MARMARA
MEDICAL JOURNAL

Review

https://dergipark.org.tr/tr/pub/marumj

Glutathione: Biosynthesis, metabolism and clinical perspectives

A. Suha YALCIN[1], Naci POLAT[2], Gokhan BICIM[1]

[1]Department of Basic Sciences – Biochemistry, Faculty of Dentistry, Istanbul Kent University, Istanbul, Turkiye
[2]Department of Physiopathology, Faculty of Medicine, Ankara University, Ankara, Turkiye
Corresponding Author: A. Suha YALCIN
E-mail: asyalcin@marmara.edu.tr

Submitted: 01.12.2025 **Accepted:** 30.01.2026

ABSTRACT
Glutathione (GSH) is the main thiol antioxidant involved in redox balance, detoxification, immune regulation, and mitochondrial function. Disturbances in GSH metabolism contribute to cancer, neurodegeneration, cardiometabolic, and hepatic/renal diseases. Mechanisms such as ferroptosis, mitochondrial transport, and S-glutathionylation highlight translational relevance of GSH metabolism. Analytical challenges in methodology are present with liquid chromatography/mass spectroscopy as the gold standard but limited by accessibility. Future priorities include randomized trials and in vivo imaging, which may pave the way for glutathione-centered personalized medicine. In this review, we have included biosynthesis and metabolism of GSH as well as its biochemical roles related to nutrition, health, and disease. Finally, we have discussed measurement methods and future therapeutic approaches based on GSH metabolism.
Keywords: Glutathione, Ferroptosis, Oxidative Stress, N-acetylcysteine, Redox balance

1. INTRODUCTION

This review was prepared using the PubMed, Web of Science, Scopus, and TR Dizin databases, which were searched with the keywords "glutathione," "redox," "clinical biochemistry," "ferroptosis," and "analytical methods." We have included studies published between 1980 and 2025 in English or Turkish, and recent publications within the last five years took precedence.

Glutathione (GSH) is the most important thiol-based antioxidant that maintains cellular redox homeostasis. This tripeptide (γ-glutamyl-cysteinyl-glycine) was discovered by Hopkins in 1921. It has functions against reactive oxygen species (ROS) and participates in a wide variety of biological activities such as detoxification mechanisms, immune response regulation, mitochondrial energy metabolism, and cellular signaling pathway control [1, 2]. Free GSH exists in high concentrations within cells (1-10 mM), making it a central molecule that acts as a "redox buffer" across several organelles including the cytoplasm, nucleus, endoplasmic reticulum, and mitochondria [3]. The oxidized form of GSH is glutathione disulfide (GSSG) (Figure 1). GSH/GSSG ratio is one of the most reliable indicators of cellular redox balance, and its disruption may lead to oxidative stress which contributes to the pathogenesis of various degenerative diseases and the aging process [4]. GSH is essential against oxidative stress, and has regulatory roles in maintaining immune cell function, epigenetic mechanisms and signaling pathways [5, 6]. Abnormal GSH metabolism seems to be important in the development of complex diseases such as cardiovascular diseases, neurodegenerative diseases, metabolic syndrome and cancer [7-11]. In this review, we have included biosynthesis and metabolism of GSH as well as its biochemical roles related to nutrition, health and disease. Finally, we have discussed measurement methods and future therapeutic approaches based on GSH metabolism.

How to cite this article: Yalcin AS, Polat N, Bicim G. Glutathione: Biosynthesis, metabolism and clinical perspectives. Marmara Med J 2026;39(2): 155-165. doi: 10.5472/marumj.1954152

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1954152
Marmara Med J 2026;39(2): 155-165

Marmara Medical Journal
Original Article

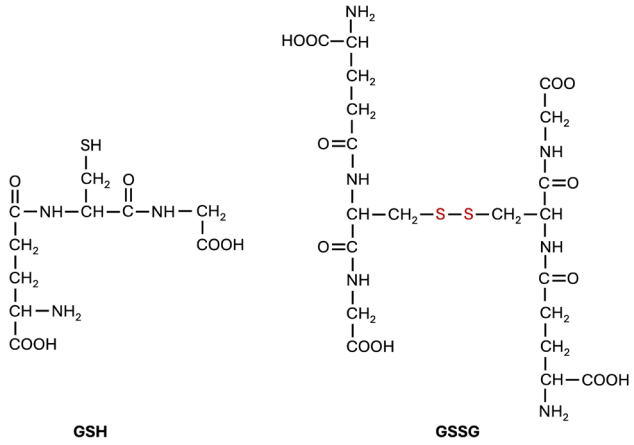

Figure 1. *Structures of glutathione (GSH) and oxidized glutathione (GSSG)*

A. Structure and Biosynthesis

Glutathione is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side chain and cysteine [1]. The carboxyl group of the cysteine residue is attached by normal peptide linkage to glycine. The presence of the γ-glutamyl bond protects GSH from proteolytic enzymes and makes it stable. Cellular concentrations of free GSH are 1-10 mM and it is essential for keeping redox balance in both the cytoplasm and mitochondria [3]. GSH is present in different cellular organelles where it has vital activities. The highest concentration of GSH is in the cytosol and needed for redox homeostasis. Mitochondria have specific carrier systems to transport GSH across their membranes. Here GSH serves as a critical defense line for cell survival by neutralizing ROS generated during energy metabolism [12, 13]. GSH is maintained through enzymatic systems that act as redox buffers during protein folding and disulfide bond formation [14, 15]. The indirect protective effect of GSH against oxidative DNA damage is part of the overall cellular antioxidant defense network.

Glutathione biosynthesis is a two-step adenosine triphosphate (ATP)-dependent process (Figure 2). The enzymes involved are: (1) γ-glutamylcysteine ligase (GCL), also known as γ-glutamylcysteine synthetase, and (2) glutathione synthetase (GS). The first reaction is the condensation of glutamate and cysteine to form γ-glutamylcysteine and represents the rate-limiting step of GSH biosynthesis. The enzyme consists of a catalytic subunit and a modulatory subunit; the latter increases substrate affinity and facilitates the response to redox stress [6]. Glutathione synthetase completes the synthesis by adding glycine to γ-glutamylcysteine. Both steps are ATP-dependent, and biosynthesis of GSH is regulated by the intracellular level of cysteine. Sulfur-containing amino acids from the diet (methionine and cysteine) are important in this regard. There are various ways of controlling GSH biosynthesis: substrate availability, signaling pathways, epigenetic mechanisms, and the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent redox flux. Oxidative stress activates the Nrf2–Keap1 axis increasing GCL and GS expression, while mTOR–ATF4 signaling provides adaptive responses during nutrient deprivation [8, 11, 16-21]. N-acetylcysteine (NAC), α-lipoic acid, and cysteine connect biosynthesis with metabolic pathways and have an important effect on intracellular GSH pools. Epigenetic modifications further control the transcription of GCL and GS [22]. This upregulation leads to therapy resistance with GCL inhibitors in cancer [11]. Genetic polymorphisms in GCL also define the interindividual redox response. Sustained biosynthesis requires an uninterrupted supply of NADPH. Even minute variations in cystine availability can markedly change GSH flux. Cells must distribute NADPH among GSH, thioredoxin, fatty acid synthesis, and other processes which make GSH flux regulation essential for both survival and adaptation [17, 23, 24].

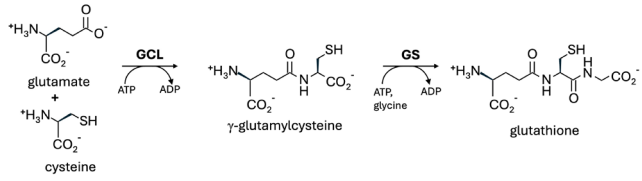

Figure 2. *Enzymatic synthesis of glutathione*

B. Mitochondrial Glutathione (mGSH)

Mitochondria use more than ninety percent of the oxygen utilized in a cell and are the main source of ROS. In this context, mGSH is important as an antioxidant because it helps to detoxify ROS, remove lipid peroxides, and protect cardiolipin. Further, it has a role in the release of cytochrome c, Bcl-2 family proteins, calcium homeostasis, and the mitochondrial permeability transition pore (mPTP), making it crucial for pathways like ferroptosis and apoptosis, as well as survival [13, 16, 25-28]. Mitochondrial GSH is not synthesized *de novo* but imported from the cytosol via inner membrane carriers, mainly the dicarboxylate carrier (DIC, SLC25A10), tricarboxylate carrier (TTC, SLC25A11), and SLC25A39 [29]. DIC mediates transport with succinate/malate, especially in liver and kidney. TTC couples GSH flux with isocitrate/malate to link energy metabolism and redox balance. Recent work identifies SLC25A39 as critical for mGSH uptake and redox maintenance [30]. Genetic or pharmacological inhibition of these carriers leads to mGSH depletion, lipid peroxide accumulation, ferroptosis-like death, and mPTP opening. Tissues with high metabolic activity, such as the liver and neurons, are particularly dependent on these systems [13, 29, 30].

C. Glutathione Dependent Enzymes

Glutathione forms the backbone of thiol-based antioxidant defense in the cell and operates through two main axes: (i) peroxide-reducing networks (particularly the glutathione peroxidase [GPX] and peroxiredoxin/thioredoxin [PRDX/TRX] systems), and (ii) reversible post-translational modifications such as protein S-glutathionylation. GPX family acts on a broad spectrum of substrates, including membrane-embedded phospholipid peroxides, whereas the PRDX/TRX system fine-tunes hydrogen

http://doi.org/10.5472/marumj.1954152
Marmara Med J 2026;39(2): 155-165

peroxide (H_2O_2) signaling. Functional "crosstalk" exists between these two networks, allowing coordinated regulation of redox balance. A recent review emphasizes that the GPX and PRDX/TRX axes act synergistically in both the detoxification of H_2O_2 and the regulation of redox signaling [31]. In mammals, a family of eight glutathione peroxidases (GPX1–GPX8) has been identified. Most of these enzymes are selenoproteins that reduce hydrogen peroxide and lipid hydroperoxides using GSH as an electron donor. The functional overview of GPXs is as follows: GPX1 (the widespread cytosolic form; primary defense in cardiovascular and metabolic tissues), GPX2 (predominantly expressed in the gastrointestinal barrier), GPX3 (soluble in plasma, involved in systemic defense), and GPX4 (directly reduces phospholipid hydroperoxides). The unique feature of GPX4 is its ability to target membrane-embedded phospholipid peroxides, making it the only enzyme that halts the chain reaction of lipid peroxidation, thereby suppressing ferroptosis. Consequently, GPX4 loss results in increased lipid peroxidation followed by ferroptosis. This process may represent a "critical biological threshold" in cancer and neurodegeneration biology. Cystine uptake via SLC7A11 and subsequent GSH synthesis are prerequisites for GPX4 activity, while NADPH is necessary to sustain the cycle for GSSG to GSH reduction by glutathione reductase (GR) and cystine to cysteine reduction. Pharmacological inhibition of GPX4 can enhance chemotherapy sensitivity in certain tumor types. However, excessive inhibition of GPX4 might damage T-cell performance and antitumor reactions. Recent reviews and experimental studies describe ferroptosis as a "double-edged sword" that must be seen in the interaction between the tumor microenvironment and immunity [32, 33]. Glutathione S-transferases (GSTs) catalyze the conjugation of electrophiles with GSH, playing key roles in detoxification and drug metabolism. Genetic polymorphisms of these enzymes contribute to interindividual differences in metabolic reactions and drug responses. For example, the GSTT1 "null" genotype has been associated with increased susceptibility to head and neck cancers [34]. GR reduces oxidized glutathione (GSSG) to its reduced form (GSH) using NADPH. Therefore, NADPH flux is directly linked to its activity. This reaction is one of the fundamental mechanisms required to maintain cellular redox balance [35, 36]. Glutathione-dependent formaldehyde dehydrogenase, also known as ADH5 or GSNOR (S-nitrosoglutathione reductase), plays a role in controlling nitrosative stress by metabolizing GSNO, thereby lowering intracellular S-nitrosothiol (SNO) levels. Dysfunction or reduced expression of ADH5/GSNOR can result in S-nitrosothiol accumulation, excessive protein S-nitrosylation, and subsequent cellular dysfunction [37]. S-glutathionylation is a reversible post-translational modification in which thiol groups (–SH) of proteins are covalently modified by GSH. This modification acts as a protective barrier during oxidative stress by preventing irreversible oxidation of reactive thiols, while also playing a regulatory role in signaling. Transcription factors that are components of signaling pathways like NF-κB, p53, and STAT3 and metabolic enzymes such as GAPDH together with ion channels can be subjected to functional and activity changes via S-glutathionylation. The cycle between S-glutathionylation and de-glutathionylation is controlled by enzymes such as GSTπ and glutaredoxins (GRX, particularly GRX1 and GRX2) [5, 31]. Accumulation of S-glutathionylation in excess under chronic oxidative stress may lead to the disruption of redox signaling networks, encourage mitochondrial dysfunction and activate cell death pathways like ferroptosis and apoptosis. It has been pointed out that S-glutathionylation acts both as a switch for enzyme activities and as a buffer/protective mechanism which protects proteins from oxidative damage [9, 38]. A study conducted on GSH-dependent formaldehyde dehydrogenase showed that the Cys174 residue of ADH5 becomes glutathionylated to reduce GSNOR activity thereby impairing cellular defense against nitrosative stress [39]. Additionally, the deficiency of ADH5 has been related to the buildup of S-nitrosoglutathione, resulting in inflammatory responses that are not properly regulated as well as cellular dysfunction [37, 40].

D. Ferroptosis and Glutathione

Ferroptosis is a regulated form of cell death that is morphologically and biochemically distinct from apoptosis and is triggered by iron-dependent lipid peroxidation of polyunsaturated phospholipid membranes, leading to membrane damage [29, 41]. The main suppressor of ferroptosis is GPX4, which is the only enzyme that can reduce phospholipid hydroperoxides and thus stop lipid peroxidation. GPX4 requires cellular GSH supply through the NADPH–cystine/cysteine–GSH axis regulated by SLC7A11 and GR for its activity. Cells become sensitive to ferroptosis when GSH, NADPH, or GPX4 are deficient [33]. The pathway connects tightly to the overall redox balance: NADPH comes from the pentose phosphate pathway and malic enzyme. Isocitrate dehydrogenase provides support for both GSH recycling as well as cystine reduction. Cancer cells use high GSH/GPX4 activity to escape therapy. However, inhibition of either GPX4 or SLC7A11 will sensitize tumors. GPX4 has an important role in T-cell immunity, thus suggesting that context-specific therapeutic strategies are required [29]. There is some tissue-specific evidence that highlights clinical relevance in the brain. GSH depletion and GPX4 loss promote ferroptosis in Alzheimer's disease and Parkinson's disease [42]. In the heart, ischemia-reperfusion injury involves lipid peroxide accumulation, degradation of GPX4, and opening of mPTP, which can be prevented by ferroptosis inhibitors [43, 44]. Hyperglycemia plus lipotoxicity deplete NADPH reserves in metabolic syndrome thereby further lowering the threshold for ferroptosis [45].

E. Systemic and Tissue-Specific Pathophysiology of Glutathione

Glutathione is the most abundant intracellular nonprotein thiol and a central regulator of redox homeostasis, detoxification, immune signaling, and cell survival. Pathophysiological alterations in GSH metabolism—either depletion, impaired recycling, or dysregulated compartmentalization—contribute to a broad spectrum of diseases, ranging from metabolic and inflammatory disorders to neurodegeneration and cancer. Levels and functions of GSH vary with cell type, tissue-specific metabolic profiles, and oxidative burden. These are important for vulnerability of redox homeostasis specific to different tissues. Table I lists disease modules and levels of evidence summarizing major diseases associated with GSH dysregulation.

http://doi.org/10.5472/marumj.1954152
Marmara Med J 2026;39(2): 155-165

Table I. Disease modules and levels of evidence associated with GSH dysregulation [6, 18, 22, 61, 72, 73, 85, 86]

Disease Module	Mechanisms	Clinical / Translational	Level of Evidence / Notes
Cancer	High GSH/GPX4 Cystine uptake via SLC7A11 NADPH flux	Ferroptosis inducers Nrf2–KEAP1 modulation Targeting GPX4 and SLC7A11	In vitro + animal models Limited clinical cohorts GPX4–GSH axis
Neurodegeneration	mGSH depletion/cardiolipin oxidation ETC dysfunction GCL/GPX4 impairment	MRS-based measurements Correlation of GSH with disease Need for standardization	In vitro + animal models Small clinical cohorts Alzheimer – Parkinson disease
Cardiometabolic	mGSH depletion – mPTP opening Ca^{2+} imbalance, NADPH consumption in hyperglycemia, lipotoxicity	Nrf2 activators NAC, α-lipoic acid Exercise/nutrition interventions GST polymorphism screening	Observational + RCT NAC supplementation is supported in DM and hypertension
Liver/Kidney	GSH depletion Acetaminophen toxicity NAFLD, NASH Impaired S-glutathionylation	NAC as clinical standard Strengthening Nrf2-dependent defense networks	Animal models + observational data NAFLD, NASH, CKD

1. Redox Homeostasis and Oxidative Stress

Systemically, GSH acts as a primary scavenger of ROS either directly or as a cosubstrate for GPXs. The GSH:GSSG ratio is widely used as a marker of cellular redox status. Chronic oxidative stress overwhelms GSH synthesis or recycling via GR, leading to sustained redox imbalance. Additionally, age-related decline in GSH synthesis exacerbates systemic vulnerability to oxidative damage. Redox homeostasis is particularly relevant in cardiovascular disease, aging, diabetes, and sepsis [46, 47].

2. Detoxification and Xenobiotic Metabolism

Conjugation via GSTs is a major phase II detoxification pathway. GSH depletion enhances susceptibility to drug-induced toxicity, particularly acetaminophen hepatotoxicity. Genetic polymorphisms in GSTs modulate disease risk and drug responses. Systemic impact is observed in liver injury, environmental toxin sensitivity, and chemotherapy resistance [48, 49].

3. Immune System Regulation and Inflammation

Glutathione is critical for T-cell proliferation and NK cell activity. NAC improves immunity in HIV/TB coinfection. Combined NAC + Vitamin D therapy reduces senescence markers in elderly peripheral blood mononuclear cells (PBMCs). Studies of COVID-19 with NAC have produced mixed results [5, 50-57]. GlyNAC supplementation in older adults improved redox and mitochondrial function. GSH also regulates immune cell proliferation, cytokine signaling, and NFκB activation. Low intracellular GSH skews immune responses towards a proinflammatory phenotype. Impaired GSH synthesis is linked to chronic inflammation and immune senescence. HIV infection, autoimmune diseases, and chronic inflammatory disorders are among the affected diseases [58, 59].

4. Liver and Kidney Disease

Liver is the main site of GSH synthesis. It contains the highest GSH concentration and serves as a systemic GSH reservoir. Hepatic GSH depletion precedes mitochondrial dysfunction and cell death. Impaired export of GSH affects systemic redox balance in nonalcoholic fatty liver disease (NAFLD), alcoholic liver disease, and drug-induced liver injury [60, 61]. GSH deficiency contributes to NAFLD, non-alcoholic steatohepatitis (NASH), and chronic kidney disease (CKD) [62-64]. Supplementation reduces oxidative stress in NAFLD models and protects against cisplatin – or sepsis-induced acute kidney injury (AKI) [65-69]. In kidney, proximal tubules rely on high GSH. GSH depletion underlies acetaminophen toxicity, for which NAC is the life-saving standard [70]. Disturbances are common in CKD, where supplementation shows variable benefit [71].

5. Central Nervous System Disorders

Neurons rely on astrocytic support for GSH precursors. GSH depletion leads to mitochondrial damage and increased neuronal vulnerability to excitotoxicity. Early GSH loss is a hallmark of neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, and schizophrenia [72]. Neurons depend on cysteine from astrocytes and their low GSH/mGSH ratio makes them susceptible to oxidative stress. Depletion of mGSH and consequent lipid peroxidation promote ferroptosis-like neuronal death. Magnetic resonance spectroscopy (MRS) studies have shown region-specific loss of GSH in Alzheimer's disease, Parkinson's disease, and schizophrenia, supporting its role as a biomarker [73-76]. NAC can partially restore the brain redox capacity [77].

6. Heart and Lung Disease

High ROS production makes mGSH essential in cardiac tissue. Ischemia–reperfusion and metabolic syndrome deplete mGSH and NADPH, thereby lowering ferroptosis thresholds

http://doi.org/10.5472/marumj.1954152
Marmara Med J 2026;39(2): 155-165

and triggering cardiolipin oxidation and mPTP opening. Potential therapies include Nrf2 activators, NAC, α-lipoic acid, and lifestyle interventions [78-80]. GST polymorphisms also modulate cardiovascular risk in Turkish cohorts [81]. Ferroptosis inhibitors and mitochondria-targeted peptides demonstrate cardio protection [29, 82]. Pulmonary epithelial lining fluid contains unusually high GSH concentrations. Oxidant exposure (i.e. smoking, pollution) depletes epithelial GSH. Susceptibility to fibrosis and infection is enhanced as a result of GSH dysregulation in chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis, and acute respiratory distress syndrome (ARDS) [83, 84].

7. Cancer

Glutathione plays a dual role in cancer. Low GSH promotes genomic instability and carcinogenesis whereas high GSH in tumors supports proliferation and chemoresistance. Tissue-specific targeting of GSH metabolism is an active therapeutic strategy in cancer treatment [85, 86]. Tumors escape therapy through maintenance of high GSH/GPX4 activity together with an uptake of cystine via SLC7A11. Inhibition of this axis, for example GPX4/SLC7A11 inhibition, or modulation of Nrf2 could drive ferroptosis, but too much loss of GPX4 might also damage T-cell function [11, 29, 33].

8. Miscellaneous

Glutathione protects mucosal integrity and regulates epithelial turnover. Reduced salivary and mucosal GSH is linked to oral diseases such as periodontitis and oral lichen planus [87]. Impaired GSH metabolism also contributes to mucositis and inflammatory bowel disease (IBD). Exercise elevates ROS, and the GSH/GSSG ratio influences performance and recovery. NAC improves antioxidant capacity but may blunt physiological adaptations with different effects depending on dose and timing [88]. In general, pathophysiological effects of the GSH network differ according to cell type, tissue microenvironment, and metabolic context. Distinct mechanisms and therapeutic implications are present in cancer as well as neurodegenerative disease, cardiometabolic disorders, hepatic and renal pathology. Table II summarizes clinical applicability of blood GSH, GSSG levels, and GSH/GSSG ratio in both healthy individuals and in pathological conditions.

F. From Laboratory to Human Studies

Glutathione is one of the most important biomolecules in human health. It does not only keep the balance of redox inside cells, but it also has very important roles in controlling immune responses, functioning of neurons, balancing cardiometabolic factors, and detoxifying harmful substances. Lower amounts of GSH or changes in the ratio between GSH and GSSG are parts of the disease process in many systemic diseases. Although GSH is very important biologically and clinically; accurate, sensitive, and consistent measurement of GSH has been a challenge. This is due to its high susceptibility to oxidation, matrix effects, and delays in processing of samples that change "true" values, leading to large variability between laboratories. Thus, pre-analytical care and proper choice of analytical platforms have become critically important. A recent review particularly emphasized these pitfalls and strategies for overcoming them [89].

Table II. *Clinical applicability of blood levels of GSH, GSSG, and GSH/GSSG ratio in healthy individuals and in pathological conditions [110-112]*

	Healthy individuals	Pathological conditions	Clinical applicability / interpretation
Reduced glutathione (GSH)	High intracellular concentrations (especially in erythrocytes) Predominant form (>95–98% of total glutathione pool) Reflects adequate antioxidant and detoxification capacity	Decreased levels reported in conditions with chronic or acute oxidative stress Neurodegenerative diseases, cardiovascular disease, diabetes, chronic liver disease, infections, cancer	Marker of antioxidant reserve and intracellular redox buffering Low GSH suggests impaired defense against oxidative stress, xenobiotics, and inflammation Useful for monitoring disease burden or response to antioxidant or metabolic interventions
Oxidized glutathione (GSSG)	Very low levels under physiological conditions Efficiently reduced back to GSH by glutathione reductase	Elevated in oxidative stress–associated disorders Inflammation, malignancies, metabolic and cardiovascular diseases, severe infections	Marker of oxidative burden and redox imbalance Increased GSSG reflects ROS overproduction or impaired recycling Particularly informative when measured relative to GSH
GSH/GSSG ratio	High ratio (commonly >100:1 at the cellular level) Indicates a strongly reducing intracellular environment	Decreased ratio in diseases characterized by oxidative stress Cancer, neurodegenerative disorders, diabetes, chronic inflammatory diseases, severe infections	Considered as the most sensitive and integrative indicator of redox status Reflects dynamic balance between antioxidant capacity and oxidative load Useful for disease staging, prognosis, and monitoring therapeutic efficacy

- *Whole blood or erythrocyte GSH better reflects intracellular antioxidant status than plasma*
- *Plasma GSSG and GSH are prone to artificial oxidation during sampling, which may affect the true GSH/GSSG ratio*

http://doi.org/10.5472/marumj.1954152
Marmara Med J 2026;39(2): 155-165

1. Spectrophotometric and Fluorimetric Assays

Assay with Ellman's reagent (dithionitrobenzoic acid, DTNB) measures free thiols. This method is easy and cheap, but not specific to GSH because other thiols give a signal and is very much exposed to pre-analytical oxidation artifacts. The fluorimetric assay based on o-phthalaldehyde (OPA) derivatization increases sensitivity but needs careful sample preparation. Protein precipitation and use of non-reducing alkylating agents like N-ethylmaleimide may reduce artifacts. Common pitfalls of these assays, including falsely elevated GSSG results as well as artifactually low GSH results, were reviewed in detail [7].

2. *Flow cytometry*

Glutathione can be measured by flow cytometry using membrane permeable, thiol-reactive fluorescent probes such as monochlorobimane, which forms a fluorescent adduct with GSH in a reaction catalyzed by glutathione S-transferase, enabling quantitative, single-cell analysis of intracellular redox status [90]. This method allows simultaneous multiparametric assessment of GSH levels alongside immunophenotypic or functional markers, revealing redox heterogeneity within cell populations. GSH depletion or dysregulation is associated with disease progression, treatment resistance, and prognosis. Flow cytometric assessment of GSH is valuable for evaluating oxidative stress, immune cell dysfunction, and altered redox balance in diseases including cancer, neurodegenerative disorders, cardiovascular disease, and inflammation [91].

3. Enzyme immunoassays (EIA)

Enzyme-linked immunosorbent assays are widely used for the quantification of GSH in biological samples such as plasma, serum, cell lysates, and tissue homogenates. These assays typically rely on the derivatization of GSH (reduced and/or oxidized), followed by detection using highly specific antibodies, enabling sensitive and reproducible measurement at low micromolar or nanomolar concentrations [92, 93]. EIA-based GSH assays are well suited for high-throughput clinical and experimental studies, allowing standardized assessment of systemic oxidative stress and redox imbalance. Clinically, GSH measurement by EIA has been applied as a biomarker in conditions including cardiovascular disease, cancer, metabolic disorders, and inflammatory diseases, where altered GSH/GSSG ratios reflect disease severity, treatment response, and antioxidant capacity.

4. Chromatographic and Mass Spectrometric Methods

High-performance liquid chromatography (HPLC) with fluorescence or electrochemical detection can separate and quantify GSH and GSSG. Specificity can be enhanced through derivatization and alkylation. Liquid chromatography tandem mass spectrometry LC/MS-MS is considered the "gold standard" for clinical and research applications [8]. It offers high specificity and sensitivity and requires small sample volumes compatible with various matrices, including plasma, erythrocytes, and tissues, ensuring interlaboratory comparability when using isotopically labeled internal standards. A review of papers from 2019-2024 systematically summarized these advantages along with typical artifacts [94]. A Turkish study comparing HPLC and spectrophotometry for measuring erythrocyte GSH has made an early contribution to methodological standardization [95].

5. Capillary Electrophoresis and Microsensors

Capillary electrophoresis allows fast separation using small sample volumes. However, instrument access is still limited. Electrochemical and biosensor-based approaches using microelectrodes permit almost real-time monitoring of GSH in cell cultures as well as tissue slices. A recent review provides a wide technological overview on these developments [96].

6. Magnetic Resonance Spectroscopy (MRS)

Magnetic resonance spectroscopy (^{1}H-MRS) allows *in vivo* quantification of brain GSH. However, the peaks of GSH overlap those from neighboring metabolites. Advanced spectral editing is required together with higher magnetic field strength and multicenter standardization. Methodological papers published between 2019–2024 have demonstrated reliable reproducibility of protocols. The clinical spectrum of applications is steadily expanding neuropsychiatric disorders and neurodegeneration [74, 75, 97].

7. Fluorescent and Biosensor Probes

The family of redox-sensitive GFP (roGFPs), most notably the Grx1-roGFP2 fusion, gives real-time specificity towards the GSH/GSSG redox couple. This can provide very high spatiotemporal resolution imaging on micro-niche dynamics when applied together with H_2O_2-sensitive probes like HyPer. Since its introduction in 2008 [98], Grx1-roGFP2 has been optimized for enhanced specificity and rapid responsiveness, with applications now spanning live-cell and tissue-level studies [99-101]. Recent studies with HyPer systems further complement this by enabling monitoring of reduction activity [102].

8. Positron Emission Tomography (PET) Tracers

System Xc–, also named cystine/glutamate antiporter, is an important intracellular antioxidant element. Positron emission tomography (PET) tracer [^{18}F] FSPG (BAY 94-9392) does not directly image GSH but visualizes System Xc– (SLC7A11) activity. Since this pathway is linked to cystine supply, GSH biosynthesis, and redox stress, it serves as an indirect biomarker of oxidative stress and therapeutic response. Studies from 2023–2024 have reported advances in production standardization, early response monitoring to radiotherapy, and examples of clinical application, with new clinical trials currently underway [103].

9. Miscellaneous

Local contributions to the analytical measurement of GSH and related enzyme activities remain relatively limited. Aktaş et

http://doi.org/10.5472/marumj.1954152
Marmara Med J 2026;39(2): 155-165

al. [95] previously compared spectrophotometric and HPLC methods for erythrocyte reduced GSH measurements. More recently, a study demonstrated HPLC-based validation in local samples [104]. Yazıcı et al. [105] evaluated redox status across different matrices. Polat et al. [106] introduced analysis of GSH level in plasma by micro-fluidic system.

10. Quality Control Issues

The most significant sources of error in GSH measurements arise during the pre-analytical phase. Hemolysis leads to the release of abundant erythrocytic GSH into plasma, resulting in falsely elevated values. Delays in sample processing or storage at room temperature accelerate spontaneous oxidation of GSH, causing artificially elevated GSSG values. It was demonstrated that when plasma preparation was delayed (e.g., 1–3 hours), both GSH and GSSG levels as well as the GSH/GSSG ratio changed linearly. Addition of alkylating agents such as N-ethylmaleimide largely prevented these shifts [90]. Samples without alkylation undergo exchange between free GSH and protein-SSG, leading to GSSG values that do not reflect true concentrations. Stabilization steps such as rapid cooling and centrifugation, protein precipitation and isotope-labeled internal standards are critical for reliable analysis. Methodological differences (Ellman's assay, HPLC, enzymatic recycling, etc.) across various tissue and fluid samples are a major source of variability in results [107]. Optimization of pre-analytical controls such as sample collection, storage, and stabilization has been strongly validated by the improvement of erythrocyte GSH measurement with HPLC-ECD to emphasize the need for this type of validation in routine laboratory practice [108]. Pre-centrifugation time and hemolysis effects on metabolomic profiles were discussed, and it was emphasized that for labile thiols like GSH, time should be kept as short as possible [109]. Standardization plays a pivotal role in assuring reliability and comparability of results obtained from GSH measurements. The application of isotopically labeled internal standards in LC–MS/MS analyses increases the accuracy by correcting recovery losses and matrix effects [94]. When establishing population-based cut-off values for multi-center studies, age, sex, diet, and comorbidities should be considered [96]. To achieve inter-laboratory consistency among different laboratories working on the same project, external quality assessment programs are very important. LC–MS/MS based panels can be used as strong internal quality assurance [94]. The decision-making process regarding how to measure GSH involves several steps: (i) matrix selection where LC–MS/MS with isotopic internal standards is preferred for clinical applications, but HPLC or OPA/DTNB may be used in exploratory studies; (ii) pre-analytical control including rapid cooling, alkylation, and protein precipitation which should be strictly applied (iii) standardized reporting and quality metrics in MRS protocols; and (iv) translational imaging approaches like [^{18}F]FSPG-PET that permit monitoring of oxidative stress as well as therapeutic response during early-phase clinical studies.

G. Future Perspectives

At the center of cellular redox homeostasis, GSH is an important element. It has a direct impact on antioxidant defense, xenobiotic detoxification, immune regulation, and mitochondrial function. Dysregulation of GSH metabolism is a common feature of many diseases that include cardiovascular disease and neurodegeneration among others. Current literature emphasizes GSH metabolism as an attractive target for clinical biochemistry and translational medicine, while underscoring the need for rigorous methodological approaches [8]. The conceptual foundations were laid by classical studies, and today GSH metabolism continues to be a central focus in personalized medicine. However, the evidence base supporting existing therapeutic strategies is heterogeneous. NAC has established clinical practice and strong evidence support. Gly-NAC and α-lipoic acid have limited but promising supporting findings, but intravenous GSH applications are controversial due to insufficient evidence. New research paths have emerged by advanced mechanism discoveries such as ferroptosis, mitochondrial GSH transport, and S-glutathionylation. However, it is important to clarify what level of clinical evidence is available. From an analytical perspective, GSH measurements are fragile because they are very sensitive to pre-analytical steps, and rapid cooling, protein precipitation, and derivatization must be done meticulously. LC–MS/MS is currently considered as the gold standard, but high costs and limited instrument accessibility prevent its integration into clinical practice. Future directions should include multicenter randomized controlled trials (NAC, GlyNAC, α-lipoic acid, and Nrf2 modulators), validation of *in vivo* imaging approaches (MRS, PET), and standardization initiatives, which may pave the way for GSH-centered personalized medicine strategies.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest.

Financial support: This study received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Authors' contributions: NP, ASY: Conceptualization, NP, GB: Methodology/Literature search, NP: Writing – original draft, ASY: Writing – review and editing, GB, ASY: Visualization. All authors have reviewed and approved the final version of the manuscript.

REFERENCES

[1] Meister A. Glutathione metabolism and its selective modification. J Biol Chem 1988; 263:17205-8.

[2] Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF. The changing faces of glutathione, a cellular protagonist. Biochem Pharmacol 2003;66:1499-503. doi: 10.1016/s0006-2952(03)00504-5

[3] Sies H. Glutathione and its role in cellular functions. Free Rad Biol Med 1999; 27: 916-21. doi: 10.1016/s0891-5849(99)00177-x

[4] Zitka O, Skalickova S, Gumulec J, et al. Redox status expressed as GSH: GSSG ratio as a marker for oxidative stress in paediatric tumour patients. Oncol Lett 2012; 4: 1247-53. doi: 10.3892/ol.2012.931

[5] Franco R, Cidlowski JA. Glutathione efflux and cell death. Antiox Redox Signal 2012; 17: 1694-713. doi: 10.1089/ars.2012.4553

[6] Lu SC. Glutathione synthesis. Biochim Biophys Acta 2013; 1830: 3143-53. doi: 10.1016/j.bbagen.2012.09.008

[7] Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med 2009; 30: 1-12. doi: 10.1016/j.mam.2008.08.006

[8] Gasmi A, Nasreen A, Lenchyk L, et al. An update on glutathione's biosynthesis, metabolism, functions, and medicinal purposes. Curr Med Chem 2024; 31: 4579-601. doi: 10.2174/0109298673251025230919105818

[9] Federici L, Masulli M, De Laurenzi V, Allocati N. The role of S-Glutathionylation in health and disease: a bird's eye view. Nutrients 2024;16:2753. doi: 10.3390/nu16162753.

[10] Tan M, Yin Y, Ma X, et al. Glutathione system enhancement for cardiac protection: pharmacological options against oxidative stress and ferroptosis. Cell Death Dis 2023; 14: 131. doi: 10.1038/s41419-023-05645-y.

[11] Kalinina E. Glutathione-dependent pathways in cancer cells. Int J Molec Sci 2024; 25: 8423. doi: 10.3390/ijms25158423.

[12] Marí M, Morales A, Colell A, García-Ruiz C, Fernández-Checa JC. Mitochondrial glutathione, a key survival antioxidant. Antiox Redox Signal 2009;11:2685-700. doi: 10.1089/ARS.2009.2695

[13] Marí M, de Gregorio E, de Dios C, et al. Mitochondrial glutathione: recent insights and role in disease. Antioxidants 2020; 9: 909. doi: 10.3390/antiox9100909

[14] Dixon BM, Heath S-HD, Kim R, Suh JH, Hagen TM. Assessment of endoplasmic reticulum glutathione redox status is confounded by extensive ex vivo oxidation. Antiox Redox Signal 2008;10:963-72. doi: 10.1089/ars.2007.1869

[15] Chakravarthi S, Jessop CE, Bulleid NJ. The role of glutathione in disulphide bond formation and endoplasmic-reticulum-generated oxidative stress. EMBO Rep 2006; 7: 271-5. doi: 10.1038/sj.embor.7400645

[16] Ribas V, Garcia-Ruiz C, Fernandez-Checa J. Glutathione and mitochondria. Front Pharmacol 2014;5:151. doi: 10.3389/fphar.2014.00151

[17] Moon D-O. NADPH dynamics: Linking insulin resistance and β-Cells ferroptosis in diabetes mellitus. Int J Molec Sci 2023; 25: 342. doi: 10.3390/ijms25010342

[18] Zoccarato A, Smyrnias I, Reumiller CM, et al. NRF2 activation in the heart induces glucose metabolic reprogramming and reduces cardiac dysfunction via upregulation of the pentose phosphate pathway. Cardiovasc Res 2025;121:339-52. doi: 10.1093/cvr/cvae250

[19] Adinolfi S, Patinen T, Deen AJ, et al. The KEAP1-NRF2 pathway: targets for therapy and role in cancer. Redox Biol 2023;63:102726. doi: 10.1016/j.redox.2023.102726

[20] Dinkova-Kostova AT, Copple IM. Advances and challenges in therapeutic targeting of NRF2. Trends Pharmacol Sci 2023; 44:137-49. doi: 10.1016/j.tips.2022.12.003

[21] Torrence ME, MacArthur MR, Hosios AM, et al. The mTORC1-mediated activation of ATF4 promotes protein and glutathione synthesis downstream of growth signals. Elife 2021;10: e63326. doi: 10.7554/eLife.63326

[22] Orhan G, Yapici N, Yuksel M, et al. Effects of N-acetylcysteine on myocardial ischemia-reperfusion injury in bypass surgery. Heart Vessels 2006; 21: 42-7. doi: 10.1007/s00380-005-0873-1.

[23] TeSlaa T, Ralser M, Fan J, Rabinowitz JD. The pentose phosphate pathway in health and disease. Nature Metab 2023; 5: 1275-89. doi: 10.1038/s42255.023.00863-2

[24] Sthijns MM, van Blitterswijk CA, LaPointe VL. Redox regulation in regenerative medicine and tissue engineering: The paradox of oxygen. J Tissue Eng Reg Med 2018; 12:2013-20. doi: 10.1002/term.2730

[25] Chen TH, Wang HC, Chang CJ, Lee SY. Mitochondrial glutathione in cellular redox homeostasis and disease manifestation. Int J Mol Sci 2024;25:1314. doi: 10.3390/ijms25021314.

[26] Liu Y, Liu S, Tomar A, Yen FS, Unlu G, Ropek N, et al. Autoregulatory control of mitochondrial glutathione homeostasis. Science 2023;382:820-8. doi: 10.1126/science.adf4154

[27] Chen X, Gan B. SLC25A39 links mitochondrial GSH sensing with iron metabolism. Molec Cell 2024;84:616-8. doi: 10.1016/j.molcel.2023.12.037

[28] Sreekumar PG, Wang M, Spee C, Sadda SR, Kannan R. Transporter-mediated mitochondrial GSH depletion leading to mitochondrial dysfunction and rescue with αB crystallin peptide in RPE cells. Antioxidants 2020;9:411. doi: 10.3390/antiox9050411

[29] Ursini F, Maiorino M. Lipid peroxidation and ferroptosis: the role of GSH and GPx4. Free Rad Biol Med 2020;152:175-85. doi: 10.1016/j.freeradbiomed.2020.02.027

[30] Ma M, Kong P, Huang Y, et al. Activation of MAT2A-ACSL3 pathway protects cells from ferroptosis in gastric cancer. Free Rad Biol Med 2022;181:288-99. doi: 10.1016/j.freeradbiomed.2022.02.015

[31] Pei J, Pan X, Wei G, Hua Y. Research progress of glutathione peroxidase family (GPX) in redoxidation. Front Pharmacol 2023;14:1147414. doi: 10.3389/fphar.2023.1147414.

[32] Liang C, Zhang X, Yang M, Dong X. Recent progress in ferroptosis inducers for cancer therapy. Adv Mater 2019;31:1904197. doi: 10.1002/adma.201904197

[33] Zhou Q, Meng Y, Li D, et al. Ferroptosis in cancer: from molecular mechanisms to therapeutic strategies. Signal Transduct Target Ther 2024;9:55. doi: 10.1038/s41392-024-01769-5.

[34] Sadafi S, Choubsaz P, Kazemeini SMM, Imani MM, Sadeghi M. Glutathione S-transferase theta 1 (GSTT1) deletion polymorphism and susceptibility to head and neck carcinoma: a systematic review with five analyses. BMC Cancer 2024;24:885. doi: 10.1186/s12885-024-12618-7.

[35] Ting KK, Jongstra-Bilen J, Cybulsky MI. The multi-faceted role of NADPH in regulating inflammation in activated myeloid cells. Front Immunol 2023;14:1328484. doi: 10.3389/fimmu.2023.1328484.

[36] Alves F, Lane D, Nguyen TPM, Bush AI, Ayton S. In defence of ferroptosis. Signal Transduct Target Ther 2025;10: 2. doi: 10.1038/s41392-024-02088-5.

[37] Jia M, Chai L, Wang J, et al. S-nitrosothiol homeostasis maintained by ADH5 facilitates STING-dependent host defense against pathogens. Nature Comm 2024;15: 1750. doi: 10.1038/s41467-024-46212-z.

[38] Matsui R, Ferran B, Oh A, et al. Redox regulation via glutaredoxin-1 and protein S-glutathionylation. Antiox Redox Signal 2020;32:677-700. doi: 10.1089/ars.2019.7963

[39] Kasamatsu S, Nishimura A, Alam MM, et al. Supersulfide catalysis for nitric oxide and aldehyde metabolism. Sci Adv 2023;9:eadg8631. doi: 10.1126/sciadv.adg8631

[40] Barnett SD, Buxton IL. The role of S-nitrosoglutathione reductase (GSNOR) in human disease and therapy. Crit Rev Biochem Molec Biol 2017;52:340-54. doi: 10.1080/10409238.2017.1304353.

[41] Peleman C, Hellemans S, Veeckmans G, et al. Ferroptosis is a targetable detrimental factor in metabolic dysfunction-associated steatotic liver disease. Cell Death Diff 2024; 31:1113-26. doi: 10.1038/s41418-024-01348-9.

[42] Ji Y, Zheng K, Li S, et al. Insight into the potential role of ferroptosis in neurodegenerative diseases. Front Cell Neurosci 2022;16:1005182. doi: 10.3389/fncel.2022.1005182.

[43] Wang X, Chen T, Chen S, et al. STING aggravates ferroptosis-dependent myocardial ischemia-reperfusion injury by targeting GPX4 for autophagic degradation. Signal Transduct Target Ther 2025;10:136. doi: 10.1038/s41392-025-02216-9

[44] Chen B, Fan P, Song X, Duan M. The role and possible mechanism of the ferroptosis-related SLC7A11/GSH/GPX4 pathway in myocardial ischemia-reperfusion injury. BMC Cardiovasc Disord 2024;24:531. doi: 10.1186/s12872-024-04220-3.

[45] Zhou D, Lu P, Mo X, et al. Ferroptosis and metabolic syndrome and complications: association, mechanism, and translational applications. Front Endocrinol 2024;14: 1248934. doi: 10.3389/fendo.2023.1248934.

[46] Jones DP. Redefining oxidative stress. Antioxid Redox Signal 2006;8:1865-79. doi: 10.1089/ars.2006.8.1865.

[47] Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med 2009;30:1-12. doi: 10.1016/j.mam.2008.08.006.

[48] Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother 2003;57:145-55. doi: 10.1016/s0753-3322(03)00043-x.

[49] Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol 2005;45:1-88. doi: 10.1146/annurev.pharmtox.45.120403.095857.

[50] Nabipur L, Mouawad M, Venketaraman V. Additive effects of glutathione in improving antibiotic efficacy in HIV–M. tb co-infection in the central nervous system: a systematic review. Viruses 2025;17:127. doi: 10.3390/v17010127

[51] De Rosa S, Zaretsky M, Dubs J, et al. N-acetylcysteine replenishes glutathione in HIV infection. Eur J Clin Invest 2000;30:915-29. doi: 10.1046/j.1365-2362.2000.00736.x

[52] Lin N, Erdos T, Louie C, et al. The role of glutathione in the management of cell-mediated immune responses in individuals with HIV. Int J Molec Sci 2024;25:2952. doi: 10.3390/ijms25052952

[53] Rastgoo S, Pourvali K, Raeissadat SA, Eslamian G, Zand H. Co-administration of vitamin D and N-acetylcysteine to modulate immunosenescence in older adults with vitamin D deficiency: a randomized clinical trial. Front Immunol 2025;16:1570441. doi: 10.3389/fimmu.2025.1570441.

[54] Labarrere CA, Kassab GS. Glutathione deficiency in the pathogenesis of SARS-CoV-2 infection and its effects upon the host immune response in severe COVID-19 disease. Front Microbiol 2022;13:979719. doi: 10.3389/fmicb.2022.979719.

[55] Rahimi A, Samimagham HR, Azad MH, Hooshyar D, Arabi M, KazemiJahromi M. The efficacy of N-Acetylcysteine in severe COVID-19 patients: A structured summary of a study protocol for a randomised controlled trial. Trials 2021;22:271. doi: 10.1186/s13063-021-05242-4.

[56] Liu T-H, Wu J-Y, Huang P-Y, et al. Clinical efficacy of N-acetylcysteine for COVID-19: A systematic review and meta-analysis of randomized controlled trials. Heliyon 2024; 10:e25179. doi: 10.1016/j.heliyon.2024.e25179

[57] Kumar P, Liu C, Suliburk J, et al. Supplementing glycine and N-acetylcysteine (GlyNAC) in older adults improves glutathione deficiency, oxidative stress, mitochondrial dysfunction, inflammation, physical function, and aging hallmarks: a randomized clinical trial. J Gerontol 2023;78:75-89. doi: 10.1093/gerona/glac135

[58] Dröge W, Breitkreutz R. Glutathione and immune function. Proc Nutr Soc 2000;59: 595-600. doi: 10.1017/s0029665100000847.

[59] Morris D Khusarany M, Nguyen T. Glutathione and infection. Biochim Biophys Acta 2013;1830:3329-49. doi: 10.1016/j.bbagen.2012.10.012.

[60] Lu SC. Regulation of hepatic glutathione synthesis. Semin Liver Dis 1998;18:331-43. doi: 10.1055/s-2007-1007168.

[61] Yuan L, Kaplowitz N. Glutathione in liver diseases. Mol Aspects Med 2009;30:29-41. doi: 10.1016/j.mam.2008.08.003.

[62] Fannin RD, Russo M, O'Connell TM, et al. Acetaminophen dosing of humans results in blood transcriptome and metabolome changes consistent with impaired oxidative phosphorylation. Hepatology 2010;51:227-36. doi: 10.1002/hep.23330

[63] Aykaç G, Uysal M, Yalçin AS, Koçak-Toker N, Sivas A, Öz H. The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. Toxicology 1985;36:71-6. doi: 10.1016/0300-483x(85)90008-3

[64] Haklar G, Yeğenağa I, Yalçin AS. Evaluation of oxidant stress in chronic hemodialysis patients: use of different parameters.

Clin Chim Acta 1995;234:109-14. doi: 10.1016/0009-8981(94)05982-x

[65] Duni A, Liakopoulos V, Rapsomanikis K-P, Dounousi E. Chronic kidney disease and disproportionally increased cardiovascular damage: does oxidative stress explain the burden? Oxid Med Cell Longev 2017;2017:9036450. doi: 10.1155/2017/9036450.

[66] Santacroce G, Gentile A, Soriano S, Novelli A, Lenti MV, Di Sabatino A. Glutathione: Pharmacological aspects and implications for clinical use in non-alcoholic fatty liver disease. Front Med 2023;10:1124275. doi: 10.3389/fmed.2023.1124275.

[67] Nguyen MT, Lian A, Guilford FT, Venketaraman V. A literature review of glutathione therapy in ameliorating hepatic dysfunction in non-alcoholic fatty liver disease. Biomedicines 2025;13:644. doi: 10.3390/biomedicines13030644

[68] Huang Y, Li Y, Xu X, Teng J, Ding X, Xu J. Protective effect of reduced glutathione on acute kidney injury in lung cancer patients treated with cisplatin: a retrospective cohort study. Renal Failure 2024;46:2411359. doi: 10.1080/0886022X.2024.2411359.

[69] Wang L, Jiang L, Wang J, et al. Reduced glutathione ameliorates sepsis-induced acute kidney injury in mouse model: Involvement of cuproptosis. Renal Failure 2025;47: 2518227. doi: 10.1080/0886022X.2025.2518227.

[70] Wong A, Isbister G, McNulty R, et al. Efficacy of a two bag acetylcysteine regimen to treat paracetamol overdose (2NAC study). EClinicalMedicine 2020;20:100288. doi: 10.1016/j.eclinm.2020.100288.

[71] Simões e Silva AC. Oxidative stress in renal health. Antioxidants (Basel) 2025; 14: 144. doi: 10.3390/antiox14020144.

[72] Dringen R. Metabolism and functions of glutathione in brain. Prog Neurobiol 2000; 62:649-71. doi: 10.1016/s0301-0082(99)00060-x.

[73] Murray AJ, Humpston CS, Wilson M, et al. Measurement of brain glutathione with magnetic resonance spectroscopy in schizophrenia-spectrum disorders – a systematic review and meta-analysis. Brain Behav Immun 2024;115:3-12. doi: 10.1016/j.bbi.2023.09.017

[74] Marjańska M, McCarten JR, Hodges JS, Hemmy LS, Terpstra M. Distinctive neurochemistry in Alzheimer's disease via 7 T in vivo magnetic resonance spectroscopy. J Alzheimer's Dis 2019; 68:559-69. doi: 10.3233/JAD-180861

[75] Do K, Trabesinger A, Kirsten-Krüger M, et al. Schizophrenia: glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. Eur J Neurosci 2000;12:3721-8. doi: 10.1046/j.1460-9568.2000.00229.x

[76] Bottino F, Lucignani M, Napolitano A, et al. In vivo brain GSH: MRS methods and clinical applications. Antioxidants 2021;10:1407. doi: 10.3390/antiox10091407

[77] Martinez-Banaclocha M. N-Acetylcysteine: Modulating the cysteine redox proteome in neurodegenerative diseases. Antioxidants 2022;11:416. doi: 10.3390/antiox11020416

[78] Fatima S, Zhou H, Chen Y, Liu Q. Role of ferroptosis in the pathogenesis of heart disease. Front Physiol 2024;15:1450656. doi: 10.3389/fphys.2024.1450656.

[79] Tian M, Huang X, Li M, et al. Ferroptosis in diabetic cardiomyopathy: from its mechanisms to therapeutic strategies. Front Endocrinol 2024;15:1421838. doi: 10.3389/fendo.2024.1421838.

[80] Dhyani N, Tian C, Gao L, Rudebush TL, Zucker IH. Nrf2-Keap1 in cardiovascular disease: which is the cart and which the horse? Physiol 2024;39:288-301. doi: 10.1152/physiol.00015.2024

[81] Koç S, Alptekin D. Glutatyon S-transferaz genindeki delesyonların (GSTT1 ve GSTM1) koroner arter hastalığı ve akut miyokart infarktüsü ile ilişkisi. (In Turkish) Çukurova Med J 2014;39:256-61. doi:10.17826/cutf.25776

[82] Zheng H, Ou J, Han H, Lu Q, Shen Y. SS-31@ Fer-1 alleviates ferroptosis in hypoxia/reoxygenation cardiomyocytes via mitochondrial targeting. Biomed Pharmacother 2025;183:117832. doi: 10.1016/j.biopha.2025

[83] Rahman I, Biswas SK, Jimenez LA, Torres M, Forman HJ. Glutathione, stress responses, and redox signaling in lung inflammation. Antioxid Redox Signal 2005;7:42-59. doi: 10.1089/ars.2005.7.42.

[84] Cantin AM, North SL, Hubbard RC, Crystal RG. Normal alveolar epithelial lining fluid contains high levels of glutathione. J Appl Physiol (1985) 1987;63:152-7. doi: 10.1152/jappl.1987.63.1.152.

[85] Traverso N, Ricciarelli R, Nitti M, et al. Role of glutathione in cancer progression and chemoresistance. Oxid Med Cell Longev 2013;2013:972913. doi: 10.1155/2013/972913.

[86] Estrela JM, Ortega A, Obrado E. Glutathione in cancer biology and therapy. Crit Rev Clin Lab Sci 2006;43:143-81. doi: 10.1080/10408360500523878

[87] Sardaro N, Della Vella F, Incalza MA, et al. Oxidative stress and oral mucosal diseases: an overview. In Vivo 2019;33:289-296. doi: 10.21873/invivo.11474.

[88] Fernandez-Lazaro D, Dominguez-Ortega C, Busto N, et al. Influence of N-acetylcysteine supplementation on physical performance and laboratory biomarkers in adult males: a systematic review of controlled trials. Nutrients 2023;15:2463. doi: 10.3390/nu15112463

[89] Giustarini D, Tsikas D, Colombo G, et al. Pitfalls in the analysis of the physiological antioxidant glutathione (GSH) and its disulfide (GSSG) in biological samples: An elephant in the room. J Chromatog 2016;1019:21-8. doi: 10.1016/j.jchromb.2016.02.015

[90] Kamencic H, Lyon A, Paterson PG, Juurlink BH. Monochlorobimane fluorometric method to measure tissue glutathione. Anal Biochem 2000;286:35-7. doi: 10.1006/abio.2000.4765.

[91] Hedley DW, Chow S. Evaluation of methods for measuring cellular glutathione content using flow cytometry. Cytometry 1994;15:349-58. doi: 10.1002/cyto.990150411.

[92] Rahman I, Kode A, SK. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nature Protoc 2006;1: 3159-65. doi:10.1038/nprot.2006.378

[93] Semenova NV, Lesnaya AS, Madaeva I, Nikitina OA, Kolesnikova L. Assessment of the glutathione system and the biomolecules oxidation products contribution to the state of free radical homeostasis in women of two ethnic groups with insomnia. Sibirskij Nauchnyj Medicinskij Zhurnal 2023;43:95-101. doi: 10.18699/ssmj20230509.

[94] Tsiasioti A, Tzanavaras PD. Determination of glutathione and glutathione disulfide using liquid chromatography: A review on recent applications. Microchem J 2023;193: 109157. doi: 10.1016/j.microc.2023.109157

[95] Aktaş M, Değirmenci U, Ercan SK, Tamer L, Atik U. Redükte glutatyon ölçümünde HPLC ve spektrofotometrik yöntemlerin karşılaştırılması. (In Turkish) Türk Klin Biyok Derg 2005;3:95-9.

[96] Hamad A, Elshahawy M, Negm A, Mansour FR. Analytical methods for determination of glutathione and glutathione disulfide in pharmaceuticals and biological fluids. Rev Anal Chem 2019;38:20190019. doi: 10.1515/revac-2019-0019

[97] Saleh MG, Rimbault D, Mikkelsen M, et al. Multi-vendor standardized sequence for edited magnetic resonance spectroscopy. Neuroimage 2019;189:425-31. doi: 10.1016/j.neuroimage.2019.01.056

[98] Gutscher M, Pauleau A-L, Marty L, et al. Real-time imaging of the intracellular glutathione redox potential. Nature Meth 2008;5:553-9. doi: 10.1038/nmeth.1212

[99] Marchetti M, Ronda L, Cozzi M, Bettati S, Bruno S. Genetically encoded biosensors for the fluorescence detection of O_2 and reactive O_2 species. Sensors 2023;23:8517. doi: 10.3390/s23208517

[100] Trautsch I, Heta E, Soong PL, et al. Optogenetic monitoring of the glutathione redox state in engineered human myocardium. Front Physiol 2019;10:272. doi: 10.3389/fphys.2019.00272

[101] Kostyuk AI, Panova AS, Kokova AD, et al. In vivo imaging with genetically encoded redox biosensors. Int J Molec Sci 2020;21:8164. doi: 10.3390/ijms21218164

[102] Zhuravlev A, Ezeriņa D, Ivanova J, et al. HyPer as a tool to determine the reductive activity in cellular compartments. Redox Biol 2024;70:103058. doi: 10.1016/j.redox.2024.103058

[103] Lin M, Ta RT, Manning HC. Simplified and highly-reliable automated production of [18F] FSPG for clinical studies. EJNMMI Radiopharm Chem 2023;8:15. doi: 10.1186/s41181-023-00200-8.

[104] Çavdar Z, Eğilmez MY, Bülbül M, Güner G, Koçtürk S. Yüksek performanslı sıvı kromatografisi ile eritrositlerde toplam ve indirgenmiş glutatyon miktarının tayini. (In Turkish) Türk Biyok Derg 2006;31:187-93.

[105] Yazıcı A, Paşaoğlu H, Yücel D, Çelebi N, Bakır F, Özkaya M. Plasma total thiol and erythrocyte reduced glutathione levels in patients with type 2 diabetes mellitus. (In Turkish) T Klin J Med Sci 2002;22:487-92.

[106] Polat N, Çetin B, Yalçın AS. Analysis of glutathione level in plasma by micro-fluidic system. 4th Eurasia Biochemical Approaches & Techniques; Nov. 3-6, 2022.

[107] Cesarini L, Grignaffini F, Alisi A, Pastore A. Alterations in glutathione redox homeostasis in metabolic dysfunction-associated fatty liver disease: a systematic review. Antioxidants 2024;13:1461. doi:10.3390/antiox13121461

[108] Lauwers S, Van Herreweghe M, Foubert K, et al. Validation and optimisation of reduced glutathione quantification in erythrocytes by means of a coulometric high-performance liquid chromatography analytical method. Biomed Chromatogr 2024;38: e6021. doi: 10.1002/bmc.6021

[109] Thachil A, Wang L, Mandal R, Wishart D, Blydt-Hansen T. An overview of pre-analytical factors impacting metabolomics analyses of blood samples. Metabolites 2024; 14:474. doi: 10.3390/metabo14090474

[110] Tomin T, Bordag N, Zügner E, et al. Blood plasma quality control by plasma glutathione status. Antioxidants (Basel) 2021;10:864. doi: 10.3390/antiox10060864

[111] Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Rad Biol Med 2001;30: 1191-212. doi: 10.1016/S0891-5849(01)00480-4

[112] Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. Biol Chem 2009: 390: 191-214. doi: 10.1515/BC.2009.033