ORIGINAL PAPER

Oxidative stress may be a contributing factor in fibromyalgia patients' pain mechanisms

H. Beyaztas[1], S. Aktas[2,3], E.M. Guler[1,4], E. Ata[5]

[1]Department of Medical Biochemistry, Hamidiye Faculty of Medicine, University of Health Sciences, Istanbul; [2]Department of Biostatistics and Medical Informatics, Hamidiye Faculty of Medicine, University of Health Sciences, Istanbul; [3]Department of Biostatistics, Istanbul Cerrahpasa University; [4]Haydarpasa Numune Health Application and Research Center, University of Health Sciences, Istanbul; [5]Department of Physical Medicine and Rehabilitation, University of Health Sciences, Sultan Abdulhamid Han Education and Research Hospital, Istanbul, Turkey

SUMMARY

Objective. The pathophysiology of fibromyalgia (FM), a continuously painful syndrome with no known origin, has been related to mitochondrial dysfunction, oxidative stress, and inflammation. Recent studies have shown that FM may be associated with an oxidative balance disorder. The objective of this study was to measure the levels of oxidative stress in FM patients and try to understand the association between FM and free radicals.

Methods. This study was performed on 100 volunteers admitted to the University of Health Sciences, Sultan 2, Abdulhamid Han Health Application and Research Center Physical Therapy and Rehabilitation Clinic, including 50 healthy controls and 50 patients with FM. To analyze oxidative stress biomarkers, total oxidant status (TOS) and total antioxidant status (TAS) levels were measured. Total thiol (TT) and native thiol (NT) concentrations were measured to determine the relationship between thiol groups. Disulfide (DIS) and oxidative stress index (OSI) were calculated with mathematical formulas.

Results. While TOS and OSI levels were statistically higher in FM patients, TAS levels were significantly lower compared to the healthy control group ($p<0.001$). In comparison to the healthy control group, FM patients had considerably decreased TT and NT levels. DIS levels were significantly higher in FM patients than in controls ($p<0.001$).

Conclusions. Reactive oxygen species have several negative impacts on the human body. As a result of the measurements we analyzed, the relationship between FM and oxidative stress should be studied in terms of disease progression and may help improve the treatment process.

Key words: *Antioxidant, fibromyalgia, oxidative stress, rheumatological diseases, thiol-disulfide.*

Reumatismo, 2023; 75 (3): 121-127

INTRODUCTION

Fibromyalgia (FM) is a complex pain condition that is difficult to diagnose and treat and is associated with many symptoms such as sleeping disorders, chronic muscle pain in certain body areas, insomnia, headache, and others (1, 2). The FM prevalence in North America and Europe is between 0.5% and 5.8% (3). FM can strike at any age, even in children. The frequency of FM is similar across countries, cultures, and ethnic groups; there is no evidence that it is more prevalent in modern countries and cultures. FM Patients frequently have a long history of persistent pain in various parts of their bodies. About 30% of the population suffers from localized or widespread chronic musculoskeletal pain (4). Stress factors causing intense pain that ordinarily lasts a few weeks are the most likely environmental variables to cause FM. Some infections or trauma can trigger FM or comparable disorders, such as chronic fatigue syndrome. FM can also be triggered by psychological stress (5). Some chronic pain diseases, such as osteoarthritis, rheumatoid arthritis, and lupus, may cause FM (6-8). Social, behavioral, and psychological issues complicate the origin and treatment of FM. Psychiatric illnesses such as post-traumatic stress dis-

Corresponding author:
Emre Ata
Department of Physical Medicine and Rehabilitation, University of Health Sciences, Sultan Abdulhamid Han Education and Research Hospital, Selimiye Mah, Tıbbiye Cad, PK.34668, Uskudar, Istanbul, Turkey
E-mail: emreata.ftr@gmail.com

order, depression, anxiety, and obsessive-compulsive disorder are more common in people with FM. This might be the outcome of early-life stress or trauma, which are typical triggers for severe mental illnesses and FM. Pain-mediating neurotransmitters may impact mood, memory, exhaustion, and sleep (5, 6). High amounts of lipid peroxidation and malondialdehyde in the blood mononuclear cells and plasma of people with chronic pain, together with reduced catalase activity, have been linked to oxidative stress (9). Reactive oxygen species (ROS) reversibly alter the action of various enzymes and transcription factors at physiological levels, most commonly by the oxidation of particular protein thiols. They also impact pathways that increase ROS synthesis by NADPH oxidase or mitochondria, both of which have an intrinsic proclivity for positive feedback and unregulated oxidant production (10). Although various inflammatory clinical diseases are linked to increased oxidative stress, new evidence suggests that oxidative stress and pain perception are connected. An imbalance between oxidation products and antioxidant defenses causes increased oxidative stress (11, 12). This study aimed to evaluate the levels of oxidative stress in FM patients and try to understand the link between FM and oxidative stress.

MATERIALS AND METHODS

Study design and participants

In 2010, the American College of Rheumatology (ACR) revised the diagnostic criteria for FM and introduced the widespread pain index (WPI) and symptom severity (SS) scales. The tender point test was removed, and FM was reclassified as a multi-symptom disorder. The WPI and SS scores are combined to create the polysymptomatic distress scale (PDS) or fibromyalgianess scale (FS). The PDS allows for categorizing FM patients into severity levels ranging from none to extremely severe (13-15).

WPI ranges from 0 to 19, and higher scores is associated with more widespread pain in FM patients. Patients were asked whether they experienced pain in specific anatomical regions during the previous week, and a score of 1 was assigned for each region where pain was reported, while a score of 0 was given for regions without pain. The scores from all regions were then summed to obtain the WPI score.

Biochemical analyses

For biochemical analyses, blood samples were taken from FM patients and healthy controls. Each blood sample was centrifuged at 3000xg for 10 minutes, and serum samples were stored at -80°C until analysis. To assess the oxidative stress markers, we measured the amounts of thiol-disulfide (DIS), native thiol (NT), and total thiol (TT) in both groups. Total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) were evaluated.

Several laboratory tests are commonly employed as screening tools to assess various health conditions, including autoimmune diseases, hypothyroidism, myopathy, and occult liver diseases. These tests include hemogram, thyroid stimulating hormone, C-reactive protein, complete urinalysis, creatine kinase, erythrocyte sedimentation rate, aspartate aminotransferase, and alanine aminotransferase. The presence of chronic pain and fatigue is often observed in individuals affected by these diseases. By analyzing the results of these laboratory tests, healthcare professionals can gain valuable insights into the potential underlying causes of symptoms and accurately diagnose and manage the respective conditions (16-18).

Measurement of thiol-disulfide, native thiol, and total thiol levels

TT (Rel Assay Diagnostics, RL0178, Mega Tip, Turkey), and NT (Rel Assay Diagnostics, RL0185, Mega Tip, Turkey) concentrations in samples were determined using a photometric technique using commercially available kits. Using the following, the number of dynamic DIS bonds was calculated by determining half the difference between the TT and NT levels:

$$DIS=((TT-NT))/2$$

Measurement of total oxidant status and total antioxidant status levels

The samples' TAS and TOS levels were determined using a photometric technique using commercially available kits. TAS results are expressed in mmol Trolox Equiv./L, and TOS results in μmol H_2O_2 Equiv./L units. TOS/TAS was used to generate the OSI.

$$\text{OSI (optional unit)} = \frac{[(TOS, \mu mol\ H2O2\ Equiv.)]}{[(TAS, mmol\ Trolox\ Equiv.)]} \times 10^{-1}$$

Statistical analysis

SPSS, version 25.0 (IBM, Armonk, NY, USA) was used to analyze the data. In contrast to categorical variables, which were reported as the number of patients, numerical data (such as oxidative/antioxidant status parameters, TOS, TAS, and OSI) were expressed as mean ± standard deviations. Chi-square and Mann-Whitney *U* tests were used to compare the patient and control groups. Spearman's rank correlation coefficient was used to analyze associations between parameters and some clinical findings. A 95% confidence interval was shown for the difference between the groups. Statistics were considered significant at $p<0.05$. This study received ethical approval from the Hamidiye Scientific Research Ethics Committee (Document Date and Number: 27.07.2022- 22/298). All procedures conducted in this study adhered to the principles outlined in the 1964 Helsinki Declaration and its subsequent revisions, or equivalent ethical standards.

RESULTS

The study included a total of 50 patients diagnosed with primary FM based on the 2010 revised ACR diagnostic criteria. These patients were recruited from the Physical Therapy and Rehabilitation (PTR) clinic at the University of Health Sciences, Sultan 2, Abdulhamid Han Health Application and Research Center, between August and December 2022. The FM patient group consisted of adults aged 18 years and older, with a confirmed FM diagnosis, chronic widespread pain for at least 3 months, and a minimum pain intensity score of 3 on a 0-10 scale.

For the healthy control group, individuals aged 18 years and older without chronic medical conditions, diagnosed diseases, mental health disorders, or smoking history within the past year were included. Exclusion criteria for both groups encompassed chronic conditions, mental health disorders, smoking, medication use, and pregnancy or breastfeeding. The healthy control group was matched with the FM patients for age. The demographic and clinical characteristics of FM patients and controls are shown in Table I.

The pain localization of the patients was distributed as follows: left-lower leg (14%), left-upper leg (14%), right-upper leg (20%), right-lower leg (20%), left-upper arm (26%), right-upper arm (22%), right-shoulder girdle (22%), left-shoulder girdle (26%), lower back (84%), upper back (72%), left hip (buttock, trochanter) (18%), chest (66%), abdomen (66%), and right jaw (22%).

The results of TAS, TOS, and OSI in FM patients are presented in Figure 1. The findings indicate significant differences in TAS, TOS, and OSI levels between FM patients and the control group. In comparison to the control group, individuals diagnosed with FM exhibited statistically lower levels of se-

Table I - Clinical and descriptive statistics of healthy control group and patients with fibromyalgia.

Variables		Control	FM Patients
Age (years)		55.12±9.97¥	55.12±7.21¥
Duration of disease (years)		–	11.84±7.46¥
BMI (kg/m^2)		27.34±3.79¥	27.36±4.19¥
WPI (score 0-19)		–	4.98±1.49¥
Gender	Female	29	29
Education	Primary school	13	12
	Junior high school	8	10
	High school	18	17
	University	1	11
Marital status	Married	42	42
Alcohol	No	50	32
Smoking	No	50	29

¥Mean ± standard deviation; FM, fibromyalgia; BMI, body mass index; WPI, widespread pain index.

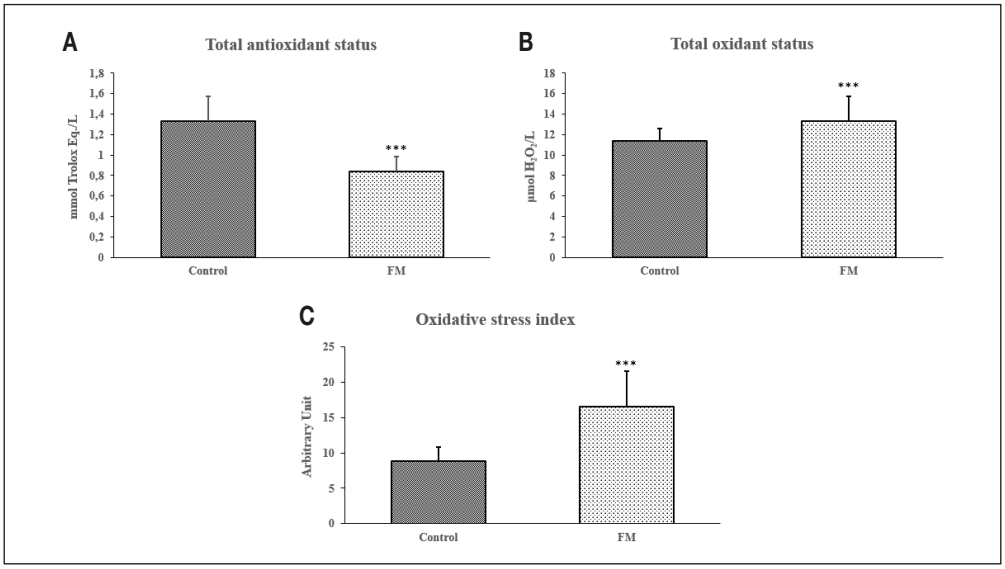

Figure 1 - A) Total antioxidant status; B) Total oxidant status; C) Oxidative stress index levels in fibromyalgia (FM) group and controls (***p<0.001).

rum total antioxidants, and higher levels of serum oxidants. Moreover, the FM patients displayed higher values of OSI when compared to the control group (p<0.001).

The concentrations of TT, NT, and DIS in FM patients and the healthy control group are shown in Table II. In our study, a statistically significant difference in NT, TT, and DIS variables emerged between patients and controls. NT and TT values were found to be higher in the control group than in the FM patient group. FM patients showed higher DIS values compared to controls (p<0.001).

No significant correlation was observed between the WPI and oxidative stress parameters in our study. Furthermore, there were no clinically significant differences identified in the routinely assessed parameters in the hospital setting.

DISCUSSION AND CONCLUSIONS

FM patients exhibited reduced antioxidant levels and elevated oxidant levels compared to the healthy control group. On the other hand, FM patients exhibited lower levels of TT and NT compared to the control group while showing higher levels of DIS. These findings provide evidence supporting the involvement of oxidative stress in the patho-

Table II - Serum thiol and disulfide concentrations (mean ± standard deviation) and ratios compared between patients with fibromyalgia and the healthy control group.

	Control (n=50)	FM (n=50)	p value
NT µM	414.88±39.98	305.66±38.65	0.001
TT µM	509.38±56.43	446.65±34.16	0.001
DIS µM	50.88±26.23	70.49±27.91	0.001
DIS/NT %	12.69±7.07	24.57±13.57	0.001
DIS/TT %	9.63±4.41	15.53±5.38	0.001
NT/TT %	78.77±8.06	68.95±10.76	0.001

FM, fibromyalgia; DIS, disulphide; NT, native thiol; TT, total thiol. Differences in FM compared to the healthy control group (independent sample t-test).

physiology of FM. The etiology of pain in fibromyalgia remains complex and multifactorial. Previous research has demonstrated an imbalance in pro-oxidant and oxidant levels among individuals experiencing specific types of pain (19).

ROS might be regarded as one of the underlying causes of most diseases because of the damage it can cause to the body. Sequential processes can be disrupted by oxygen radicals produced as a result of mitochondrial dysfunction. In FM disease, these disruptions might be the source of symptoms, including pain, sleep disorders, and chronic physical soreness. Despite the growing body of information about the role of oxidative stress in various conditions, measuring ROS remains a challenge (20, 21). In some circumstances, excess oxidants cause a decrease in antioxidants in organisms, resulting in an oxidation-reduction imbalance, which is thought to be the underlying cause of many disorders and causes oxidative stress in cells (21-23). Low to intermediate ROS concentrations affect cell signaling cascades, whereas high ROS concentrations can harm lipids, enzymes, and nucleic acids in an unspecific manner. Ascorbate creates a unique relationship between ROS production/elimination and cell death. It is an effective antioxidant at low concentrations and plays a key role in ROS removal (24). In their study, Bagis et al. found that oxidant/antioxidant balances were altered and elevated free radical levels were linked to FM. They supported the idea that oxidative stress causes FM (25). In their study, Cetinkaya et al. stated that while TAS levels were not significant, TOS and OSI values were significantly higher than those in the control group (26). Additionally, in the study by Karatas et al., NT levels were found to be high in patients with FM (27).

It is worth noting that in numerous diseases, the presence of reactive oxygen species leads to the generation of oxidative stress. However, research is ongoing to elucidate the role of oxidative stress in the pathophysiology of FM. Mercedes et al. showed that FM patients have oxidative dysfunction. Weak enzymatic antioxidant activity can cause oxidative stress by oxidizing DNA and proteins, negatively impacting FM patients' health (28). In the study by Tuzcu et al., NT levels were found to be low in FM patients, and DIS ratios were found to be high (29).

Natural compounds frequently include thiol and DIS groups. They can be present in proteins (hormones, enzymes, antibiotics), cysteine-containing peptides, and tiny compounds like lipoic acid, glutathione, and thiamine. Thiol and DIS groups are unmistakably linked to either strong chemical reactivity or the stabilization of peptide and protein structures in all of these compounds. The thiol and DIS groups are often not employed as substituents in quantitative structure-activity relationship investigations in medicinal chemistry because they are highly reactive. On occasion, aromatic rings are subjected to methylthio substitution, although even then, the resulting thioethers are very reactive. They can quickly be transformed into sulfoxides, or the other way around. Copper, mercury, zinc, and lead are all efficiently chelated by penicillamine (D-β,β-dimethylcysteine, a drug that contains thiol groups), which encourages the excretion of these metals in urine. Rheumatoid arthritis, Wilson's disease, and heavy metal toxicity are among the conditions for which it is clinically administered (30). This knowledge can be used to create novel compounds that target or include thiol groups. These chemicals can be examined for their role in the mechanism of pain.

Transient receptor potential (TRP) channels may play a role in a variety of activities, including the production of inflammatory mediators and the activity of neurotransmitters in the spinal cord. These features clearly imply that pain relief will be facilitated by the targeted and selective reduction of TRP channel activity. Given that TRP channels serve as receptors for irritants originating from plants, such as capsaicin, menthol, and the spicy components in mustard and garlic plants, isothiocyanates, and thiosulfinates, their significant functions are probably not unexpected. TRPA1 is a particularly intriguing member of this group in terms of environmental irritants. This is due to the fact that TRPA1 reacts with substances that are

structurally different, but are all capable of forming covalent adducts with thiol groups. For instance, membrane-permeable electrophiles like allyl isothiocyanate or allicin can activate TRPA1 by covalently altering cysteine residues in the channel's amino-terminal cytoplasmic domain (31, 32). In order to reduce the severity of pain, these channels might be considered. Therefore, research on thiol groups and TRP channels is thought to potentially provide a pathway for pain management and treatment strategies in FM patients.

In conclusion, there are some findings indicating the presence of oxidative stress and an imbalance in thiol-disulfide levels in FM patients. However, the exact role of oxidative stress and thiol groups in the pathophysiology of FM is still not fully understood. Further research and data collection are needed in this regard. Understanding the involvement of thiol groups and oxidative stress in the mechanisms underlying FM can help identify new targets for pain management and treatment strategies.

Contributions

HB, writing-original draft, review and editing, visualization, investigation; SA, formal analysis, writing-review and editing; EA, EMG, conceptualization, methodology, writing-review and editing, project administration, resources.

Conflict of interest

The authors declare no potential conflict of interest.

Ethics approval and consent to participate

This study received ethical approval from the Hamidiye Scientific Research Ethics Committee (Document Date and Number: 27.07.2022 - 22/298). All procedures conducted in this study adhered to the principles outlined in the 1964 Helsinki declaration and its subsequent revisions, or equivalent ethical standards.

Availability of data and materials

Data and materials are available from the corresponding author upon request.

REFERENCES

1. Neumeister MW, Neumeister EL. Fibromyalgia. Clin Plast Surg. 2020; 47: 203-13.
2. Araújo FM, Melo DeSantana J. Physical therapy modalities for treating fibromyalgia. F1000Res 2019; 8: F1000 Faculty Rev-2030.
3. Kia S, Choy E. Update on treatment guideline in fibromyalgia syndrome with focus on pharmacology. Biomedicines. 2017; 5: 20.
4. McBeth J, Jones K. Epidemiology of chronic musculoskeletal pain. Best Pract Res Clin Rheumatol. 2007; 21: 403-25.
5. Clauw DJ. Fibromyalgia: a clinical review. JAMA. 2014; 311: 1547-55.
6. Phillips K, Clauw DJ. Central pain mechanisms in the rheumatic diseases: future directions. Arthritis Rheum. 2013; 65: 291-302.
7. Wolfe F. Fibromyalgianess. Arthritis Rheum. 2009; 61: 715-6.
8. Wolfe F, Häuser W, Hassett AL, Katz RS, Walitt BT. The development of fibromyalgia - I: examination of rates and predictors in patients with rheumatoid arthritis (RA). Pain. 2011; 152: 291-9.
9. Cordero MD, Cano-García FJ, Alcocer-Gómez E, De Miguel M, Sánchez-Alcázar JA. Oxidative stress correlates with headache symptoms in fibromyalgia: coenzyme Q10 effect on clinical ımprovement. PLoS One. 2012; 7: e35677.
10. Ward JPT. From physiological redox signalling to oxidant stress. Adv Exp Med Biol. 2017; 967: 335-42.
11. Wang ZQ, Porreca F, Cuzzocrea S, Galen K, Lightfoot R, Masini E, et al. A newly ıdentified role for superoxide in ınflammatory pain. J Pharmacol Exp Ther. 2004; 309: 869-78.
12. Chung CP, Titova D, Oeser A, Randels M, Avalos I, Milne GL, et al. Oxidative stress in fibromyalgia and its relationship to symptoms. Clin Rheumatol. 2009; 28: 435-8.
13. Wolfe F, Häuser W. Fibromyalgia diagnosis and diagnostic criteria. Ann Med. 2011; 43: 495-502.
14. Wolfe F, Walitt BT, Rasker JJ, Katz RS, Häuser W. The use of polysymptomatic distress categories in the evaluation of fibromyalgia (FM) and FM severity. J Rheumatol. 2015; 42: 1494-501.
15. Littlejohn GO, Guymer EK. In clinical practice, the term "central sensitivity score" ıs more useful than the term "polysymptomatic distress scale": comment on the editorial by Wolfe. Arthritis Rheumatol. 2015; 67: 2553.
16. Wolfe F. New American college of rheumatology criteria for fibromyalgia: a twenty-year journey. Arthritis Care Res. 2010; 62: 583-4.
17. Siracusa R, Di Paola R, Cuzzocrea S, Impellizzeri D. Fibromyalgia: pathogenesis, mechanisms, diagnosis and treatment options update. Int J Mol Sci. 2021; 22: 3891.
18. Staud R. Biology and therapy of fibromyalgia:

pain in fibromyalgia syndrome. Arthritis Res Ther. 2006; 8: 208.
19. Hendrix J, Nijs J, Ickmans K, Godderis L, Ghosh M, Polli A. The ınterplay between oxidative stress, exercise, and pain in health and disease: potential role of autonomic regulation and epigenetic mechanisms. Antioxidants. 2020; 9: 1166.
20. Ito F, Sono Y, Ito T. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic ınflammation. Antioxidants. 2019; 8: 72.
21. Brieger K, Schiavone S, Miller Jr, Krause KH. Reactive oxygen species: from health to disease. Swiss Med Wkly. 2012; 142: w13659.
22. Singh A, Kukreti R, Saso L, Kukreti S. Oxidative stress: a key modulator in neurodegenerative diseases. Molecules. 2019; 24: 1583.
23. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005; 38: 1103-11.
24. Szarka A, Lőrincz T, Hajdinák P. Friend or foe: the relativity of (anti)oxidative agents and pathways. Int J Mol Sci. 2022; 23: 5188.
25. Bagis S, Tamer L, Sahin G, Bilgin R, Guler H, Ercan B, et al. Free radicals and antioxidants in primary fibromyalgia: an oxidative stress disorder? Rheumatol Int. 2005; 25: 188-90.
26. Çetinkaya FN, Koçak FA, Kurt EE, Güçlü K, Tuncay F, Şaş S, et al. The effects of balneotherapy on oxidant/antioxidant status in patients with fibromyalgia: an observational study. Arch Rheumatol. 2020; 35: 506-14.
27. Karatas G, Gunduz R, Haskul I, Ustun B, Neselioglu S, Karatas F, et al. Dynamic thiol and disulphide homoeostasis in fibromyalgia. Arch Med Sci. 2020; 16: 597-602.
28. La Rubia M, Rus A, Molina F, Del Moral ML. Is fibromyalgia-related oxidative stress implicated in the decline of physical and mental health status? Clin Exp Rheumatol. 2013; 31: S121-7.
29. Tuzcu A, Baykara RA, Alışık M, Omma A, Acet GK, Dogan E, et al. Alteration of thiol-disulfide homeostasis in fibromyalgia syndrome. Acta Medica. 2019; 62: 12-8.
30. Bazzini P, Wermuth CG. Substituent groups. In: Wermuth CG (ed). The practice of medicinal chemistry. Amsterdam, The Netherlands: Elsevier. 2008. 319-57.
31. Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. Cell. 2009; 139: 267-84.
32. Cortright DN, Krause JE, Broom DC. TRP channels and pain. Biochim Biophys Acta. 2007; 1772: 978-88.