanced age and higher BMI are independent risk factors for elevated pulse pressure.

A final model obtained for LA as a dependent variable was:

$$LA = 38.929 + 0.045\ \text{age} + 0.004\ \text{BMI} + 1.762\ \text{male gender} + 0.111\ \text{PP}$$

Based on the obtained regression model, it was shown that more advanced age, higher BMI and male gender represented independent risk factors for greater left atrium diameter.

A final model obtained for LVH was:

$$P\ (LVH) = 0.079 - 1.448\ \text{antihypertensive drugs} + 1.210\ \text{OSA} + 0.043\ \text{PP}$$

The obtained regression model indicated that no antihypertensive drug therapy, diagnosis of obstructive sleep apnoea and elevated pulse pressure represented independent risk factors for left ventricular hypertrophy.

Detailed results of regression analysis in the study group are presented in Table 9. Figure 1 shows a diagram summarizing the results of the regression analysis. This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

Table 9. Results of regression analysis in the study group.

Multivariable Stepwise Backward Regression Analysis Model for: PP (mmHg)			
	Rc	SEM of Rc	*p*
intercept	44.801	8.308	< 0.001
age (years)	0.118	0.043	<0.05
BMI (kg/m^2)	0.355	0.133	<0.05
antihypertensives drugs	−3.757	1.250	<0.01
SELENOP (ng/mL)	−1.876	0.636	<0.01
renalase (ng/mL)	−0.011	0.004	<0.05
	$p < 0.01$		
Multivariable Stepwise Backward Regression Analysis Model for: LA (mm)			
	Rc	SEM of Rc	*p*
intercept	38.929	3.254	<0.001
age (years)	0.045	0.022	<0.05
BMI (kg/m^2)	0.004	0.001	<0.01
male gender	1.762	0.792	<0.05
PP (mmHg)	0.111	0.037	<0.01
	$p < 0.01$		
Logistic Regression Analysis Model for:			
Probability of LVH			
	Rc	SEM of Rc	*p*
BMI (kg/m^2)	0.079	0.021	<0.01
antihypertensives drugs	−1.448	0.321	<0.01
OSA	1.210	0.514	<0.05
PP (mmHg)	0.043	0.020	<0.05
	$p < 0.05$		

BMI—body mass index, LA—left atrium diameter, LVH—left ventricular hypertrophy, OSA—obstructive sleep apnoea, PP—pulse pressure, Rc—regression coefficient, SEM—standard error of mean.

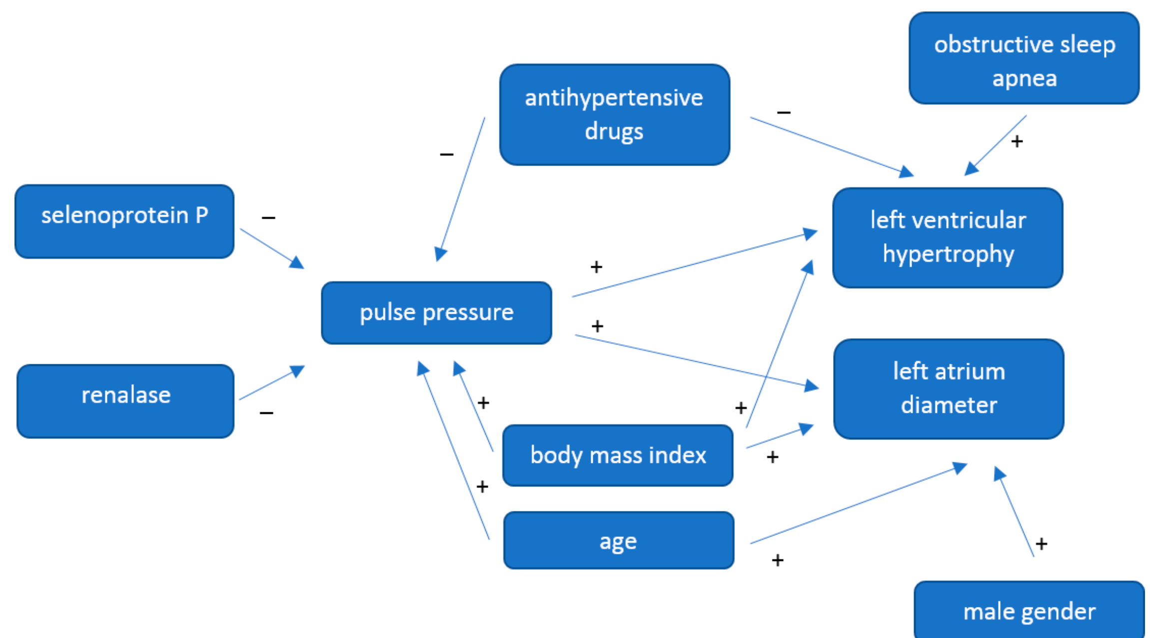

Figure 1. Diagram summarizing the regression analysis.

4. Discussion

The key finding of this study is that both selenoprotein-P and renalase levels correlated significantly with different ABPM and ECHO parameters. We found no correlation between the peroxiredoxin-5 level and the tested parameters.

Peroxiredoxins (Prdxs) are a superfamily of selenium-free and haeme-free peroxidases able to catalyse the reduction of hydrogen peroxide, alkyl hydroperoxides and peroxini- trite [29]. In humans, the PRDXs family comprises six isoforms (Prdx1–6), of which all have been proposed to be involved in CVD pathogenesis [11]. Prdx 1, 2 and 4 were described to play protective roles in the development of atherosclerosis [11]. The overexpression of Prdx3 was found to protect the heart against left ventricular remodelling and failure after myocardial infarction. Prdx6-deficient mice had increased susceptibility to ischemia- reperfusion injury [11]. Prdx5 was the last family member to be identified. It is thought to exert antioxidative and cytoprotective properties; however, the exact mechanism of its function and its importance remain to be fully elucidated [30]. Hu C. et al. described that Prx5 expression was upregulated in hypertrophic hearts and cardiomyocytes. In addition, Prx5 knockdown accelerated pressure overload-induced cardiac hypertrophy and dysfunc- tion in mice by activating oxidative stress and cardiomyocyte apoptosis [31]. Kunze A et al. reported on decreased Prdx5 levels in severe stroke [32]. In this study, we failed to find any significant correlations between PRDx5 plasma level and the tested parameters. Thus, at this point, we do not recommend the use of Prdx5 plasma level measurement to be used in selecting patients with an increased risk of cardiovascular complications in hypertensive patients.

In our recent work, we have reported that a higher SELENOP level was independently associated with higher plasma TAS, and TAS was further associated with lower mean blood pressure values. The obtained results suggested an indirect connection between the SELENOP level and blood pressure. In this study, we report that hypertensive patients had significantly lower SELENOP levels than normotensive patients ($p < 0.05$). In the light of our previous results, the association between SELENOP and mean blood pressure may possibly be explained by SELENOP's involvement in maintaining the right plasma TAS and keeping the redox balance.

In terms of the ABPM parameters, patients with SELENOP levels higher than the median had a notably lower mean blood pressure and pulse pressure when compared to the patients with lower SELENOP levels. We also found a negative linear correlation

between SELENOP and mean systolic blood pressure (r = −0.33, p < 0.05), mean diastolic blood pressure (r = −0.21, p < 0.05), mean blood pressure (r = −0.28), p < 0.05) and pulse pressure (r = −0.32, p < 0.05). Furthermore, regression analysis revealed that low SELENOP levels was an independent risk factor for higher PP with a p-value under 0.01. In sum, a lower SELENOP level was associated with unfavourable cardiovascular consequences measured on ABPM–higher MBP, MDBP, MSBP and PP. This result points to the possible applicability of SELENOP measurement in the initial selection of high cardiovascular-risk patients, especially if access to more advanced examinations is limited. Proper cardiovascular risk stratification remains a fundamental step in the effort to reduce morbidity and mortality from cardiovascular diseases (CVDs) [33]. With a constantly growing number of people suffering from CVDs, it is challenging to choose patients at the highest risk of premature death and to tailor the right treatment. Interestingly, our results stay in line with the findings of a study carried out by Schomburg et al. in which SELENOP deficiency predicted cardiovascular morbidity and mortality [34]. It was a population-based prospective cohort study, which included 4366 subjects during a median follow-up time of 9.3 (8.3–11) years. The 20% of subjects with the lowest SELENOP concentrations without a history of cardiovascular disease had markedly increased risk of cardiovascular morbidity and mortality. It is worth noting that the mean concentration of SELENOP within our study group was quite low and would be considered the lowest quantile from the Schomburg study.

In terms of ECHO parameters, we have found a negative linear correlation between SELENOP levels and both LVMI (r = −0.33) and RWT (r = 0.20). Moreover, patients with SELENOP levels higher than the median had notably lower left atrium diameter and were less likely to develop left ventricular hypertrophy (p < 0.05). Based on the information provided, we suggest SELENOP measurement as a possible indicator of patients at increased LVH risk who should be of particular interest and may benefit from ECHO testing. The application of SELENOP measurement in the risk stratification seems more achievable than aiming to increase its level with therapy. Selenium supplementation may be a promising strategy in preventing hypertension and its cardiovascular consequences; nevertheless, this issue is more complex. Data on selenium supplementation are inconclusive as both beneficial and harmful effects have been reported [35–40]. It is suggested that subjects with low selenium levels at baseline could benefit from supplementation; on the contrary, those with an adequate or high status might be negatively affected [40].

The notion that renalase serves to degrade catecholamines has been dismissed; however, many studies still point to its involvement in blood pressure regulation. The reports on this subject are inconclusive. Many studies indicate higher renalase concentration in hypertensive patients [41,42], and many studies show contrary results [43,44]. Our analysis revealed that patients with a renalase level ≥ median (subgroup I) had significantly lower PP and LA diameter values than patients in the subgroup with a lower renalase level (subgroup J). Moreover, renalase levels correlated in a linear negative manner with PP (r = −0.22, p < 0.05) and a low renalase level was an independent risk factor for higher pulse pressure in the regression analysis with a p-value under 0.05. We thus hypothesise that renalase may be more involved in keeping the balance between systolic and diastolic blood pressure rather than in regulating crude blood pressure values. However, taking into consideration the complexity of renalase activity and its enzymatic and non-enzymatic properties, many more studies are needed to elucidate this issue.

In our study, independent risk factors for LVH included a higher pulse pressure on ABPM, higher body mass index (BMI), lack of antihypertensive treatment and the diagnosis of obstructive sleep apnoea (OSA). Interestingly, mean blood pressure values were less effective in predicting LVH than pulse pressure; thus, we propose PP as the preferable parameter to be used in CV risk stratification and selecting patients who require further care and treatment. Independent risk factors for left atrium enlargement included higher PP and higher BMI, but also more advanced age and male gender. These results stay consistent with other studies on LA diameter, BMI and gender [45].

Obstructive sleep apnoea was an independent risk factor for LVH. It should be under- lined that when dividing patients by the diagnosis of OSA (groups C and D), we did not find any differences in MBP values; however, patients with OSA had significantly higher
PP. We indicate OSA patients as the group of special interest and recommend their PP to be checked even if blood pressure remains within the normal range. Interestingly SELENOP levels did not differ significantly between patients with and without OSA, suggesting that SELENOP may rather be involved in the pathomechanism of primary than secondary hypertension.

This study has some limitations. In terms of the study group, the limitations are the small size of the group and the relatively small percentage of patients without OSA and without abnormal body weight. In terms of the research methodology, the limitations of the ABPM analysis include the absence of separate measurements from the hours of daily activity and the measurements from the hours of night rest, the lack of assessment of the diastolic function of the left ventricle in the ECHO examination and the subjective selection of antioxidants.

5. Conclusions

In our study, both selenoprotein-P (SELENOP) and renalase levels correlated significantly with different ambulatory blood pressure monitoring (ABPM) and echocardiography (ECHO) parameters. We found no correlation between the peroxiredoxin-5 level and the tested parameters. A lower SELENOP level was associated with unfavourable cardiovascu- lar consequences measured on ABPM–higher mean blood pressure, mean diastolic blood pressure, mean systolic blood pressure and pulse pressure, as well as on ECHO–increased left ventricle mass index and relative wall thickness. We point to the possible application of SELENOP plasma-level testing in the initial selection of high cardiovascular-risk patients, especially if access to more advanced examinations is limited. We further suggest SELENOP measurement as a possible indicator of patients at increased left ventricular hypertrophy risk who should be of particular interest and may benefit from ECHO testing. In this study group, renalase levels did not differ significantly between subjects with or without diagnosed hypertension. However, our analysis revealed that renalase level correlated in a linear negative manner with pulse pressure, and low renalase level was an independent risk factor for higher pulse pressure in the regression analysis. Given that reports on renalase are inconsistent, its clinical use appears to be delayed until its exact function is recognised.

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OPINIA KOMISJI BIOETYCZNEJ

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 39/2020

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

prof. dr hab. Jacek Daroszewski (choroby wewnętrzne, endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód, prof. nadzw. (duchowny)
mgr Luiza Müller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
prof. dr hab. Leszek Szenborn, (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.

„Znaczenie aktywności selenoproteinaz i stężenia renalazy we krwi w patogenezie nadciśnienia tętniczego i obturacyjnego bezdechu sennego"

zgłoszonym przez **lek. Karolinę Czerwińską** uczestniczkę szkoły doktorskiej w Katedrze i Zakładzie Higieny Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła wyrazić zgodę na przeprowadzenie badania w Klinice Chorób Wewnętrznych, Zawodowych, Nadciśnienia Tętniczego i Onkologii Klinicznej Uniwersyteckiego Szpitala Klinicznego im. Jana Mikulicza-Radeckiego we Wrocławiu oraz Katedrze i Zakładzie Higieny Uniwersytetu Medycznego we Wrocławiu pod nadzorem dr. hab. Pawła Gacia **pod warunkiem zachowania anonimowości uzyskanych danych**.

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności:

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu

Opinia powyższa dotyczy: projektu badawczego będącego podstawą rozprawy doktorskiej

Wrocław, dnia 27 stycznia 2020 r.

BW

Uniwersytet Medyczny we Wrocławiu
KOMISJA BIOETYCZNA
przewodniczący
prof. dr hab. Jan Kornafel

OŚWIADCZENIA WSPÓŁAUTORÓW

Wrocław, 16.08.2023r.

lek. Karolina Czerwińska

Katedra Zdrowia Populacyjnego
Zakład Zdrowia Środowiskowego i Medycyny Pracy
Uniwersytetu Medycznego we Wrocławiu
ul. Jana Mikulicza-Radeckiego
50-345 Wrocław

OŚWIADCZENIE O WSPÓŁAUTORSTWIE

Oświadczam, że w pracy:

1. Karolina Czerwińska, Rafał Poręba, Paweł Gać. **Renalase—A new understanding of its enzymatic and non- enzymatic activity and its implications for future research.** Clin Exp Pharmacol Physiol. 2022 Jan;49(1):3-9. doi: 10.1111/1440-1681.13594. Epub 2021 Oct 19. PMID: 34545616.

   Mój udział polegał na opracowaniu koncepcji i metodologii badania, analizie dostępnego piśmiennictwa, przygotowaniu manuskryptu oraz rycin.

2. Karolina Czerwińska, Lidia Januszewska, Iwona Markiewicz-Górka, Aleksandra Jaremków, Helena Martynowicz, Krystyna Pawlas, Grzegorz Mazur, Rafał Poręba, Paweł Gać. **Selenoprotein P, peroxiredoxin-5, renalase, and total antioxidant status in patients with suspected obstructive sleep apnea**. Sleep Breath. 2023 Jul 26. doi: 10.1007/s11325-023-02880-7. Epub ahead of print. PMID: 37495908.

   Mój udział polegał na opracowaniu koncepcji badania, tworzeniu bazy danych, przeglądzie piśmiennictwa związanego z tematem pracy, analizie i interpretacji uzyskanych danych, przygotowaniu i korektach manuskryptu.

3. Karolina Czerwińska, Lidia Januszewska, Iwona Markiewicz-Górka, Aleksandra Jaremków, Helena Martynowicz, Krystyna Pawlas, Grzegorz Mazur, Rafał Poręba, Paweł Gać. **Selenoprotein P, peroxiredoxin-5, renalase and selected cardiovascular consequences tested in ambulatory blood pressure monitoring and echocardiography.** Antioxidants 2023, 12(6), 1187; doi: 10.3390/antiox12061187.

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Karolina Czerwińska

Uniwersytet Medyczny we Wrocławiu
Katedra Zdrowia Populacyjnego
ZAKŁAD ZDROWIA ŚRODOWISKOWEGO
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Wrocław, 16.08.2023r.

Dr hab. n. med. Paweł Gać, prof. UMW

Katedra Zdrowia Populacyjnego
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Uniwersytet Medyczny we Wrocławiu
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OŚWIADCZENIE O WSPÓŁAUTORSTWIE

Oświadczam, że w pracy:

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Wrocław, 28.08.2023 r.

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Helena Martynowicz

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Wrocław, 16.08.2023r.

Prof. dr hab. n. med. Krystyna Pawlas

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Wrocław, 16.08.2023r.

Dr n. o zdr. Aleksandra Jaremków

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Uniwersytetu Medycznego we Wrocławiu
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Uniwersytet Medyczny we Wrocławiu
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adiunkt

dr Aleksandra Jaremków

Uniwersytet Medyczny we Wrocławiu
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Wrocław, 16.08.2023r.

Dr n. biol. med. Iwona Markiewicz-Górka

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Wrocław, 16.08.2023r.

Mgr Lidia Januszewska

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Zakład Zdrowia Środowiskowego i Medycyny Pracy
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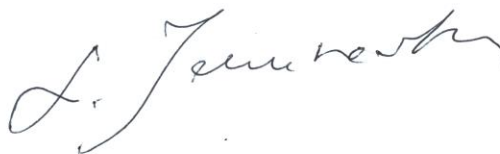
OŚWIADCZENIE O WSPÓŁAUTORSTWIE

Oświadczam, że w pracy:

1. Karolina Czerwińska, Lidia Januszewska, Iwona Markiewicz-Górka, Aleksandra Jaremków, Helena Martynowicz, Krystyna Pawlas, Grzegorz Mazur, Rafał Poręba, Paweł Gać. **Selenoprotein P, peroxiredoxin-5, renalase, and total antioxidant status in patients with suspected obstructive sleep apnea**. Sleep Breath. 2023 Jul 26. doi: 10.1007/s11325-023-02880-7. Epub ahead of print. PMID: 37495908.
   Mój udział polegał na wykonywaniu analiz laboratoryjnych oraz wsparciu w tworzeniu bazy danych.

2. Karolina Czerwińska, Lidia Januszewska, Iwona Markiewicz-Górka, Aleksandra Jaremków, Helena Martynowicz, Krystyna Pawlas, Grzegorz Mazur, Rafał Poręba, Paweł Gać. **Selenoprotein P, peroxiredoxin-5, renalase and selected cardiovascular consequences tested in ambulatory blood pressure monitoring and echocardiography.** Antioxidants 2023, 12(6), 1187; doi: 10.3390/antiox12061187.
   Mój udział polegał na wykonywaniu analiz laboratoryjnych oraz wsparciu w tworzeniu bazy danych.

Ponadto wyrażam zgodę na wykorzystanie w/w prac w cyklu artykułów stanowiących podstawę rozprawy doktorskiej lek. Karoliny Czerwińskiej.



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