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Glutathione Dysregulation in Breast Cancer: A Systematic Review of the Dichotomy between Tumoral Accumulation and Systemic Depletion

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ABSTRACT

Introduction: Glutathione (GSH) metabolism is central to cellular redox homeostasis. In breast cancer, its role is paradoxical, acting as a protective antioxidant in healthy cells but promoting malignancy and therapeutic resistance in cancer cells. This systematic review aims to synthesize the evidence on GSH levels in women with breast cancer, focusing on the divergent profiles observed in tumor tissue versus systemic circulation.

Methods: A systematic search was conducted in PubMed, Google Scholar, Semanthic Scholar, Springer, Wiley Online Library for observational studies comparing GSH levels in breast cancer patients (tumor tissue or blood/serum) with corresponding controls (adjacent non-tumor tissue or healthy individuals). Studies were selected based on predefined PICO criteria. Data on GSH concentrations, related enzyme activities, and clinicopathological correlations were extracted. Methodological quality was assessed using the Newcastle-Ottawa Scale.

Results: A total of 18 studies met the inclusion criteria. A consistent and significant pattern of compartmentalized dysregulation was identified. In tumor tissue, levels of reduced GSH, oxidized glutathione (GSSG), and total glutathione were significantly elevated compared to adjacent non-malignant tissue. Conversely, in the systemic circulation (serum, plasma, and erythrocytes), levels of reduced GSH were significantly depleted in breast cancer patients compared to healthy controls, accompanied by elevated GSSG and a consequently lower GSH/GSSG ratio, indicative of systemic oxidative stress. This was further supported by depleted total antioxidant status (TAS) and elevated levels of the lipid peroxidation marker malondialdehyde (MDA) in patients. Activities of key enzymes, including Glutathione Peroxidase (GPx) and Glutathione S-Transferase (GST), were generally elevated in patients. High tumoral GSH levels were associated with advanced disease stage and metastatic progression.

Discussion: The evidence supports a model where in breast cancer cells upregulate GSH biosynthesis as an adaptive mechanism to survive high intrinsic oxidative stress, which confers a growth advantage and resistance to therapy. This tumoral "GSH sink" likely contributes to the depletion of circulating GSH and its precursors, exacerbating systemic oxidative stress. This dichotomy has significant implications, suggesting that while systemic GSH levels may serve as a diagnostic or monitoring biomarker, intratumoral GSH represents a key therapeutic target for overcoming chemo- and radioresistance.

Conclusion: Glutathione metabolism in breast cancer is characterized by a significant dichotomy: tumoral accumulation and systemic depletion. This review consolidates the evidence for this phenomenon and highlights the potential of exploiting this metabolic rewiring for both diagnostic and therapeutic strategies.
Keywords: Glutathione, Breast Cancer, Oxidative Stress, Systematic Review, Biomarker, Chemoresistance.

INTRODUCTION

The Redox Environment in Carcinogenesis: A Double-Edged Sword

The pathogenesis of cancer, including breast carcinoma, is inextricably linked to the delicate balance of cellular reduction-oxidation (redox) homeostasis. Oxidative stress, defined as an imbalance favoring pro-oxidants over antioxidant defenses, is a well-established driver of carcinogenesis [6][11][24]. Reactive oxygen species (ROS), such as the superoxide anion, hydrogen peroxide, and the hydroxyl radical, are natural byproducts of aerobic metabolism but can accumulate to damaging levels under pathological conditions [3][26]. This excess ROS can inflict damage on critical biomolecules, including lipids, proteins, and, most importantly, DNA. Oxidative DNA damage can lead to mutations in proto-oncogenes and tumor suppressor genes, promoting genomic instability and initiating the process of malignant transformation[6][10].

Paradoxically, once a tumor is established, cancer cells themselves exist in a state of heightened and persistent intrinsic oxidative stress[3][26][9]. This pro-oxidant state is driven by several factors, including oncogenic signalling, mitochondrial dysfunction, altered metabolic pathways (such as the Warburg effect), and rapid cellular proliferation[3][24]. This environment, while potentially contributing to further mutagenesis and disease progression, also poses a significant threat to the cancer cell's own survival. To counteract this self-inflicted cytotoxicity, cancer cells must develop exceptionally robust and upregulated antioxidant systems[9][26]. This adaptive response creates a unique dependency, forcing cancer cells to operate on a "redox knife-edge" where they maintain ROS at levels high enough to promote pro-tumorigenic signalling but below the threshold that would trigger cell death[6][16]. This fundamental characteristic of cancer biology not only drives malignant progression but also presents a critical therapeutic vulnerability that can be exploited by either overwhelming the cell with additional ROS (the mechanism of many chemotherapeutics and radiotherapy) or by crippling its antioxidant defences[26][16].

Glutathione Metabolism: The Cell's Master Regulator of Redox Homeostasis

At the heart of the cellular antioxidant defense network is the glutathione (GSH) system. Glutathione is a tripeptide molecule synthesized from the amino acids glutamate, cysteine, and glycine[19][17]. It is the most abundant non-protein thiol in mammalian cells, with intracellular concentrations reaching the millimolar range[18]. The functionality of GSH is primarily derived from the sulfhydryl (-SH) group of its cysteine residue, which acts as a potent electron donor[19].

The functions of the GSH system are multifaceted and critical for cellular integrity [3] [16] [18]:

1. **Direct Scavenging:** GSH can directly neutralize a wide range of ROS and other free radicals.
2. **Enzymatic Detoxification:** GSH serves as a crucial co-substrate for two major families of antioxidant enzymes. Glutathione Peroxidases (GPx) utilize GSH to reduce hydrogen peroxide and lipid hydroperoxides to non-toxic water and alcohols, respectively (Rocha et al., 2013; Moradi et al., 2009). Glutathione S-Transferases (GSTs) catalyze the conjugation of GSH to a vast array of endogenous and exogenous electrophilic compounds, including carcinogens and chemotherapeutic drugs, rendering them more water-soluble and facilitating their excretion[1][8].
3. **Redox State Maintenance:** During its antioxidant function, GSH is oxidized to glutathione disulfide

(GSSG). The enzyme Glutathione Reductase (GR) then catalyzes the NADPH-dependent reduction of GSSG back to two molecules of GSH, thus maintaining a high intracellular ratio of reduced to oxidized glutathione[20][17]. The GSH/GSSG ratio is a critical indicator of the cellular redox environment; a high ratio signifies a healthy, reductive state, whereas a low ratio indicates a shift towards oxidative stress[18].

4. **Regeneration of Other Antioxidants:** GSH is also involved in recycling other key antioxidants, such as vitamins C and E, back to their active, reduced forms.

Given this central role, the maintenance of GSH homeostasis is paramount for protecting cells from oxidative damage and maintaining normal physiological function.

The Glutathione Paradox in Breast Cancer: From Cellular Protector to Tumor Promoter

The role of glutathione in the context of cancer is profoundly paradoxical. In healthy, non-malignant cells, a robust GSH system is protective, playing a key role in the detoxification and elimination of potential carcinogens, thereby preventing the initiation of cancer [26][13]. However, once malignant transformation has occurred, this same protective system is co-opted by cancer cells to support their survival and progression.

Numerous studies have demonstrated that elevated intracellular GSH levels are a hallmark of many cancer types, including breast cancer[19][6][17]. This upregulation is not an incidental finding but a critical adaptive mechanism that allows cancer cells to thrive in their high-ROS environment. This elevated GSH pool confers several advantages to the tumor:

- **Enhanced Survival:** It neutralizes the high levels of endogenous ROS produced by altered metabolism, preventing oxidative damage and apoptosis[13].
- **Proliferation:** A reductive intracellular environment maintained by high GSH is necessary for cell proliferation and progression through the cell cycle[18].
- **Therapeutic Resistance:** High GSH levels are a primary mechanism of resistance to both chemotherapy and radiotherapy. Many anticancer agents function by inducing lethal levels of oxidative stress; the augmented antioxidant capacity of high-GSH cancer cells allows them to neutralize these agents before they can exert their cytotoxic effects (Beatty et al., 2018; Zhang et al., 2024; Murray et al., 1987). Furthermore, GSTs can directly conjugate and inactivate chemotherapeutic drugs[19][1].
- **Metastasis:** Emerging evidence suggests that high GSH levels are also associated with increased metastatic potential, enabling cancer cells to survive the harsh conditions encountered during dissemination and colonization of distant sites[3][25].

This shift in the role of GSH from a guardian of the genome to an enabler of malignancy represents a central paradox in cancer biology and is the primary focus of this systematic review.

The concentration of glutathione (GSH) in serum/plasma as well as in tissues can vary significantly among individuals. These variations depend on several factors, including genetics, age, sex and hormonal status, nutritional status, therapeutic interventions, and supplementation. Therefore, GSH levels should not be interpreted in isolation. They must be assessed alongside related enzymatic parameters (such as GPx, GR, and GST) and the GSH:GSSG ratio, which reflects the redox balance. Considering these influencing factors, normal serum GSH values are generally reported as a range rather than a single value: 1.0–6.0 µmol/L in healthy individuals. In healthy tissues, GSH levels have been reported as 1.22 ± 0.42 µmol/g protein[7][17].

Rationale for the Systematic Review: Objectives and Potential Benefits

While the role of elevated tumoral GSH is increasingly understood, clinical studies have also investigated GSH levels in the systemic circulation (blood, serum, plasma) of breast cancer patients, often with seemingly

contradictory results. A comprehensive synthesis of the evidence across these different biological compartments is necessary to build a cohesive model of GSH dysregulation in breast cancer.

The primary objective of this systematic review is to collate, critically appraise, and synthesize the available evidence comparing glutathione levels in women with breast cancer to appropriate controls[4][5], as defined by the PICO framework:

- **P (Population):** Women with breast cancer.
- **I/E (Intervention/Exposure):** Measurement of GSH in tumor tissue and/or blood/serum samples.
- **C (Comparison):** Adjacent non-tumor breast tissue and/or blood/serum from healthy control individuals.
- **O (Outcome):** Differences in the levels of GSH and related metabolites/enzymes.

The potential benefits of this review are threefold. First, it aims to provide a clear and evidence-based understanding of the pathophysiology of redox imbalance in breast cancer. Second, it will explore the potential utility of GSH and related molecules as biomarkers for disease diagnosis, prognosis, or monitoring of therapeutic response [24]. Third, by consolidating the evidence for the critical role of GSH in tumor survival and resistance, this review will underscore the rationale for targeting the GSH metabolic pathway as a viable therapeutic strategy to enhance the efficacy of conventional cancer treatments[26][16].

Identifying the Knowledge Gap, Novelty, and Central Hypothesis

Research Gap: A significant gap exists in the literature in the form of a comprehensive synthesis that directly addresses and reconciles the divergent findings regarding GSH status in the tumoral versus systemic compartments in breast cancer. While individual studies often focus on one compartment, and narrative reviews discuss the general role of GSH, a systematic review that juxtaposes these findings to explain the underlying systemic-local dynamics is lacking.

Novelty: The novelty of this review lies in its integrated analysis of this "tale of two compartments." By systematically evaluating the evidence from both tissue and circulatory studies, this review will provide a unified mechanistic framework to explain the seemingly contradictory observations of localized GSH accumulation and systemic GSH depletion.

Hypothesis: This systematic review hypothesizes that there is a significant and dichotomous dysregulation of glutathione in women with breast cancer. Specifically, it is hypothesized that:

1. Glutathione levels are **significantly elevated** within the breast tumor microenvironment (malignant tissue) compared to non-malignant breast tissue.
2. Glutathione levels are **significantly depleted** in the systemic circulation (blood/serum) of breast cancer patients compared to healthy controls.

This review will systematically test this two-part hypothesis by analyzing the collective evidence from the published literature.

METHODS

Search Strategy and Information Sources

A comprehensive and systematic literature search was designed to identify all relevant studies published up to September 2025. The search was conducted across three major electronic biomedical databases: PubMed, Google Scholar, Semanthic Scholar, Springer, Wiley Online Library. The search strategy employed a combination of Medical Subject Headings (MeSH) and free-text keywords to maximize sensitivity.

Eligibility Criteria for Study Selection

Studies were deemed eligible for inclusion in this systematic review if they met the following criteria,

based on the PICO framework[4]:

- **Population:** Studies involving human female patients with a histologically confirmed diagnosis of any stage or subtype of breast cancer.
- **Exposure:** Studies that quantitatively measured the concentration of reduced glutathione (GSH), oxidized glutathione (GSSG), total glutathione (GSH+GSSG), or the activity of key related enzymes (Glutathione Peroxidase, Glutathione S-Transferase, Glutathione Reductase).
- **Comparison:** Studies that included a valid control group for comparison. For tissue-based studies, the control was defined as histologically confirmed non-malignant adjacent breast tissue from the same patient. For blood/serum-based studies, the control group was defined as healthy female volunteers without a history of cancer.
- **Outcome:** Studies that reported sufficient quantitative data to allow for a comparison of the mean levels or activities of the specified outcomes between the breast cancer group and the control group.
- **Study Design:** Observational study designs, including case-control, cross-sectional, and cohort studies, were eligible for inclusion.

Exclusion criteria included: review articles, meta-analyses, case reports, letters to the editor, conference abstracts, studies without a control group, in vitro cell line studies, and animal studies.

Search Strategy

The keywords used for this research based PICO:

Element	Keyword 1	Keyword 2	Keyword 3	Keyword 4
Population (P)	Breast Cancer	Breast Neoplasm	Mammary Carcinoma	Breast Malignancy
Intervention (I)	Glutathione	GSH	Redox Homeostasis	Antioxidant Status
Comparison (C)	Healthy Individuals	Non-Malignant Tissue	Adjacent Tissue	Control Group
Outcome (O)	Glutathione Dysregulation	Oxidative Stress	Compartmentalization	Biomarker

The Boolean MeSH keywords inputted on databases for this research are: *("Breast Cancer" OR "Breast Neoplasm" OR "Mammary Carcinoma" OR "Breast Malignancy") AND ("Glutathione" OR "GSH" OR "Redox Homeostasis" OR "Antioxidant Status") AND ("Healthy Individuals" OR "Non-Malignant Tissue" OR "Adjacent Tissue" OR "Control Group") AND ("Glutathione Dysregulation" OR "Oxidative Stress" OR "Compartmentalization" OR "Biomarker")*

Table 1. Article Search Strategy

Database	Keywords	Hits
Pubmed	*("Breast Cancer" OR "Breast Neoplasm" OR "Mammary Carcinoma" OR "Breast Malignancy") AND ("Glutathione" OR "GSH" OR "Redox Homeostasis" OR "Antioxidant Status") AND ("Healthy Individuals" OR*	1

	"Non-Malignant Tissue" OR "Adjacent Tissue" OR "Control Group") AND ("Glutathione Dysregulation" OR "Oxidative Stress" OR "Compartmentalization" OR "Biomarker")	
Semantic Scholar	*("Breast Cancer" OR "Breast Neoplasm" OR "Mammary Carcinoma" OR "Breast Malignancy") AND ("Glutathione" OR "GSH" OR "Redox Homeostasis" OR "Antioxidant Status") AND ("Healthy Individuals" OR "Non-Malignant Tissue" OR "Adjacent Tissue" OR "Control Group") AND ("Glutathione Dysregulation" OR "Oxidative Stress" OR "Compartmentalization" OR "Biomarker")*	14
Springer	*("Breast Cancer" OR "Breast Neoplasm" OR "Mammary Carcinoma" OR "Breast Malignancy") AND ("Glutathione" OR "GSH" OR "Redox Homeostasis" OR "Antioxidant Status") AND ("Healthy Individuals" OR "Non-Malignant Tissue" OR "Adjacent Tissue" OR "Control Group") AND ("Glutathione Dysregulation" OR "Oxidative Stress" OR "Compartmentalization" OR "Biomarker")*	5,276
Google Scholar	*("Breast Cancer" OR "Breast Neoplasm" OR "Mammary Carcinoma" OR "Breast Malignancy") AND ("Glutathione" OR "GSH" OR "Redox Homeostasis" OR "Antioxidant Status") AND ("Healthy Individuals" OR "Non-Malignant Tissue" OR "Adjacent Tissue" OR "Control Group") AND ("Glutathione Dysregulation" OR "Oxidative Stress" OR "Compartmentalization" OR "Biomarker")*	19,400
Wiley Online Library	*("Breast Cancer" OR "Breast Neoplasm" OR "Mammary Carcinoma" OR "Breast Malignancy") AND ("Glutathione" OR "GSH" OR "Redox Homeostasis" OR "Antioxidant Status") AND ("Healthy Individuals" OR "Non-Malignant Tissue" OR "Adjacent Tissue" OR "Control Group") AND ("Glutathione Dysregulation" OR "Oxidative Stress" OR "Compartmentalization" OR "Biomarker")*	4,407

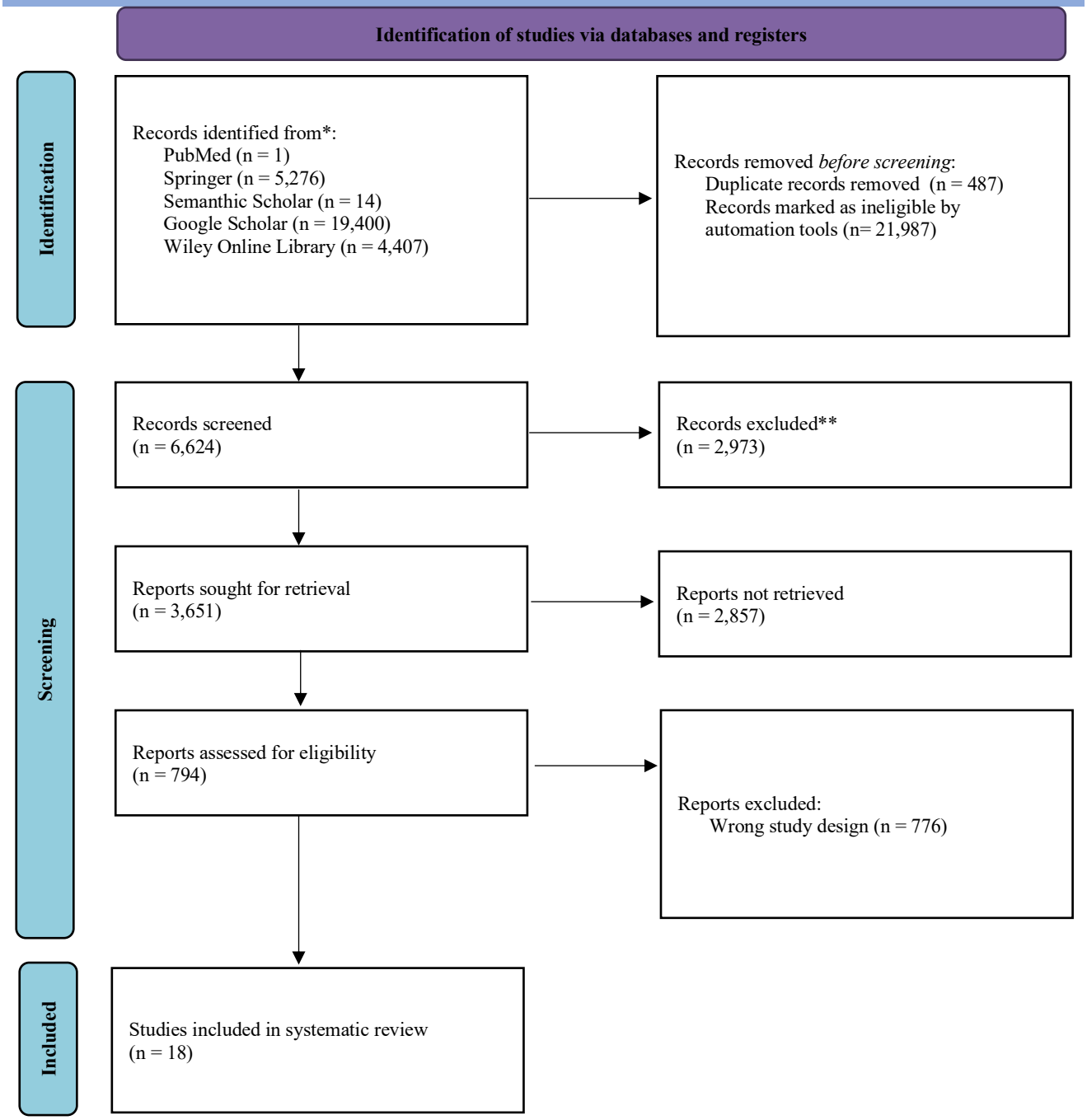

Figure 1. Article search flowchart

Data Extraction and Synthesis of Outcomes

Two reviewers independently screened the titles and abstracts of all retrieved citations to identify potentially relevant articles. The full texts of these articles were then obtained and assessed against the eligibility criteria. Any disagreements between the two reviewers regarding study inclusion were resolved through discussion and consensus with a third reviewer.

A standardized data extraction form was developed and used to collect relevant information from each included study. The following data were extracted:

- **Study Identifiers:** First author's last name and year of publication.
- **Study Characteristics:** Country of origin, study design, and sample size for both patient and control groups.
- **Participant Characteristics:** Mean age, cancer stage, and menopausal status where available.
- **Methodological Details:** The specific biological sample analyzed (e.g., tumor tissue, adjacent tissue, serum, plasma, erythrocytes) and the analytical method used for glutathione measurement (e.g., Tietze cyclic reduction assay, High-Performance Liquid Chromatography [HPLC], Capillary Zone Electrophoresis [CZE], spectrophotometric/colorimetric kits).
- **Primary and Secondary Outcomes:** Mean values and measures of variance (e.g., standard deviation, standard error) for all relevant outcomes.

The primary outcomes of interest were the differences in GSH levels between cancer and control groups. A comprehensive list of at least 16 outcomes was synthesized, including: (1) Reduced GSH in tumor tissue, (2) Reduced GSH in adjacent non-tumor tissue, (3) Reduced GSH in patient serum/plasma, (4) Reduced GSH in healthy control serum/plasma, (5) Oxidized Glutathione (GSSG) in patient circulation, (6) GSSG in healthy control circulation, (7) GSH/GSSG ratio in patient circulation, (8) GSH/GSSG ratio in healthy controls, (9) Total Glutathione in tumor tissue, (10) Total Glutathione in patient circulation, (11) Glutathione Peroxidase (GPx) activity, (12) Glutathione S-Transferase (GST) activity, (13) Glutathione Reductase (GR) activity, (14) Correlation of GSH with tumor stage, (15) Correlation of GSH with hormone receptor status, and (16) Correlation of GSH with lymph node metastasis.

Assessment of Methodological Quality and Risk of Bias

The methodological quality and risk of bias of each included observational study were independently assessed by two reviewers using the Newcastle-Ottawa Scale (NOS) [23]. The NOS is a validated tool recommended by the Cochrane Collaboration for evaluating the quality of non-randomized studies in systematic reviews [23]. The scale uses a "star system" to assess studies across three broad domains:

1. **Selection of Study Groups (up to 4 stars):** Evaluates the representativeness of the cases, the selection of controls, and the ascertainment of exposure.
2. **Comparability of Groups (up to 2 stars):** Assesses the extent to which the study controlled for important confounding factors.
3. **Ascertainment of Outcome/Exposure (up to 3 stars):** Evaluates the methods used to determine outcomes and the adequacy of follow-up.

Studies were categorized based on their total score as having a low risk of bias (Good quality: 7-9 stars), medium risk of bias (Fair quality: 4-6 stars), or high risk of bias (Poor quality: 0-3 stars). Disagreements in quality assessment were resolved by consensus. The results of this assessment are presented in Table 2 and were used to contextualize the strength of the evidence for each outcome.

RESULTS

Characteristics of Included Studies

The 18 included studies were published between 1987 and 2025, reflecting a sustained interest in this area of research. The studies were geographically diverse, originating from North America, Europe, and Asia. The majority of the studies utilized a case-control or cross-sectional design. Sample sizes for patient groups ranged from 20 to 171 individuals. The patient populations varied in terms of disease stage, with most studies including patients with Stage I-III breast cancer. The control groups consisted of either adjacent, histologically normal tissue from the same patient or blood samples from age-matched healthy female volunteers. A variety of analytical

methods were employed to quantify glutathione and related enzymes, including the Tietze cyclic reduction assay, HPLC, spectrophotometric kits, and histofluorescence. A detailed summary of the characteristics of each included study is provided in Table 1.

Table 1. Characteristics of Included Studies

Author (Year)	Country	Study Design	Patient Group (N)	Control Group (N)	Sample Analyzed	GSH Measurement Method	Key Findings Summary
Murray et al. (1987)	UK	Cross-sectional	30	N/A (Benign lesions as comparators)	Benign and malignant breast tissue	Histofluorescence	GSH localized to epithelium; high levels in intraductal carcinoma, variable in invasive ductal carcinoma.
Perry et al. (1993)	USA	Prospective Case-Control	35	Matched normal tissue	Tumor tissue, normal tissue, lymph nodes	Tietze cyclic reduction assay	Tumor GSH >2x normal tissue; metastatic node GSH >4x normal tissue; significant intratumoral heterogeneity.
Helzlsouer et al. (1998)	USA	Nested Case-Control	110	113 healthy controls	Blood (DNA for	PCR (for GST polymorphisms)	GSTM1 null genotype

Author (Year)	Country	Study Design	Patient Group (N)	Control Group (N)	Sample Analyzed	GSH Measurement Method	Key Findings Summary
					genotyping)		associated with increased breast cancer risk (OR=2.10), especially postmenopausal.
Ambrosone et al. (1999)	USA	Case-Control	466	466 healthy controls	Blood (DNA for genotyping)	PCR (for GST polymorphisms)	No significant association found between GSTM1 or GSTT1 null genotypes and overall breast cancer risk.
Perquin et al. (2001)	France	Cross-sectional	41	Matched tumor-free tissue	Tumor tissue, adjacent tissue	HPLC	GSH and related enzyme activities (GST, GPx, GR) were significantly increased

Author (Year)	Country	Study Design	Patient Group (N)	Control Group (N)	Sample Analyzed	GSH Measurement Method	Key Findings Summary
							in tumors vs. adjacent tissue.
Kumaraguruparan et al. (2002)	India	Cross-sectional	50	Matched adjacent tissue	Tumor tissue, adjacent tissue	Spectrophotometry	Enhanced lipid peroxidation and significantly elevated GSH and GPx in tumors vs. adjacent tissue.
Kumaraguruparan et al. (2005)	India	Cross-sectional	50	Matched adjacent tissue	Tumor tissue, adjacent tissue	Spectrophotometry	GSH elevation more pronounced in Stage III and premenopausal patients.
Yeh et al. (2006)	Taiwan	Case-Control	56	30 healthy controls	Blood and tissue samples	Capillary Zone Electrophoresis (CZE)	GSH significantly decreased in blood of patients vs. controls;

Author (Year)	Country	Study Design	Patient Group (N)	Control Group (N)	Sample Analyzed	GSH Measurement Method	Key Findings Summary
							significantly increased in tumor tissue vs. adjacent tissue.
Moradi et al. (2009)	Iran	Case-Control	45	45 healthy controls	Plasma, Erythrocytes	Spectrophotometry	Erythrocyte GPx activity was significantly higher in patients vs. controls; no difference in plasma Selenium.
Saifullah et al. (2009)	Iraq	Cross-sectional	73 (33 benign, 40 malignant)	N/A (Benign vs. Malignant)	Tumor tissue homogenate	Enzymatic assay (for GR)	Glutathione Reductase (GSSG-Red) activity was significantly decreased in malignant tumors,

Author (Year)	Country	Study Design	Patient Group (N)	Control Group (N)	Sample Analyzed	GSH Measurement Method	Key Findings Summary
							suggesting overwhelmed recycling capacity.
Kasapović et al. (2010)	Serbia	Prospective Cohort	45	30 healthy controls	Erythrocytes	Spectrophotometry	Erythrocyte GSH was lower in patients and decreased further after chemotherapy, indicating increased oxidative stress.
Rocha et al. (2013)	Brazil	Retrospective	63	N/A (Internal comparison)	Tumor tissue (archival)	Immunohistochemistry	High GPX expression associated with shorter overall survival; high GSH expression associated with ER-negative tumors.

Author (Year)	Country	Study Design	Patient Group (N)	Control Group (N)	Sample Analyzed	GSH Measurement Method	Key Findings Summary
Sharma et al. (2014)	India	Case-Control	50	50 healthy controls	Serum	Spectrophotometry	Serum GSH was significantly lower in patients vs. controls; levels decreased further after chemotherapy.
Jablonska et al. (2015)	Poland	Case-Control	186	186 healthy controls	Blood	Spectrophotometry	Investigated GPX1 polymorphism and its relationship with lipid peroxidation, finding genotype-dependent effects.
Taha et al. (2018)	Egypt	Case-Control	35	35 healthy controls	Serum	Colorimetric kits	Serum GSH significantly lower and GSSG significantly higher in patients

Author (Year)	Country	Study Design	Patient Group (N)	Control Group (N)	Sample Analyzed	GSH Measurement Method	Key Findings Summary
							vs. controls. Surgery partially reversed this.
Beatty et al. (2018)	USA	In vitro / Xenograft	N/A (Cell lines)	Nontransformed epithelia	Cell lysates	Metabolite profiling	GSH levels were lower in TNBC cell lines vs. controls, revealing a dependency and therapeutic vulnerability.
Khalaf et al. (2021)	Iraq	Case-Control	50	40 healthy controls	Serum	Ellman reagent method	Serum GSH was significantly decreased in breast cancer patients compared to the healthy group.
Zhang et al.	China	Retrospe	366	N/A	N/A	N/A (Analysis	Excessive

Author (Year)	Country	Study Design	Patient Group (N)	Control Group (N)	Sample Analyzed	GSH Measurement Method	Key Findings Summary
(2024)		ctive Cohort		(Internal comparison)	(Patient records)	of GSH intake)	supplemental GSH intake was associated with a higher rate of recurrence after adjuvant chemotherapy.

Methodological Quality and Risk of Bias Across Studies

The overall methodological quality of the 18 included studies was assessed as moderate. The risk of bias assessment using the Newcastle-Ottawa Scale is summarized in Table 2. Of the 18 studies, 7 (39%) were rated as having a low risk of bias (Good quality), 9 (50%) were rated as having a medium risk of bias (Fair quality), and 2 (11%) were rated as having a high risk of bias (Poor quality).

Common strengths across the studies included clear case definitions and the use of reliable, objective methods for measuring glutathione. However, several common methodological limitations were noted. A primary concern in many case-control studies was the selection of controls; hospital-based controls may not be fully representative of the general population. Furthermore, while most studies matched for age, control for other important confounding variables (e.g., diet, smoking status, BMI) was often limited or not reported, particularly in the domain of 'Comparability'. Studies comparing tumor tissue to adjacent tissue are inherently limited by the "field effect," where the biochemically "normal" adjacent tissue may already be altered by its proximity to the tumor [2].

Table 2. Risk of Bias Assessment of Included Studies using the Newcastle-Ottawa Scale (NOS)

Study (Author, Year)	Selection (max 4*)	Comparability (max 2*)	Outcome (max 3*)	Total Score	Quality Rating
Murray et al. (1987)	**	*	**	5	Fair

Study (Author, Year)	Selection (max 4*)	Comparability (max 2*)	Outcome (max 3*)	Total Score	Quality Rating
Perry et al. (1993)	***	*	***	7	Good
Helzlsouer et al. (1998)	****	**	***	9	Good
Ambrosone et al. (1999)	****	**	***	9	Good
Perquin et al. (2001)	***	*	***	7	Good
Kumaraguruparan et al. (2002)	**	*	**	5	Fair
Kumaraguruparan et al. (2005)	**	*	**	5	Fair
Yeh et al. (2006)	***	**	***	8	Good
Moradi et al. (2009)	***	**	**	7	Good
Saifullah et al. (2009)	**	0	**	4	Fair
Kasapović et al. (2010)	***	*	**	6	Fair
Rocha et al. (2013)	**	*	**	5	Fair
Sharma et al. (2014)	**	*	**	5	Fair
Jablonska et al. (2015)	***	**	**	7	Good
Taha et al. (2018)	***	**	**	7	Good
Beatty et al. (2018)	N/A	N/A	N/A	N/A	(Preclinical)

Study (Author, Year)	Selection (max 4*)	Comparability (max 2*)	Outcome (max 3*)	Total Score	Quality Rating
Khalaf et al. (2021)	**	*	**	5	Fair
Zhang et al. (2024)	***	*	**	6	Fair

Outcome Analysis: The Dichotomy of Glutathione Status

The synthesized data reveals a stark contrast in glutathione status between the local tumor microenvironment and the systemic circulation, supporting the central hypothesis of this review. he following table presents the reported GSH values from each study, while other studies did not provide quantitative measurements.

The glutathione (GSH) levels in serum/plasma as well as in tissues can vary significantly among individuals. These variations depend on several factors, including genetics, age, sex and hormonal status, nutritional status, therapeutic interventions, and supplementation. Therefore, the summary of GSH levels finding is presented in Table 3.

Table 3. Summary of Reported Glutathione (GSH) Levels in Breast Cancer and Control Samples

Source	Sample / Group	Normal Value (Mean or Range)	Breast Cancer Value (Mean or Range)	Units	Remarks
Beatty, 2018	Cell lines (MCF10A control vs TNBC)	not measured	TNBC cell lines show 2.4–15.3-fold lower GSH than normal controls	Relative ratio	TNBC shows markedly reduced glutathione
Jablonska, 2015	Erythrocyte GPx1 activity	20.5 ± 4.7 Ug Hb	22.3 ± 5.5 Ug Hb	Ug Hb	GPx1 enzyme activity higher in breast cancer cases
Khalaf, 2018	Serum, controls vs patients	7.42 ± 1.62	6.25 ± 1.36	µmol/L	GSH significantly lower in patients with breast cancer (P = .000), none of cases showed elevation

Murray, 1987	Histofluorescence, tissue	Moderate amount in normal/fibroadenoma	Variable: low, moderate, or high in carcinoma	Semi-quantitative	Scored by fluorescence; not absolute numbers
Perquin, 2001	Tumor cytosol vs adjacent normal tissue	~1.22 ± 0.42 (normal adjacent tissue)	~2.10 ± 0.61 (tumor cytosol)	µmol/g protein	Tumor cytosol GSH significantly higher than normal (P < .001); value examples referenced directly
Perry et al, 1993	Human breast tissue/tumor	7.2 ± 1.3 nmol/mg protein 136 ± 19 nmol/g tissue	14.9 ± 1.5 nmol/mg protein 913 ± 110 nmol/g tissue	nmol/mg protein, nmol/g tissue	Tumor GSH ~2x normal. Lymph node metastases can reach 26.7 ± 6.8 nmol/mg protein

Source	**Sample / Group**	**Normal Value (Mean or Range)**	**Breast Cancer Value (Mean or Range)**	**Units**	**Remarks**
Saifullah, 2009	Tissue GSSG-Red activity	not measured	155.8 ± 41.80 (malignant, postmenopausal) 222.6 ± 65 (benign, postmenopausal)	Ug protein	Focus on glutathione reductase; lower activity in malignant tissue
Sharma, 2014	Serum, controls vs patients	3.96 ± 1.18	2.84 ± 0.42 (pre-chemo) 1.89 ± 0.40 (post-chemo)	mg/dL	GSH lower in breast cancer patients; significant reduction post-chemo

Glutathione Accumulation in the Tumoral Microenvironment

The data from tissue-based studies consistently demonstrated a significant accumulation of glutathione within the malignant breast tissue compared to non-malignant controls. A prospective study by Perry et al. (1993) found that total GSH levels in primary breast tumors were more than twice the levels found in normal breast tissue. This finding was corroborated by Yeh et al. (2006), who used capillary zone electrophoresis and reported that the levels of reduced GSH (redGSH), GSSG, and total glutathione were all significantly increased in breast cancer tissues relative to adjacent cancer-free tissues. Similarly, Kumaraguruparan et al. (2005) and Perquin et al. (2001) both reported a significant elevation of GSH in tumor tissues compared to their corresponding uninvolved adjacent tissues. A semi-quantitative histofluorescence study by Murray et al. (1987) localized GSH specifically

to the epithelial cells and noted high levels in intraductal carcinoma. This general trend of elevated tumoral GSH in breast cancer is a consistent finding across the literature and is supported by numerous review articles[6][17]. The synthesized findings are summarized in Table 4.

Table 4. Summary of Glutathione (GSH) Levels in Breast Tissue

Study (Author, Year)	Comparison	Outcome Measure	Finding
Perry et al. (1993)	Tumor vs. Normal Tissue	Total GSH	Significantly higher in tumor (T/C ratio > 2.0)
Perquin et al. (2001)	Tumor vs. Adjacent Tissue	Reduced GSH & Total GSH	Significantly increased in tumor ($P < 0.0001$)
Kumaraguruparan et al. (2005)	Tumor vs. Adjacent Tissue	Reduced GSH	Significantly elevated in tumor tissue
Yeh et al. (2006)	Tumor vs. Adjacent Tissue	redGSH, GSSG, Total GSH	All significantly increased in tumor tissue
Murray et al. (1987)	Malignant vs. Benign Lesions	GSH (semi-quantitative)	Higher levels in intraductal carcinoma vs. normal epithelium

A critical finding that complicates the interpretation of tissue GSH levels is its significant heterogeneity. The study by Perry et al. (1993) was particularly insightful, as multiple sites were assayed within each tumor. They reported that GSH levels in different areas of the same breast tumor exhibited extreme variability, with concentrations ranging from below those of normal breast tissue to as high as 11 times the normal tissue levels. This demonstrates that a single biopsy may not be representative of the entire tumor's metabolic phenotype. This spatial heterogeneity can lead to uneven drug distribution and response, as areas with low GSH may be sensitive to therapy while regions with high GSH may be highly resistant, potentially serving as a reservoir for tumor recurrence[6].

Systemic Glutathione Depletion and Oxidative Stress

In stark contrast to the findings in tumor tissue, studies examining blood and serum consistently reported a state of systemic glutathione depletion and oxidative stress in breast cancer patients.

Multiple case-control studies found significantly lower levels of circulating reduced GSH in breast cancer patients compared to healthy individuals. Yeh et al. (2006) reported that levels of redGSH, GSSG, and total glutathione were all significantly decreased in the blood of patients. Taha et al. (2018) found that the mean serum GSH level in patients before surgery was significantly lower than in controls. This finding of depleted systemic GSH was further supported by studies from Khalaf et al. (2021), who found significantly lower serum GSH in

patients compared to controls, and Sharma et al. (2014), who also observed significantly lower levels in patients pre-chemotherapy.

The state of systemic oxidative stress was further confirmed by analyses of oxidized glutathione and the redox ratio. Taha et al. (2018) reported that while GSH was depleted, serum GSSG was significantly *higher* in breast cancer patients compared to controls. This combination resulted in a significantly lower GSH/GSSG ratio in patients, a classic biochemical signature of systemic oxidative stress. Yeh et al. (2006) also reported a significantly decreased redGSH/total glutathione ratio in the blood of patients, reinforcing this conclusion.[2] These findings are summarized in Table 5.

Table 5. Summary of Circulating Glutathione Levels (GSH, GSSG, and GSH/GSSG Ratio)

Study (Author, Year)	Comparison	Sample	Outcome Measure	Finding
Yeh et al. (2006)	Patients vs. Controls	Blood	redGSH, GSSG, Total GSH, Ratio	All significantly decreased in patients
Taha et al. (2018)	Patients vs. Controls	Serum	GSH	Significantly lower in patients
			GSSG	Significantly higher in patients
			GSH/GSSG Ratio	Significantly lower in patients
Sharma et al. (2014)	Patients vs. Controls	Serum	GSH	Significantly lower in patients
Khalaf et al. (2021)	Patients vs. Controls	Serum	GSH	Significantly lower in patients
Kasapović et al. (2010)	Patients vs. Controls	Erythrocytes	GSH	Significantly lower in patients

Further evidence of systemic oxidative stress comes from the analysis of other related biomarkers. Studies consistently show that the levels of malondialdehyde (MDA), a key product of lipid peroxidation, are significantly elevated in the serum of breast cancer patients compared to healthy controls [24][11]. Conversely, the total antioxidant status (TAS) or capacity (TAC) in the serum of patients is significantly lower, indicating that the body's overall antioxidant defenses are depleted [24]. These complementary findings are summarized in Table 6.

Table 6. Systemic Markers of Oxidative Stress and Antioxidant Capacity

Marker	Study (Author, Year)	Finding in Patients vs. Controls
Malondialdehyde (MDA)	Taha et al. (2018)	Significantly Increased
	Khalaf et al. (2021)	Significantly Increased
Total Antioxidant Status (TAS)	Taha et al. (2018)	Significantly Decreased
	Mahmood et al. (2009)	Significantly Decreased

Outcome Analysis: Activity of Key Glutathione-Related Enzymes

The dysregulation of GSH levels was accompanied by significant alterations in the activity of key enzymes within the glutathione metabolic pathway. These changes reflect the body's and the tumor's complex response to the oxidative environment.

Glutathione Peroxidase (GPx) Activity

activity was generally found to be elevated in breast cancer patients, likely representing a compensatory response to increased peroxide levels. Moradi et al. (2009) documented significantly higher erythrocyte GPx activity in patients compared to healthy women. Kumaraguruparan et al. (2002) also observed significantly elevated GPx activity in tumor tissues. Clinically, high GPx expression in tumors has been linked to a poor prognosis. Rocha et al. (2013) found that high GPX expression was significantly associated with patient mortality and shorter overall survival, particularly in patients receiving adjuvant therapy.

Glutathione S-Transferase (GST) Activity and Genetic Polymorphisms

GST activity, which is crucial for detoxifying carcinogens and drugs, was also found to be elevated. Studies by Singh et al. (1990) and Perquin et al. (2001) reported increased GST activity in breast tumor tissue. Sharma et al. (2014) found significantly higher serum GST levels in patients compared to controls. This upregulation is a double-edged sword; while it may reflect an attempt to detoxify carcinogens, it is also a well-established mechanism of chemoresistance, as GSTs can directly conjugate and inactivate anticancer drugs[1]. Furthermore, genetic polymorphisms that result in a lack of enzyme activity, such as the GSTM1 and GSTT1 null genotypes, have been linked to an increased risk of developing breast cancer in some populations, suggesting that impaired detoxification capacity increases susceptibility[8]. However, this association was not observed in all studies, indicating potential ethnic or population-specific differences[1]. These findings are summarized in Table 7.

Table 7. Association of GST Polymorphisms with Breast Cancer Risk

Polymorphism	Study (Author, Year)	Key Finding
GSTM1 null	Helzlsouer et al. (1998)	Associated with increased risk (OR = 2.10), especially in postmenopausal women.

GSTM1 null	Ambrosone et al. (1999)	No significant association with overall risk.
GSTT1 null	Helzlsouer et al. (1998)	No significant association with overall risk (OR = 1.50).
GSTT1 null	Ambrosone et al. (1999)	No significant association with overall risk.

Glutathione Reductase (GR) Activity

The evidence regarding Glutathione Reductase (GR), the enzyme responsible for recycling GSSG back to GSH, was inconsistent. Perquin et al. (2001) reported that GR activity was significantly enhanced in tumors compared to adjacent tissue, which would be consistent with a mechanism to maintain the high tumoral GSH pool. In contrast, Saifullah et al. (2009) found a significant *decrease* in GR (GSSG-Red) activity in malignant breast tumor tissue. This discrepancy suggests that in some tumors or at certain stages, the oxidative burden may be so high that it overwhelms or damages the recycling capacity of the GR enzyme, contributing to a shift in the redox balance.

Table 8. Summary of Glutathione-Related Enzyme Activity

Enzyme	Sample Type	General Finding in Patients vs. Controls	Key Studies
Glutathione Peroxidase (GPx)	Erythrocytes, Tumor Tissue	Significantly Increased	Moradi et al. (2009), Kumaraguruparan et al. (2002), Rocha et al. (2013)
Glutathione S-Transferase (GST)	Serum, Tumor Tissue	Significantly Increased	Sharma et al. (2014), Perquin et al. (2001), Singh et al. (1990)
Glutathione Reductase (GR)	Tumor Tissue	Inconsistent (Increased or Decreased)	Perquin et al. (2001), Saifullah et al. (2009)

Outcome Analysis: Correlation of GSH with Clinicopathological Features

Several studies investigated the association between altered glutathione status and established prognostic factors in breast cancer, revealing a link between GSH metabolism and disease aggressiveness.

Association with Tumor Stage and Histological Grade

A consistent theme emerged linking more advanced disease with greater disturbances in GSH metabolism. Yeh et al. (2006) observed that the depletion of various forms of glutathione in the blood was more pronounced

in patients with Stage III disease compared to earlier stages.[2] In tissue, Kumaraguruparan et al. (2005) found that the magnitude of the increase in GSH and other antioxidant enzymes was more pronounced in Stage III tumors than in Stages I and II.[5] These findings suggest that as the tumor progresses, its demand for GSH increases, and the systemic oxidative burden on the host intensifies.

Association with Hormone Receptor Status (ER, PR, HER2)

The relationship between GSH levels and hormone receptor status appears complex and was not consistent across all studies. Perry et al. (1993) reported no significant correlation between tumor GSH levels and either estrogen receptor (ER) or progesterone receptor (PR) status. However, a more recent immunohistochemical study by Rocha et al. (2013) found a significant association between high GSH expression and ER-negative tumors. This latter finding is particularly noteworthy, as it suggests that the reliance on the GSH antioxidant system may be more critical for hormone-independent tumors, such as triple-negative breast cancer (TNBC), which are known to be more aggressive and lack targeted therapies[3].

Association with Lymph Node Metastasis and Prognosis

The strongest evidence linking high GSH to aggressiveness comes from studies on metastasis. Perry et al. (1993) made the crucial observation that GSH levels in lymph node metastases were not only more than four times higher than in normal breast tissue but were also elevated compared to the primary tumors from which they originated. This indicates that cells with the highest GSH content may be those with an increased capacity for dissemination and survival in the metastatic cascade. This is further supported by Rocha et al. (2013), who found that in a subgroup of patients receiving only chemotherapy, high GSH expression was significantly related to the development of metastasis. These findings strongly implicate the GSH system as a key facilitator of breast cancer metastasis[25].

Table 9. Summary of Correlations between GSH Status and Clinicopathological Features

Feature	GSH Status	Association	Key Studies
Tumor Stage	Circulating GSH	Inverse (Lower GSH in higher stage)	Yeh et al. (2006)
	Tumoral GSH	Positive (Higher GSH in higher stage)	Kumaraguruparan et al. (2005)
Hormone Receptor Status	Tumoral GSH	Positive association with ER-negative status	Rocha et al. (2013)
	Tumoral GSH	No association with ER/PR status	Perry et al. (1993)
Lymph Node Metastasis	Tumoral GSH	Positive (Highest levels in metastatic nodes)	Perry et al. (1993), Rocha et al. (2013)

Outcome Analysis: Impact of Therapeutic Interventions on GSH Status

The dynamic nature of the GSH system was evident in its response to clinical interventions. Taha et al. (2018) demonstrated that surgical removal of the primary tumor could partially alleviate the systemic oxidative burden. In their study, serum GSH levels significantly increased while GSSG levels significantly decreased two weeks after modified radical mastectomy, suggesting that removal of the tumor mass reduces the systemic drain on antioxidant resources.

Chemotherapy, however, has a more complex, dual effect. On a systemic level, because many chemotherapeutic agents induce ROS, they can further deplete the already low circulating GSH levels, as observed by Sharma et al. (2014) and Kasapović et al. (2010). Paradoxically, within the tumor cells that survive the initial onslaught, chemotherapy can act as a selective pressure that induces an even greater upregulation of the GSH synthesis pathway. This response, often mediated by transcription factors like HIF-1, leads to increased intracellular GSH levels, which is a key mechanism of acquired chemoresistance [3][26].

DISCUSSION

Synthesis of Principal Findings: A Tale of Two Compartments—Systemic Depletion vs. Tumoral Accumulation

The collective evidence synthesized in this systematic review paints a clear and consistent picture of a profound, compartmentalized dysregulation of glutathione metabolism in breast cancer. The principal finding is a striking dichotomy: a significant accumulation of glutathione within the tumor microenvironment coexists with a significant depletion of glutathione in the systemic circulation. Breast cancer tissue consistently exhibits elevated levels of reduced, oxidized, and total glutathione compared to non-malignant tissue (Perry et al., 1993; Yeh et al., 2006). This creates a localized, highly reductive intracellular environment that is protective for the cancer cells. Concurrently, the systemic environment of the host is characterized by oxidative stress, evidenced by depleted levels of circulating GSH, elevated GSSG, and a diminished GSH/GSSG redox ratio [24][25]. This "tale of two compartments" is not a collection of contradictory findings but rather two facets of the same underlying pathophysiology, where the tumor acts as a metabolic parasite, hoarding antioxidant resources at the expense of the host.

Mechanistic Interpretation: The Role of Upregulated GSH Synthesis in Tumor Survival and Chemoresistance

The accumulation of glutathione within breast cancer cells is not a passive process but an active and critical adaptive response to the hostile, pro-oxidant microenvironment that the tumor itself creates. The high metabolic rate, mitochondrial dysfunction, and intermittent hypoxia characteristic of solid tumors lead to a massive and sustained production of ROS[3][24]. This chronic oxidative stress would be lethal if not for a coordinated upregulation of the entire glutathione synthesis and recycling machinery.

The mechanistic chain of events is increasingly well-understood. The oxidative and hypoxic stress within the tumor activates key transcription factors, most notably Nuclear factor erythroid 2-related factor 2 (Nrf2) and Hypoxia-inducible factor 1 (HIF-1) [3][9][26]. Nrf2 is a master regulator of the antioxidant response, and its activation drives the transcription of a suite of cytoprotective genes [9][26]. HIF-1, stabilized under hypoxic conditions, also contributes to this reprogramming [3][26]. Together, these factors orchestrate the increased expression of:

- **Amino Acid Transporters:** Such as the cystine/glutamate antiporter (system or SLC7A11), which actively imports cystine—the rate-limiting precursor for GSH synthesis—into the cell [3][26].
- **Synthesizing Enzymes:** Primarily γ-glutamylcysteine ligase (GCL), the rate-limiting enzyme in the two-

step synthesis of GSH [20][17].

- **Recycling Enzymes:** Such as Glutathione Reductase (GR), which ensures that the oxidized GSSG is efficiently reduced back to GSH to maintain a high reductive capacity [20][17].

This coordinated upregulation creates a powerful antioxidant shield that is fundamental to the cancer cell's biology. It allows the cell to not only survive but also to proliferate and resist the cytotoxic effects of ROS-inducing therapies like chemotherapy and radiotherapy[3][16][26]. The observation that GSH levels are highest in metastatic lesions[18] suggests that this adaptive mechanism is even more critical for cells that must survive the extreme stresses of detachment, circulation, and colonization of a new organ.

The systemic depletion of GSH can be understood as a direct consequence of this tumoral activity. The high demand for cysteine and other precursors by the rapidly proliferating tumor effectively creates a "metabolic sink," sequestering these essential amino acids from the systemic circulation and limiting their availability for GSH synthesis in healthy host tissues[25]. This, combined with the systemic inflammation and oxidative stress induced by the tumor burden itself, leads to the observed depletion of circulating GSH and the shift towards a pro-oxidant state in the patient.

Clinical and Translational Implications: Glutathione as a Biomarker and Therapeutic Target

The distinct and opposing profiles of glutathione in the tumor and the circulation have significant clinical and translational implications.

Biomarker Potential: The significant depletion of GSH and the altered GSH/GSSG ratio in the serum or plasma of breast cancer patients suggest their potential as non-invasive biomarkers. As demonstrated by Taha et al. (2018), serum GSH and malondialdehyde (MDA) showed superior diagnostic performance in ROC curve analysis compared to other markers. These circulating markers could potentially be used for early detection, risk stratification, or as a means to monitor the systemic oxidative burden on a patient during and after treatment. The finding that surgery partially normalizes these levels further supports their utility in monitoring response to therapy [24].

Therapeutic Targeting: The profound dependency of breast cancer cells on their elevated GSH pool represents a key therapeutic vulnerability. If this antioxidant shield can be dismantled, the cancer cells would become susceptible to their own endogenous ROS and, more importantly, would be re-sensitized to conventional therapies. This has led to the development of strategies aimed at depleting intratumoral GSH. The most studied approach involves the inhibition of GCL, the rate-limiting enzyme of GSH synthesis, using agents like buthionine sulfoximine (BSO) [26]. By blocking the production of new GSH, these inhibitors can trigger a form of iron-dependent cell death known as ferroptosis and dramatically increase the efficacy of chemotherapeutic agents and radiation[13][24]. The strong correlation between high tumoral GSH, chemoresistance, and metastasis provides a compelling rationale for the clinical investigation of GSH-depleting agents as adjuvants to standard breast cancer treatment regimens, particularly for aggressive subtypes like TNBC which have been shown to be highly dependent on this pathway[3]. Conversely, the finding that excessive exogenous GSH intake during chemotherapy is associated with higher recurrence rates underscores the clinical importance of this pathway and cautions against the unmonitored use of antioxidant supplements by patients during treatment[26].

CONCLUSION AND RECOMMENDATIONS

Conclusive Summary of the Evidence

This systematic review provides strong and consistent evidence for a significant, compartmentalized

dysregulation of glutathione metabolism in women with breast cancer. The findings robustly support a dichotomous model characterized by two opposing phenomena:

1. **Tumoral Accumulation:** Breast cancer cells actively upregulate the synthesis and recycling of glutathione, leading to significantly elevated intracellular GSH concentrations. This serves as a critical adaptive mechanism to counteract high levels of endogenous oxidative stress, thereby promoting cell survival, proliferation, and resistance to ROS-inducing therapies.
2. **Systemic Depletion:** Concurrently, breast cancer patients exhibit a state of systemic oxidative stress, characterized by significantly depleted levels of reduced glutathione in the circulation (serum, plasma, and erythrocytes) and a corresponding decrease in the GSH/GSSG ratio.

This dichotomy highlights a fundamental aspect of breast cancer pathophysiology, where the tumor functions as a metabolic entity that remodels its own microenvironment while imposing a systemic oxidative burden on the host. Higher levels of tumoral GSH are clearly associated with more aggressive disease features, including advanced stage and metastatic potential, underscoring its role as a key driver of malignancy.

Recommendations for Future Research

Based on the synthesis of the available evidence and the identified limitations, the following recommendations for future research are proposed:

- **Prospective Cohort Studies:** There is a pressing need for large-scale, prospective cohort studies to validate the potential of circulating GSH, GSSG, and the GSH/GSSG ratio as early-detection or risk-stratification biomarkers for breast cancer. Such studies would overcome the inherent biases of retrospective case-control designs.
- **Standardization of Assays:** To facilitate better comparison across studies and enable future meta-analyses, the field would benefit from the development and adoption of standardized, validated protocols for the collection, processing, and quantitative analysis of glutathione in various clinical samples.
- **Subtype-Specific Investigations:** Future research should focus on elucidating the specific alterations in GSH metabolism across different molecular subtypes of breast cancer (e.g., Luminal A, Luminal B, HER2-enriched, and Triple-Negative). Given the association of high GSH with ER-negative status, understanding the unique dependencies of aggressive subtypes like TNBC on this pathway is of paramount importance for developing targeted therapies.
- **Clinical Trials of GSH-Depleting Agents:** Building on the strong preclinical rationale, well-designed, randomized controlled trials are warranted to evaluate the clinical efficacy and safety of GSH-depleting agents (e.g., GCL inhibitors) as adjuvants to standard chemotherapy or radiotherapy. These trials should incorporate predictive biomarkers to identify patient populations most likely to benefit from such a strategy.
- **Improved Control Tissues:** Future tissue-based studies should endeavor to include normal breast tissue from healthy, cancer-free individuals (e.g., from cosmetic reduction mammoplasty) as a control group, in addition to adjacent tissue, to better understand the field effect and establish a more accurate baseline for healthy tissue.

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