

Biomarkers of Oxidative Stress in Fibromyalgia and Their Diagnostic Implications

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Abstract

Introduction: Fibromyalgia is a chronic, widespread pain disorder accompanied by fatigue, nonrestorative sleep, cognitive difficulties, and psychiatric comorbidity. Diagnosis remains clinical, and no objective biomarker has been established, yet growing evidence implicates oxidative stress in its pathophysiology. **Objective:** To synthesize current evidence on oxidative stress biomarkers and their relationship to the clinical presentation of fibromyalgia, and to evaluate their potential diagnostic and therapeutic relevance. **Methods:** A narrative review of the literature indexed in PubMed, Scopus, and Google Scholar (2005–2025, English language) was conducted, focusing on enzymatic and non-enzymatic antioxidants, oxidative damage markers, and validated symptom measures. **Results:** Reduced activities of superoxide dismutase, glutathione peroxidase, and catalase, together with increased markers of lipid and DNA oxidation (malondialdehyde, 4-hydroxynonenal, 8-oxo-dG), are associated with greater symptom severity on validated clinical scales such as the

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Fibromyalgia Impact Questionnaire. Composite indices (total oxidant status, total antioxidant status, oxidative stress index) and multi-marker panels provide more robust assessments than single analytes, given compensatory changes within the antioxidant network and pre-analytical influences such as diet and sample handling. Methodological constraints of common assays (TBARS, immunoassays) argue for chromatographic and mass-spectrometric approaches for symptom-specific quantification and for standardized sampling protocols. Conclusion: Redox imbalance is mechanistically linked to the core clinical features of fibromyalgia, including pain, fatigue, and impaired quality of life. Validated, multi-layered biomarker panels combined with standardized laboratory practice may improve patient phenotyping and stratification and inform interventions targeting oxidative mechanisms, although rigorous controlled studies are required.

Keywords: fibromyalgia, oxidative stress, biomarkers, lipid peroxidation, malondialdehyde, antioxidants

1. Introduction

Fibromyalgia is a chronic, widespread pain condition accompanied by sleep disruption, cognitive difficulties, psychiatric symptoms, and multiple somatic complaints, frequently with overlapping conditions and a substantial impact on quality of life [1,2,3]. Diagnosis is currently clinical and relies on the American College of Rheumatology (ACR) criteria. The original 1990 classification required widespread pain for at least three months combined with a specified number of tender points on physical examination [1,3,4]. Subsequent revisions in 2010 and 2011, together with a 2016 update, introduced the Widespread Pain Index and the Symptom Severity Scale to better capture somatic and cognitive symptoms and to reduce misclassification, although no objective biomarkers are yet available [5,6,7]. These developments have improved practicality but still pose challenges, because phenotypic heterogeneity and comorbid conditions complicate precise diagnosis and standardization [1,3,8]. Against this background, fibromyalgia is recognized as a highly prevalent and disabling condition.

2. Materials and Methods

This article was prepared as a narrative review of the literature concerning the relationship between oxidative stress biomarkers and the clinical presentation of fibromyalgia. It was conducted and is reported according to

the principles of a narrative review; it was not designed as a systematic review and was therefore not conducted or reported in accordance with the PRISMA 2020 guidelines, which are intended for systematic reviews and meta-analyses [9]. Accordingly, no registered protocol, formal risk-of-bias assessment, quantitative synthesis, or PRISMA flow diagram is presented.

A search of the electronic databases PubMed, Scopus, and Google Scholar was performed to identify relevant publications. The search terms included "fibromyalgia," "oxidative stress," "diagnostic criteria for fibromyalgia," "fibromyalgia questionnaires," "Fibromyalgia Impact Questionnaire (FIQ)," "Widespread Pain Index," "pathophysiology," and "etiology of fibromyalgia," used individually and in combination. Articles published between 2005 and 2025 and written in English were considered eligible. The final literature search was conducted in June 2026. Of the records identified, approximately 56 were screened on the basis of title and abstract, and approximately 43 were ultimately included in the synthesis. Publications were selected on the basis of their relevance to the central theme of the review, namely the association between oxidative stress biomarkers – including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), total antioxidant status (TAS), and total oxidant status (TOS) – and the clinical features of fibromyalgia, encompassing its neurological and psychiatric manifestations as well as chronic widespread pain. The reference lists of selected articles were additionally screened to identify further relevant sources.

As a narrative review, this work did not follow a registered protocol, and no formal systematic search algorithm, study-selection flow diagram, or quantitative risk-of-bias assessment was undertaken. Articles were selected according to topical relevance at the authors' discretion, and the resulting synthesis is interpretive rather than exhaustive. The limitations arising from this design are discussed in Section 4.

3. Overview of the Evidence

3.1 Fibromyalgia Overview

Fibromyalgia is defined as a chronic pain disorder with widespread musculoskeletal pain accompanied by fatigue, cognitive dysfunction, multiple somatic complaints, and psychiatric disturbances such as depression and anxiety [1,2,10]. Patients typically report diffuse pain persisting for more than three months, together with sleep disturbances and mood or cognitive symptoms that substantially impair quality of life. Routine laboratory and imaging investigations are usually normal and serve

primarily to exclude alternative diagnoses rather than to establish fibromyalgia itself [2,3,10].

Current diagnostic practice relies on clinical criteria. The ACR criteria issued in 2010 define fibromyalgia as chronic widespread pain associated with somatic symptoms and rely on the Widespread Pain Index and the Symptom Severity Scale as core measures [3,4,11]. A 2011 modification combined those indices into the fibromyalgia symptom scale, later termed the polysymptomatic distress scale. The 2016 ACR revision added a generalized pain criterion requiring pain in at least four of five body regions, specified key somatic symptoms, and explicitly stated that a diagnosis of fibromyalgia remains valid irrespective of other coexisting illnesses [4,5,8].

Clinically, fibromyalgia presents a characteristic symptom constellation extending beyond pain. Patients often report profound fatigue, nonrestorative sleep, cognitive difficulties sometimes described as "fibro fog," and diverse somatic complaints such as headaches and gastrointestinal symptoms. The high burden of chronic fatigue has likewise been documented in regional cohorts, where it ranks among the most prevalent and most treatment-resistant symptoms of the disorder [12]. Psychiatric comorbidities are frequent; more than half of affected individuals meet criteria for depression, and other mental health conditions – including generalized anxiety, bipolar disorder, and substance use disorders – commonly coexist [3,10,13]. Many patients exhibit overlapping chronic pain syndromes, including temporomandibular pain, irritable bowel syndrome, interstitial cystitis, chronic tension headaches, migraines, and myalgic encephalomyelitis or chronic fatigue syndrome. Restless legs syndrome may further exacerbate sleep disturbances, compounding daytime fatigue and pain. These overlapping conditions contribute to marked disability and complicate both recognition and management [3,14,15].

Epidemiological data indicate that fibromyalgia affects approximately 2–8% of the population [13,14]. Prevalence increases with age, and the highest rates are observed in women aged 20 to 55 years. In adults, females are reported to be roughly twice as likely as males to develop fibromyalgia, and the condition represents a leading cause of generalized musculoskeletal pain in women [16]. Patients frequently experience substantial functional impairment, with reported disability rates between 10% and 30% and an increased risk of suicide compared with the general population. Those treated at tertiary care centers tend to have a worse prognosis than patients

managed in community primary care, reflecting both greater baseline severity and complex psychosocial contributors [2,17].

3.2. Etiology and Pathogenesis

Fibromyalgia likely arises from an interplay of inherited susceptibility and neurobiological abnormalities that may converge on altered redox homeostasis. Family and twin studies indicate a substantial genetic contribution, with polymorphisms affecting pain-modulation systems (e.g., serotonergic, noradrenergic, and catecholaminergic pathways) and genes governing mitochondrial function and antioxidant defenses predisposing individuals to heightened pain sensitivity [18,19,20]. Several of these polymorphisms – particularly those affecting catecholaminergic (e.g., catechol-O-methyltransferase) and serotonergic (e.g., the serotonin transporter) signaling – have also been implicated in the shared genetic architecture of chronic pain and affective disorders, which may contribute to the frequent co-occurrence of mood symptoms in fibromyalgia [21]. Implicated neurobiological mechanisms include central sensitization, dysfunctional descending inhibitory control, microglial and astrocyte activation, and mitochondrial impairment; each of these can amplify reactive oxygen species (ROS) production or reduce ROS clearance, thereby promoting oxidative stress [10,22,23]. Oxidative stress, in turn, can modify ion channel function, neurotransmitter signaling, and synaptic plasticity, potentially perpetuating nociceptive amplification and neuroinflammation [24]. Fibromyalgia may therefore be conceptualized as a gene–environment syndrome in which genetic predisposition to impaired antioxidant capacity and dysregulated pain pathways interacts with neurobiological processes that generate and sustain oxidative damage, potentially contributing to chronic widespread pain [8,15,25].

It should be emphasized that the directionality of these relationships remains uncertain. Because much of the supporting evidence is derived from cross-sectional studies, it is not yet possible to determine whether oxidative abnormalities are causal contributors to fibromyalgia, downstream consequences of chronic pain, stress, and physical deconditioning, or epiphenomena. The associations described throughout this review should therefore be interpreted as correlative rather than firmly causal.

Central to the clinical picture of fibromyalgia is a state of altered pain processing within the central nervous system, and this shift in sensory modulation appears to result from an interplay between neurophysiological changes and immune-mediated processes.

Dysfunctions in monoaminergic neurotransmission have been consistently observed, with elevations in excitatory neurotransmitters such as glutamate and substance P occurring alongside decreased levels of serotonin and norepinephrine in descending antinociceptive pathways [24,26]. This imbalance tips the spinal-cord sensory environment toward facilitation rather than inhibition of nociceptive signals, making normally non-painful inputs more likely to be perceived as painful. Dopamine dysregulation and altered endogenous opioid activity in cerebral structures may compound these effects, potentially affecting both pain modulation and reward-related aspects of symptom persistence [19,26]. These central alterations do not operate in isolation. Persistent peripheral inputs from structures such as muscles or viscera can reinforce central sensitization over time, and conditions such as irritable bowel syndrome or interstitial cystitis, often comorbid with fibromyalgia, may contribute to ongoing nociceptive drive [14,22].

From an immunological standpoint, increasing attention has been paid to subtle inflammatory patterns that could influence neural excitability. Low-grade systemic inflammation may arise when disruption of the gut microbiota compromises intestinal barrier function, leading to translocation of lipopolysaccharide (LPS) into the circulation [3,10,27].

Oxidative stress adds another layer by linking immune dysfunction with neuronal sensitization. Reactive oxygen species such as superoxide radicals may promote both peripheral and central nervous system hyperexcitability while activating cytokines including tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), and interleukin-6 (IL-6). These cytokines may not only worsen symptom intensity but could also interfere with muscle nociceptor function if oxidative damage reduces nociceptor number or functionality, lowering pain thresholds further [19,27,28].

3.3 Oxidative Stress in Fibromyalgia

Reactive oxygen and nitrogen species are central to current models of redox disturbance in fibromyalgia. Inflammatory or stress-related triggers increase the production of superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^-$), particularly within mitochondria and activated immune cells [20]. Lipid-rich tissues such as the central nervous system are especially vulnerable: lipid peroxidation there gives rise to hydroperoxides and aldehydes, including malondialdehyde (MDA) and 4-hydroxynonenal, which serve as markers of oxidative injury and correlate

positively with fibromyalgia severity scores on the Fibromyalgia Impact Questionnaire (FIQ) [29,30].

Endogenous antioxidant systems attempt to counterbalance this oxidant load. Superoxide dismutase 1 (SOD1) converts $O_2\bullet^-$ to H_2O_2 , which is then detoxified to water by catalase (CAT) and glutathione peroxidase (GPx) [31]. In fibromyalgia, reduced activity of SOD1, CAT, glutathione reductase, and GPx has been documented, with lower enzyme activities correlating with greater pain, fatigue, psychological distress, and sleep disturbance as quantified by FIQ domains. Concomitantly, increased nitric oxide (NO), MDA, and other lipid peroxidation products are observed in red-blood-cell lysates, and serum nitrite has been highlighted as a pro-oxidant marker linked to pain sensation [29]. These alterations indicate that diminished enzymatic defenses and enhanced pro-oxidant activity coexist, and that higher lipid peroxidation is positively associated with worse overall functioning on the FIQ [24,29]. The principal biomarkers discussed in this review, their typical direction of change in fibromyalgia, and their reported associations with clinical severity are summarized in Table 1.

Table 1. Oxidative stress biomarkers in fibromyalgia and their reported associations with clinical severity.

Biomarker / parameter	Category	Typical direction in fibromyalgia vs. controls	Reported association with clinical measures	Common assay	Ref(s)
Superoxide dismutase (SOD)	Enzymatic antioxidant	Decreased	Negative correlation with FIQ domains (pain, function, distress, sleep)	Spectrophotometric	[24,30,32]
Catalase (CAT)	Enzymatic antioxidant	Decreased	Negative correlation with FIQ	Spectrophotometric	[24,30,32]
Glutathione peroxidase (GPx)	Enzymatic antioxidant	Decreased	Negative correlation with FIQ	Spectrophotometric	[24,29]
Glutathione reductase	Enzymatic antioxidant	Decreased	Negative correlation with FIQ domains	Spectrophotometric	[24]

Total antioxidant capacity / status (TAC/TAS)	Global antioxidant	Decreased / altered	Lower values associated with worse health status; correlate with SOD and CAT	Colorimetric	[8,15,32]
Malondialdehyde (MDA)	Lipid peroxidation product	Increased	Positive correlation with FIQ severity	TBARS, HPLC, immunoassay	[24,29,33]
4-Hydroxynonenal (4-HNE)	Lipid peroxidation product	Increased	Positive correlation with severity	HPLC, immunoassay	[24,29]
Nitric oxide (NO) / serum nitrite	Reactive nitrogen species	Increased	Linked to pain sensation	Colorimetric	[29]
Protein carbonyls	Protein oxidation	Increased	Marker of cumulative oxidative damage	Spectrophotometric, ELISA	[8,29]
8-oxo-dG	DNA oxidation	Increased	Negative correlation with antioxidants; associated with mental-health measures	HPLC-MS/MS	[23,29,30]
Total oxidant status (TOS)	Global oxidant	Increased	Associated with symptom severity and pain	Colorimetric	[33,34]
Oxidative stress index (OSI = TOS/TAS)	Composite index	Increased	Associated with severity	Calculated ratio	[15,33]

Legend: Directions reflect the most commonly reported findings; results vary across studies owing to differences in patient populations, disease severity, comorbidities, and assay methods, and most underlying data are cross-sectional (see Section 4). Abbreviations: ELISA, enzyme-linked immunosorbent assay; FIQ, Fibromyalgia Impact Questionnaire; HPLC, high-performance liquid chromatography; MS/MS, tandem mass spectrometry; TBARS, thiobarbituric acid reactive substances.

Mechanistically, mitochondrial dysfunction constitutes a critical source and target of these radicals. In fibromyalgia and chronic fatigue syndrome, oxidative and nitrosative stress leads to overproduction of ROS and peroxynitrite, which damage mitochondrial enzymes and respiratory-chain complexes. Peroxynitrite inactivates key mitochondrial proteins, resulting

in impaired energy metabolism and a vicious circle in which mitochondria both generate and are injured by ROS and reactive nitrogen species [23,35]. Evidence for increased oxidized low-density lipoproteins, protein carbonyls, and IgM antibodies against lipid peroxidation products such as MDA and phosphatidylinositol supports ongoing oxidative damage to membranes and intracellular signaling components. These processes are proposed to contribute to peripheral nociceptive input and central sensitization, helping to explain chronic widespread pain in these syndromes [8].

Beyond these enzymatic and damage-related markers, attention has increasingly turned to mitochondrial biomarkers in fibromyalgia. Reduced coenzyme Q10 (CoQ10) levels, alterations in mitochondrial DNA, and markers of impaired mitophagy have been reported and may reflect the bioenergetic dysfunction implicated in symptom generation, although these findings require confirmation in larger, prospectively designed cohorts [20,28,35]. CoQ10 is of particular interest because it functions both as an essential electron carrier in the mitochondrial respiratory chain and as a lipid-soluble antioxidant. Its distribution in blood appears altered in fibromyalgia, with reduced concentrations in blood mononuclear cells – despite elevated plasma levels – accompanied by increased mononuclear reactive oxygen species production [36]. In the same cell type, CoQ10 deficiency has been reported together with reduced catalase activity, lower ATP content, and increased lipid peroxidation, with lower CoQ10 and catalase levels associated with greater headache severity [37]. CoQ10 deficiency and the accompanying mitochondrial dysfunction have additionally been linked to activation of the NOD-like receptor pyrin domain-containing 3 (NLRP3) inflammasome and to elevated proinflammatory cytokines, including interleukin-1 β (IL-1 β) and interleukin-18 (IL-18), offering a potential mechanistic bridge between mitochondrial impairment and the low-grade inflammation described above [38]. Preliminary intervention data, comprising an open-label study and a small placebo-controlled trial, suggest that CoQ10 supplementation can normalize several of these biochemical markers, reduce inflammasome activation and circulating IL-1 β and IL-18, and improve clinical scores [37,38]. These findings are promising but derive largely from a single research group and from small samples, so larger independent, placebo-controlled trials are needed before therapeutic conclusions can be drawn.

Multi-marker approaches further refine the understanding of these mechanisms. Total oxidant status (TOS) captures the aggregate oxidant burden, while total antioxidant status (TAS) reflects global antioxidant

capacity. The oxidative stress index (OSI), defined as the ratio of TOS to TAS, integrates these measures into a single indicator of redox balance [34]. Elevated TOS, OSI, and MDA, together with altered TAS, have been documented in fibromyalgia, and oxidative stress and MDA levels are associated with symptom severity and pain amplification. Oxidative processes have also been associated with anxiety and depression, suggesting that redox imbalance may relate to both nociceptive and affective dimensions of the disorder [33].

Interpretation of individual oxidative markers is complicated by compensatory dynamics within the antioxidant network. Long-term excess ROS can directly damage antioxidant enzymes such as SOD and CAT, leading to a progressive decline in their activity that perpetuates oxidative injury [39]. Conversely, increased catalase or peroxiredoxin activity can maintain low H_2O_2 concentrations despite substantial oxidative stress, while reduced SOD activity may decrease H_2O_2 yet allow accumulation of superoxide [29,31]. Similarly, glutathione reductase and related enzymes can sustain high levels of reduced glutathione even in the presence of damage. These considerations demonstrate that reliance on single biomarkers such as H_2O_2 or glutathione can yield false-negative or false-positive assessments of oxidative status, and they support combined measurement of enzyme activities, peroxidation products such as MDA, and composite indices such as the OSI in fibromyalgia research [23,33]. Taken together, current evidence outlines a mechanistic framework in which mitochondrial ROS generation, impaired antioxidant enzymes, lipid peroxidation, and complex compensatory responses interact to shape the oxidative milieu in fibromyalgia, with associations to pain intensity, fatigue, and psychological symptomatology [17,23].

3.4 Evidence from Clinical Studies

Clinical investigations of oxidative imbalance in fibromyalgia have employed a range of enzymatic, non-enzymatic, and damage-related biomarkers together with standardized symptom scales. One early approach quantified total antioxidant capacity (TAC) and total peroxide levels in plasma, capturing global antioxidative and oxidative status, respectively, in patients fulfilling ACR criteria compared with pain-free controls [8,32].

More extensive profiling in larger female cohorts has combined multiple oxidative and antioxidative markers within the same individuals. Multiple correlations were observed among TAC, enzymatic activities, and non-

enzymatic antioxidants: TAC was strongly and positively related to SOD, catalase, and albumin; SOD correlated with catalase and albumin; and oxidative DNA damage correlated negatively with TAC, SOD, and catalase. These patterns are consistent with the view that diminished endogenous antioxidant enzyme activity is associated with oxidative damage to proteins and DNA, and that this damage is linked to poorer health status in fibromyalgia [30,32].

Elevated lipid peroxidation products such as MDA and 4-hydroxynonenal have been documented, with levels of lipid peroxidation showing a positive correlation with fibromyalgia severity evaluated by the FIQ. Mitochondrial dysfunction and a decreased capacity to remove free radicals are proposed to increase free-radical synthesis and to promote inflammatory cytokine-mediated pain generation in these patients [22,24,27]. Some reports have noted contradictory findings for individual biomarkers, such as unchanged lipid peroxidation in certain cohorts or a lack of correlation between lipid peroxidation and pain or FIQ scores. However, even in these settings, lipid peroxidation has been found to correlate with fibromyalgia impact, and oxidative DNA damage has maintained robust associations with mental health measures [30]. Such variability has been attributed to disease stage and individual patient characteristics, but across studies an imbalance between oxidant and antioxidant markers emerges with reasonable consistency (Table 1). It should be noted, however, that most of these studies are cross-sectional and frequently of modest sample size, which limits the strength of the inferences that can be drawn (see Section 4). On this basis, oxidant and antioxidant parameters – particularly SOD, GPx, and catalase activities, TAC, lipid peroxidation, and oxidative DNA damage – have been proposed as candidate biomarkers to aid diagnosis and guide therapy, with antioxidant strategies suggested as potential co-treatments pending evaluation in rigorous controlled trials [24,30,32].

3.5 Biomarkers of Oxidative Stress

Assessment of oxidative damage in fibromyalgia relies on quantifying ROS-induced modifications of lipids, proteins, and DNA, predominantly in accessible matrices such as venous blood and urine [23,30]. These specimens are favored in clinical and translational research because they provide low-cost, minimally invasive access to systemic oxidative processes, in contrast to the more invasive sampling of the spinal cord or tissue compartments that is generally restricted to specific disease conditions [25,39].

Lipid peroxidation products constitute a major group of oxidative damage markers. MDA and related alkenals and alkadienals are routinely quantified using spectrophotometric or fluorimetric thiobarbituric acid reactive substances (TBARS) assays, as well as by high-performance liquid chromatography (HPLC) with ultraviolet or fluorescence detection, or by immunoassays [25,40,41]. However, TBARS-based methods lack specificity, since sugars, amino acids, bilirubin, albumin, and hemolysis can all interfere, and up to 98% of the measured MDA may be generated artifactually during the high-temperature reaction step, raising concerns about their validity as in vivo oxidative stress indices [25,29,41].

Protein oxidation is captured through several complementary endpoints. Carbonyl content is considered a general indicator of protein oxidation and is typically measured by spectrophotometric assays or enzyme-linked immunosorbent assay (ELISA) after derivatization, although the structural heterogeneity of oxidation products and antibody-specificity issues can complicate interpretation [25,29]. Nitrotyrosine, formed largely via peroxynitrite and related pathways, can be detected in tissues by ELISA or by chromatographic techniques coupled to ultraviolet, thermal-energy, electrochemical, or mass-spectrometric detection [41]. Advanced oxidation protein products (AOPP) and oxidized low-density lipoproteins (oxLDL) are additional protein-based markers detectable by colorimetric assays and immunodetection, yet their measurement is influenced by antibody specificity and sample handling [29].

DNA oxidation markers provide a further layer of information on cumulative oxidative injury. The oxidized guanine lesion 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG, also referred to as 8-OHdG) is the most widely used biomarker and can be quantified in DNA from blood or tissues, or as an excreted metabolite in urine. Analytical platforms include HPLC with electrochemical detection, HPLC coupled to gas chromatography-mass spectrometry, and HPLC-tandem mass spectrometry, the last of which currently represents the preferred technique for specific lesion detection and has been correlated with chronic inflammatory states in liver and kidney disease [23,27,29].

Taken together, these markers demonstrate that no single assay captures the full spectrum of oxidative damage. Research in systemic oxidative disorders, including fibromyalgia, therefore increasingly adopts multi-marker panels that integrate lipid peroxidation indices, protein oxidation products, DNA lesions, and measures of antioxidant defenses to obtain a more robust and clinically interpretable picture of redox imbalance

[23,29,30]. Recognizing these analytical challenges, recent consensus recommendations provide detailed guidance on the pre-analytical handling, assay selection, and interpretation of ROS and oxidative-damage measurements; broader adherence to such standards would improve comparability and reproducibility across studies [25]. Composite measures such as TOS, TAS, and the OSI have been developed to capture the balance between oxidant production and antioxidant defenses at the systems level. TOS reflects the overall oxidant load in serum and indicates the degree of oxidative stress, whereas TAS represents cumulative antioxidant capacity, integrating both enzymatic and non-enzymatic components. The OSI is calculated as the ratio of TOS to TAS, usually expressed as a percentage, and provides a single indicator of redox equilibrium that is particularly useful when individual markers change in opposite directions [15,33]. These global indicators are attractive in clinical research because measuring each oxidant and antioxidant separately is impractical.

3.6 Link Between Oxidative Stress and Clinical Presentation

Redox imbalance intersects with nociceptive processing at several levels that are directly relevant to the pain experience in fibromyalgia. Brain tissue, with its high lipid content, is particularly vulnerable to free-radical injury; lipid peroxidation in the central nervous system generates hydroperoxides and aldehydes such as MDA and 4-hydroxynonenal, and levels of these products show a positive correlation with fibromyalgia severity as quantified by the FIQ [24,33]. In peripheral compartments, reduced activity of key enzymatic antioxidants – including SOD, CAT, GPx, and glutathione reductase – accompanies increased NO and MDA in red-blood-cell lysates. Activities of CAT, SOD, glutathione reductase, and GPx correlate negatively with FIQ domains that capture overall functioning, pain, psychological distress, and sleep, whereas lipid peroxidation exhibits a significant positive association with FIQ scores, linking diminished antioxidant defense and enhanced oxidative damage to worse pain-related outcomes (Table 1) [24]. Taken together, these data suggest a close association between oxidative stress markers and pain perception, in which enzymatic antioxidant deficiencies, lipid peroxidation, and reactive nitrogen intermediates may contribute to peripheral and central sensitization mechanisms relevant to the intensity and persistence of fibromyalgia pain [17,24,33].

Cognitive complaints such as impaired memory, reduced attention, and mental fatigue are frequently reported in fibromyalgia and are often grouped under the term "fibro fog." Oxidative mechanisms have been proposed to intersect with these cognitive symptoms through several

converging pathways involving neuronal vulnerability, redox-sensitive signaling, and antioxidant defense systems [10]. Neurons are intrinsically susceptible to free-radical damage because of their high content of polyunsaturated fatty acids, elevated oxygen utilization, and comparatively weak endogenous antioxidant capacity. In brain tissue, lipid peroxidation leads to accumulation of hydroperoxides and aldehydes such as MDA and 4-hydroxynonenal, and levels of these products positively correlate with fibromyalgia severity measured by the FIQ, which includes domains related to cognitive difficulty and mental fatigue [24,33]. Altered activities of SOD, CAT, GPx, and glutathione reductase have been documented in fibromyalgia, with reduced enzyme function accompanying increased NO and MDA in red-blood-cell lysates [24]. These antioxidant enzymes show negative associations with FIQ domains, including psychological distress and sleep disturbance, whereas lipid peroxidation exhibits a significant positive correlation with the FIQ. It should be emphasized, however, that direct evidence linking specific oxidative stress biomarkers to objective neurocognitive testing outcomes remains limited; much of the proposed relationship with "fibro fog" is inferred from associations with composite symptom scales rather than from dedicated cognitive assessment, and this connection should therefore be regarded as hypothesis-generating.

4. Limitations of the Review and Critical Appraisal of the Evidence

Several limitations should be considered when interpreting this synthesis. First, this is a narrative review rather than a systematic review: literature was selected on the basis of topical relevance at the authors' discretion, without a registered protocol, a reproducible search algorithm, dual independent screening, a formal quality or risk-of-bias assessment of the included studies, or a quantitative synthesis. The review was therefore not conducted or reported in accordance with the PRISMA 2020 guidelines [9], and no PRISMA flow diagram is presented. As with any narrative review, this approach carries a risk of selection and publication bias. In addition, the restriction to English-language publications may have introduced language bias and excluded relevant evidence published in national or regional journals, including Bulgarian and other Eastern European studies of fibromyalgia and of overlapping pain–mood phenotypes [12,21].

Second, the primary literature underlying this field has important methodological constraints. Many of the cited studies are cross-sectional and therefore cannot establish causality; as noted in Section 3.2, it remains

unresolved whether oxidative abnormalities are causal contributors to fibromyalgia, consequences of chronic pain, stress, and physical deconditioning, or epiphenomena. Sample sizes are frequently small, and there is substantial heterogeneity across studies in patient populations, disease severity, comorbidity profiles, and the diagnostic criteria used to define fibromyalgia [42]. Potential confounding factors – including obesity, smoking, dietary habits, physical activity, sleep quality, medication use, and psychological comorbidities – are inconsistently controlled, yet each can independently influence redox status [43]. Finally, biomarker assessment methods vary considerably between laboratories, and the limited standardization and cross-laboratory reproducibility of these assays further complicate the comparison and pooling of results. These considerations argue for cautious interpretation of the associations described in this review.

5. Conclusions

Fibromyalgia emerges as a persistent, multisystem condition in which chronic widespread pain is accompanied by sleep disturbance, fatigue, cognitive symptoms, and psychiatric comorbidity. Clinical heterogeneity and overlapping disorders complicate case ascertainment and outcome prediction, and current diagnostic instruments – based on symptom counts and severity scales – capture important clinical dimensions but can yield variable case mixes across settings. Reliable molecular readouts could therefore improve phenotyping, help stratify patients, and inform mechanistic trials.

A coherent body of evidence associates an oxidant-shifted milieu with many affected individuals. Excess formation of reactive oxygen and nitrogen species, mitochondrial respiratory-chain dysfunction, and depletion of key cofactors such as coenzyme Q10 appear linked to higher levels of lipid peroxidation and oxidized nucleic acids, while activities of endogenous antioxidant enzymes – including SOD, GPx, and CAT – are frequently reduced. Lipid peroxidation markers (for example, MDA and 4-hydroxynonenal) and oxidized DNA species (for example, 8-oxo-dG) correlate with clinical indices of pain, fatigue, and impaired quality of life, supporting a possible mechanistic link between redox imbalance, peripheral nociceptor input, and central sensitization, while acknowledging that the available evidence cannot establish causality [24].

Despite these consistent associations, a substantial gap remains between biomarker research and clinical application. The diagnostic sensitivity and

specificity of individual oxidative stress markers are largely unknown, and no single biomarker has yet demonstrated sufficient discriminatory ability to support routine diagnosis. Reproducibility across laboratories is limited, and prospective validation studies are lacking. Establishing clinical utility will require adequately powered, prospective studies that use standardized pre-analytical and analytical protocols, predefined diagnostic thresholds, and external validation across independent cohorts. In parallel, the redox and mitochondrial abnormalities reviewed here raise the possibility of therapeutic strategies that target oxidative stress directly; early data on coenzyme Q10 supplementation, including a small placebo-controlled trial, are encouraging but preliminary, and adequately powered, independent randomized trials will be needed to determine whether such interventions produce meaningful and durable clinical benefit [37,38].

Designing biomarker panels for fibromyalgia requires explicit consideration of assay characteristics, redox-pathway coverage, and clinical interpretability. Classical spectrophotometric and fluorometric assays for lipid peroxidation (TBARS), protein carbonyls, and DNA oxidation (8-oxo-dG) are inexpensive and widely used but suffer from important limitations in specificity, stability, and susceptibility to artifacts [25,30,40].

In summary, the convergence of mitochondrial dysfunction, enhanced reactive-species formation, depleted antioxidant defenses, and correlated measures of macromolecular damage provides a coherent mechanistic framework that may link redox disturbance to the core clinical features of fibromyalgia, although current evidence cannot establish the direction of these relationships [35]. Implementing validated, multilayered biomarker strategies in concert with standardized laboratory practice offers a feasible pathway toward more precise patient stratification and toward interventions that target the biological processes most closely tied to symptom generation and persistence. Continued interdisciplinary collaboration and methodological harmonization will be required to translate these insights into clinically useful and reproducible diagnostics.

Declarations

Author Contributions

Conceptualization: G.M.; Methodology: G.M., M.G.; Formal analysis: I.Y., S.D.; Investigation: G.M., I.Y., I.S., V.H.; Literature search and data curation: S.D., V.H.; Writing – original draft: G.M., K.K.; Writing – review & editing: all

authors; Supervision: S.P.-B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

This article is a narrative review based exclusively on previously published studies and did not involve any new research on human participants or animals conducted by the authors. Ethics approval and written informed consent were therefore not required, given the literature-based nature of the study design.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are available on reasonable request from the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest, financial or non-financial, relevant to this work.

References

1. Bonomi S, Oltra E, Alberio T. In search of molecular correlates of fibromyalgia: the quest for objective diagnosis and effective treatments. *Int J Mol Sci.* 2025;26(19):9762. doi:10.3390/ijms26199762
2. Bhargava J, Goldin J. Fibromyalgia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK540974/>
3. Winslow BT, Vandal C, Dang L. Fibromyalgia: diagnosis and management. *Am Fam Physician.* 2023;107(2):137–144.
4. Salaffi F, Di Carlo M, Farah S, et al. Diagnosis of fibromyalgia: comparison of the 2011/2016 ACR and AAPT criteria and validation of the modified fibromyalgia assessment status. *Rheumatology (Oxford).* 2020;59(10):3042–3049.
5. Wolfe F, Clauw DJ, Fitzcharles MA, et al. Revisions to the 2010/2011 fibromyalgia diagnostic criteria. *Semin Arthritis Rheum.* 2016;46(3):319–329. doi:10.1016/j.semarthrit.2016.08.012
6. Wolfe F, Clauw DJ, Fitzcharles MA, et al. Fibromyalgia criteria and severity scales for clinical and epidemiological studies: a modification of the ACR preliminary diagnostic

- criteria for fibromyalgia. *J Rheumatol.* 2011;38(6):1113–1122. doi:10.3899/jrheum.100594
7. Wolfe F, Clauw DJ, Fitzcharles MA, et al. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res (Hoboken)*. 2010;62(5):600–610. doi:10.1002/acr.20140
 8. Galvez-Sánchez CM, Reyes del Paso GA. Diagnostic criteria for fibromyalgia: critical review and future widespread pain perspectives. *J Clin Med.* 2020;9(4):1219. doi:10.3390/jcm9041219
 9. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* 2021;372:n71. doi:10.1136/bmj.n71
 10. Siracusa R, Di Paola R, Cuzzocrea S, et al. Fibromyalgia: pathogenesis, mechanisms, diagnosis and treatment options update. *Int J Mol Sci.* 2021;22(8):3891. doi:10.3390/ijms22083891
 11. Galvez-Sánchez CM, De la Coba P, Duschek S, et al. Reliability, factor structure and predictive validity of the widespread pain index and symptom severity scales of the 2010 American College of Rheumatology criteria of fibromyalgia. *J Clin Med.* 2020;9(8):2460. doi:10.3390/jcm9082460
 12. Reshkova V. Assessment of chronic fatigue in patients with fibromyalgia. *Rheumatology (Bulgaria)*. 2026;33(3):3–10. doi:10.35465/rbj.rbj.430
 13. Sarzi-Puttini P, Giorgi V, Marotto D, et al. Fibromyalgia: an update on clinical characteristics, aetiopathogenesis and treatment. *Nat Rev Rheumatol.* 2020;16(11):645–660. doi:10.1038/s41584-020-00506-w
 14. Clauw DJ. Fibromyalgia and related conditions. *Mayo Clin Proc.* 2015;90(5):680–692. doi:10.1016/j.mayocp.2015.03.014
 15. Fatima G, Das SK, Mahdi AA. Oxidative stress and antioxidative parameters and metal ion content in patients with fibromyalgia syndrome: implications in the pathogenesis of disease. *Clin Exp Rheumatol.* 2013;31(6 Suppl 79):S128–S133.
 16. Spaeth M. Epidemiology, costs, and the economic burden of fibromyalgia. *Arthritis Res Ther.* 2009;11(3):117. doi:10.1186/ar2715
 17. Meeus M, Nijs J, Hermans L, et al. The role of mitochondrial dysfunctions due to oxidative and nitrosative stress in the chronic pain or chronic fatigue syndromes and fibromyalgia patients: peripheral and central mechanisms as therapeutic targets? *Expert Opin Ther Targets.* 2013;17(9):1081–1089. doi:10.1517/14728222.2013.818657
 18. Park DJ, Lee SS. New insights into the genetics of fibromyalgia. *Korean J Intern Med.* 2017;32(6):984. doi:10.3904/kjim.2016.207
 19. Singh L, Kaur A, Bhatti MS, et al. Possible molecular mediators involved and mechanistic insight into fibromyalgia and associated co-morbidities. *Neurochem Res.* 2019;44:1517–1532. doi:10.1007/s11064-019-02805-5
 20. Marino Y, Inferrera F, D'Amico R, et al. Role of mitochondrial dysfunction and biogenesis in fibromyalgia syndrome: molecular mechanism in central nervous system. *Biochim Biophys Acta Mol Basis Dis.* 2024;1870(7):167301. doi:10.1016/j.bbadis.2024.167301
 21. Bogdanova S, Dimitrov S, Gerganov G, et al. Genetic and molecular insights linking mood disorders and pain. *Rheumatology (Bulgaria)*. 2026;34(1):43–64. doi:10.35465/7825v194

22. Paroli M, Gioia C, Accapezzato D, et al. Inflammation, autoimmunity, and infection in fibromyalgia: a narrative review. *Int J Mol Sci.* 2024;25(11):5922. doi:10.3390/ijms25115922
23. Poljšak B, Jamnik P, Milisav I. The importance of a multifaceted approach for accurate and comprehensive evaluation of oxidative stress status in biological systems. *Antioxidants (Basel).* 2025;14(9):1083. doi:10.3390/antiox14091083
24. Assavarittirong C, Samborski W, Grygiel-Górniak B. Oxidative stress in fibromyalgia: from pathology to treatment. *Oxid Med Cell Longev.* 2022;2022:1582432. doi:10.1155/2022/1582432
25. Murphy MP, Bayir H, Belousov V, et al. Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. *Nat Metab.* 2022;4(6):651–662. doi:10.1038/s42255-022-00591-z
26. Jurado-Priego LN, Cueto-Ureña C, Ramírez-Expósito MJ, et al. Fibromyalgia: a review of the pathophysiological mechanisms and multidisciplinary treatment strategies. *Biomedicines.* 2024;12(7):1543. doi:10.3390/biomedicines12071543
27. Russo M, Santarelli D, Georgius P, et al. A review of etiological biomarkers for fibromyalgia and their therapeutic implications. *Pain Physician.* 2024;27(8):495–506.
28. Inferrera F, Marino Y, D'Amico R, et al. Impaired mitochondrial quality control in fibromyalgia: mechanisms involved in skeletal muscle alteration. *Arch Biochem Biophys.* 2024;758:110083. doi:10.1016/j.abb.2024.110083
29. Marrocco I, Altieri F, Peluso I. Measurement and clinical significance of biomarkers of oxidative stress in humans. *Oxid Med Cell Longev.* 2017;2017:6501046. doi:10.1155/2017/6501046
30. La Rubia M, Rus A, Molina F, et al. Is fibromyalgia-related oxidative stress implicated in the decline of physical and mental health status? *Clin Exp Rheumatol.* 2013;31(6 Suppl 79):S121–S127.
31. Saxena P, Selvaraj K, Khare SK, et al. Superoxide dismutase as a multipotent therapeutic antioxidant enzyme: role in human diseases. *Biotechnol Lett.* 2021;44(1):1–22. doi:10.1007/s10529-021-03200-3
32. Altindag O, Celik H. Total antioxidant capacity and the severity of the pain in patients with fibromyalgia. *Redox Rep.* 2006;11(3):131–135. doi:10.1179/135100006X116628
33. Uslu EY, Uslu MF, Yildiz S, et al. Evaluating oxidative stress in fibromyalgia: diagnostic utility and its relationship with clinical and psychological parameters. *Medicina (Kaunas).* 2025;61(7):1248. doi:10.3390/medicina61071248
34. Beyaztas H, Aktas S, Guler EM, et al. Oxidative stress may be a contributing factor in fibromyalgia patients' pain mechanisms. *Reumatismo.* 2023;75(3):1550. doi:10.4081/reumatismo.2023.1550
35. Cordero MD, De Miguel M, Moreno Fernández AM, et al. Mitochondrial dysfunction and mitophagy activation in blood mononuclear cells of fibromyalgia patients: implications in the pathogenesis of the disease. *Arthritis Res Ther.* 2010;12(1):R17. doi:10.1186/ar2918
36. Cordero MD, Moreno-Fernández AM, de Miguel M, et al. Coenzyme Q10 distribution in blood is altered in patients with fibromyalgia. *Clin Biochem.* 2009;42(7-8):732–735. doi:10.1016/j.clinbiochem.2008.12.010

37. Cordero MD, Cano-García FJ, Alcocer-Gómez E, et al. Oxidative stress correlates with headache symptoms in fibromyalgia: coenzyme Q10 effect on clinical improvement. *PLoS One*. 2012;7(4):e35677. doi:10.1371/journal.pone.0035677
38. Cordero MD, Alcocer-Gómez E, Culic O, et al. NLRP3 inflammasome is activated in fibromyalgia: the effect of coenzyme Q10. *Antioxid Redox Signal*. 2014;20(8):1169–1180. doi:10.1089/ars.2013.5198
39. Mas-Bargues C, García-Domínguez E, Borrás C. Recent approaches to determine static and dynamic redox state-related parameters. *Antioxidants (Basel)*. 2022;11(5):864. doi:10.3390/antiox11050864
40. De Leon JAD, Borges CR. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *J Vis Exp*. 2020;(159):e61122. doi:10.3791/61122
41. Frijhoff J, Winyard PG, Zarkovic N, et al. Clinical relevance of biomarkers of oxidative stress. *Antioxid Redox Signal*. 2015;23(14):1144–1170. doi:10.1089/ars.2015.6317
42. Bazzichi L, Giacomelli C, Consensi A, et al. One year in review 2020: fibromyalgia. *Clin Exp Rheumatol*. 2020;38(1):3–8.
43. Häuser W, Ablin J, Fitzcharles MA, et al. Fibromyalgia. *Nat Rev Dis Primers*. 2015;1:15022. doi:10.1038/nrdp.2015.22

Биомаркери за оксидативен стрес при пациенти с фибромиалгия и тяхната диагностична приложимост

Резюме

Въведение: Фибромиалгията е хронично, дифузно болково разстройство, придружено от умора, нересторативен сън, когнитивни затруднения и психиатрична коморбидност. Диагнозата остава клинична и не е установен обективен биомаркер, но все повече данни сочат участието на оксидативния стрес в нейната патофизиология. **Цел:** Да се обобщят съвременните данни за биомаркерите на оксидативния стрес и тяхната връзка с клиничната изява на фибромиалгията, както и да се оцени потенциалното им диагностично и терапевтично значение. **Методи:** Извършен е наративен обзор на литературата, индексирана в PubMed, Scopus и Google Scholar (2005–2025 г., на английски език), с акцент върху ензимните и неензимните антиоксиданти, маркерите за оксидативно увреждане и валидираните мерки за оценка на симптомите. **Резултати:** Намалената активност на супероксид дисмутаза, глутатион пероксидаза и каталаза, заедно с повишените маркери за липидно и ДНК окисление (малондиалдехид, 4-хидроксиноненал, 8-оксо-dG), се свързват с по-голяма тежест на симптомите по валидирани клинични скали, като Въпросника за въздействието на фибромиалгията (FIQ). Композитните индекси (общ оксидантен статус, общ антиоксидантен статус, индекс на оксидативен стрес) и мултимаркерните панели осигуряват по-надеждна оценка от единичните маркери, предвид компенсаторните промени в антиоксидантната мрежа и преданалитичните влияния, като хранене и обработка на пробите. Методологичните ограничения на често използваните анализи (TBARS, имуноанализи) обуславят прилагането на хроматографски и маспектрометрични подходи за специфично за конкретните симптоми количествено определяне, както и на стандартизирани протоколи за вземане на проби. **Заклучение:** Редокс дисбалансът е механизтично свързан с основните клинични характеристики на фибромиалгията, включително болка, умора и влошено качество на живот. Валидираните многослойни биомаркерни панели, съчетани със стандартизирана лабораторна практика, могат да подобрят фенотипизирането и стратификацията на пациентите и да насочат интервенции, въздействащи върху оксидативните механизми, въпреки че са необходими строги контролирани проучвания.

Ключови думи: фибромиалгия, оксидативен стрес, биомаркери, липидна пероксидация, малондиалдехид, антиоксиданти