Original Article

Assessment of the anti-oxidant reduced glutathione in oral squamous cell carcinoma – Systematic review and meta-analysis

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Abstract

Background: The excess reactive oxygen species or free radicals reaction leads to oxidative injury to the biological components such as cells and tissues, which would result in the initiation and progression of carcinogenesis. The magnitude of oxidative damage depends primarily on the balance between free radicals (pro-oxidants) and antioxidant system activity.

Aim: To assess antioxidant status by evaluating the reduced glutathione (GSH) levels in various biological samples of patients with oral squamous cell carcinoma (OSCC) using available literature.

Materials and Methods: An electronic literature search was carried out in PubMed (MeSH), Science Direct, Scopus and Cross Reference by using specific keywords.

Results: The systematic electronic search identified 704 articles. After studying the articles' titles and abstracts, 657 articles were excluded for the following reasons; duplicated articles, animal studies, studies of low quality and not relevant to the research question. The remaining 47 articles were selected for full-text assessment. After eliminating the articles that did not match the objectives, the present qualitative synthesis finally included 27 articles for evaluation. The ten studies, which showed coherent data, were included in quantitative analysis. The GSH levels in OSCC groups are significantly decreased ($P < 0.001$) in plasma and erythrocyte samples compared to healthy controls.

Conclusion: The selected studies showed significantly lower levels of GSH in various biological samples of OSCC. Hence, future studies are required to validate the expression of GSH as a prognostic biomarker in oral cancer.

Keywords: An antioxidant enzyme, oral cancer, oral squamous cell carcinoma, reduced glutathione

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INTRODUCTION

Along with pharyngeal cancer, oral cancer is considered the ninth-most common cause of malignancy globally[1] and the third-leading cause of mortality in developing countries.[2] The oxidative stress (OS) induced by free radicals has been implicated in the pathogenesis of several diseases, including oral cancer.[3] Free radicals (Pro oxidants) are molecular species capable of independent existence with an unpaired electron in the outer shell. They are unstable and highly reactive oxygen species (ROS).[4] Free radicals target biomolecules and induce irreparable DNA, Lipid and Protein change, causing cellular/tissue damage.[5] The antioxidants defense system scavenges the free oxygen radicals, suppresses free radical chain reaction and lipid peroxidation. Thus, antioxidants play protects the human body from the harmful effects of ROS.[6] Excessive production of ROS by oxidative phosphorylation or suppression of the antioxidant system resulting in an imbalance between the Pro-oxidants (ROS) and antioxidants in favor of pro-oxidants is called OS. These oxidative modifications may also lead to mutations in DNA and transfer the normal cell into a malignant cell.[7]

Literature has expressed that antioxidants exert their protective effect by decreasing oxidative DNA damage and inhibiting the initiation and promotion of carcinogenesis. Reduced glutathione (GSH) is a ubiquitous tripeptide thiol compound that acts as a nonenzymatic antioxidant found in virtually all cells. GSH has a redox buffering capacity due to its ability to regenerate the essential antioxidants back to their active forms.[8] It plays a critical role in protecting organisms against toxicity by detoxifying deleterious hydrogen peroxide and alleviating OS caused by enhanced free radical production and providing a reducing capacity for several reactions.[9] GSH also maintains immune function by regulating mitogenic responses and cell proliferation.[10] It is a valuable marker for assessing the antioxidant defense mechanism in malignancy.

MATERIALS AND METHODS

Protocol and registration

The present protocol has adhered strictly to the PRISMA guidelines. The systematic review has been registered in the PROSPERO database (CRD42021265189).

Focused question

Is there any significant difference in antioxidant GSH levels between oral squamous cell carcinoma (OSCC) patients and the control group?

Based on the focus of the present systematic review, the formulated research question includes the following components of the PECOS framework:

i. Population: Patients with oral cancer
ii. Exposure or Prognostic factor: Evaluation of GSH
iii. Comparison: Between patients with oral cancer and healthy control group
iv. Outcome: GSH standardized mean difference value in various biological samples of patients with OSCC
v. Study: Identify prospective, cross-sectional and case–control studies investigating the oral cancer group's antioxidant GSH level.

Electronic search identification

The literature search was performed for published articles in electronic databases, including PubMed (MeSH), Science Direct, Scopus and Cross Reference. The articles that addressed the antioxidant defense status in OSCC using GSH levels from 1998 to 2020 were selected. The works only in English were selected, using the following keywords, "OSCC," "antioxidant status," and "GSH."

Screening for relevance

The articles that discussed antioxidant GSH levels in OSCC were collected and shortlisted. The titles and objectives of all the extracted materials were screened for applicability and duplication.

Inclusion criteria

a. Papers assessed antioxidant status by GSH level in OSCC
b. Studies evaluated different biological samples and expressed the GSH level in mean, standard deviation along with *P* value for freshly diagnosed OSCC patients and control group
c. Papers delivered sufficient data for comparison of OSCC and control groups.

Exclusion criteria

a. Articles displayed the unmatched objective and abstract
b. Being animal studies, literature or systematic reviews and case reports
c. The articles that had displayed Oral cancer under the Head-and-Neck Malignancy or oropharyngeal group had not provided specific data for OSCC
d. Studies utilized other markers of nonenzymatic or enzymatic antioxidants for evaluation
e. The works provided inadequate data for comparison between groups or articles expressed only pre- and post-treatment changes in OSCC without the control group evaluation.

Retrieval of full-text articles and evaluation

Three reviewers carried out an electronic search of the articles independently. If there is any disagreement, the consensus was reached based on the formulated criteria. The initial screening was accomplished by examining the titles and abstracts of the articles. The articles with matched objectives were carefully chosen for full-text review. Three observers independently assessed all the full-text papers against the New Castle Ottawa Scale and other specifications such as selection bias, missing and incomplete or imprecision data (e.g., inadequate sample size) and quality measures (e.g., ethics approval, informed consent, funding and conflicts of interest statement). After assessing all the particulars, the authors finally selected the eligible articles for the present systematic review.

Data extraction

The extracted data from full-text articles were author, publication year, sample size, GSH estimation method and measurements in specific units with statistical significance *P* value for OSCC and control groups. The segregated data were tabulated using the specified format.

Statistical analysis

The Forest plot was derived by the standard difference in the mean method by referring to the articles included in quantitative synthesis with the help of the comprehensive meta-analysis software. The overall standardized mean difference of GSH levels in OSCC was evaluated at a 95% confidence interval (CI). Due to significant heterogeneity between the articles, the random-effects model was used for quantitative synthesis.

RESULTS

From the methodology specified, we recovered 704 articles. Science Direct yielded 552 papers; Scopus search yielded 58 papers; PubMed search yielded 88 papers and Cross-reference yielded six papers. After fine-tuning, 657 articles were excluded because of irrelevant abstract and duplication. A full-text assessment was performed for 47 articles. After the final screening, the articles with inadequate data ($n = 4$) and reviews ($n = 16$) were excluded. After checking eligibility criteria, 27 articles with matched objectives were involved for qualitative synthesis. The articles containing incoherent data were not included for meta-analysis. Finally, 11 articles were included for quantitative synthesis [Figure 1]. We used the Newcastle-Ottawa quality assessment scale to weigh the selected studies [Table 1]. We tabulated all the derived data expressed in the eligible articles [Table 2].

Meta-analysis result

Compared to healthy tissues, GSH levels are significantly decreased ($P < 0.001$) in OSCC in plasma and erythrocyte samples. The plasma samples showed an overall standard mean difference of −7.191 with 95% CI (−9.45 to −4.94) [Figure 2 and Table 3]. The erythrocyte samples showed an overall standardized mean difference of −2.04 with 95% CI (−2.57 to −1.51) [Figure 3 and Table 4]. The meta-analysis in plasma samples exhibited high heterogeneity, reflected by the I^2 values 95.368 [Table 3]. In contrast, in erythrocyte samples, the I^2 value was 41.528, presented in Table 4. The diverse methodologies employed to assess the GSH levels could cause the high heterogeneity in plasma samples. Few studies compared the GSH level of OSCC for progressing clinical stages in various biological samples [Table 5]. The analysis of GSH levels between advancing histopathological grades is recorded in Table 6.

Publication bias

Studies included in the meta-analysis of erythrocyte samples were showed Begg and Mazumdar rank correlation; Kendall's tau without continuity correction gave a Z value for tau 0.522 with a two-tailed $P = 0.60$, indicating the absence of publication bias. Whereas studies included in the Meta-analysis of plasma samples were showed Begg and Mazumdar rank correlation, Kendall's tau without continuity correction gave a Z value for tau 2.47 with a two-tailed $P = 0.01$, which indicates a risk of publication bias.

DISCUSSION

The free radicals (Oxidants) responsible for important diseases are Superoxide anion radical (O_2•-), Singlet oxygen (O_2), Hydroxyl radical (•OH), Hydroperoxyl radical (HOO•), Hydrogen peroxide (H_2O_2), Lipid peroxide radical (ROO•), hypochlorite, nitric oxide (NO•) and Peroxynitrite (ONOO-•).[44] In a healthy human, the balance is maintained between oxidants and antioxidants. However, in an abnormal condition, it produces an excess of oxidizing species. It suppresses the antioxidant defense, which causes a shift in the ratio toward pro-oxidants and induces OS.[45] OS is the situation, initiates biomolecular damage, stimulates abnormal cell division and results in a malignant change of the tissue.

Several antioxidant compounds and enzymes may function to protect cellular components from oxidative damages.[5] Some research has indicated that OSCC people tend to

Table 1: New castle ottawa scale for studies included in the systematic review

Study (reference number)	Selection	Comparability	Exposure	Total score
Nagini *et al*., 1998[11]	4	1	2	7
Saroja *et al*., 1999[12]	4	1	3	8
Balasenthil *et al*., 2000[13]	4	1	2	7
Subapriya *et al*., 2002[14]	4	2	2	8
Subapriya *et al*., 2003[15]	4	1	2	7
Kolanjiappan *et al*., 2003[16]	4	1	2	7
Beevi *et al*., 2004[17]	4	2	2	8
Manoharan *et al*., 2005[18]	4	2	3	9
Fiaschi *et al*., 2005[19]	4	1	2	7
Elango *et al*., 2006[20]	4	2	3	9
Rasheed *et al*., 2007[21]	4	1	3	8
Sharma *et al*., 2009[22]	4	2	2	8
Bathi *et al*., 2009[23]	4	2	3	9
Raghavendra *et al*., 2010[24]	4	1	3	8
Srivastava K *et al*., 2012[8]	4	1	2	7
Rasool *et al*., 2014[25]	4	1	2	7
Shetty *et al*., 2014[26]	4	1	3	8
Metgud *et al*., 2014[9]	4	1	3	8
Bhat *et al*., 2015[27]	4	1	2	7
Thomas and Sethupathy 2015[28]	4	1	2	7
Srivastava K *et al*., 2016[3]	4	2	3	9
Banerjee *et al*., 2017[29]	4	2	3	9
Madhulatha *et al*., 2017[30]	4	1	2	7
Khan *et al*., 2017[31]	4	2	3	9
Basu *et al*., 2018[32]	4	1	1	6
Babiuch *et al*., 2019[33]	4	2	3	9
Shahi *et al*., 2020[34]	4	2	3	9

Selection - Case definition, case selection, control definition and selection, Comparability - Consideration of Matching known and potential confounding factors, Exposure - Securing patient records, interviewer blindness to groups, similarity ascertainment between groups and nonresponse rate

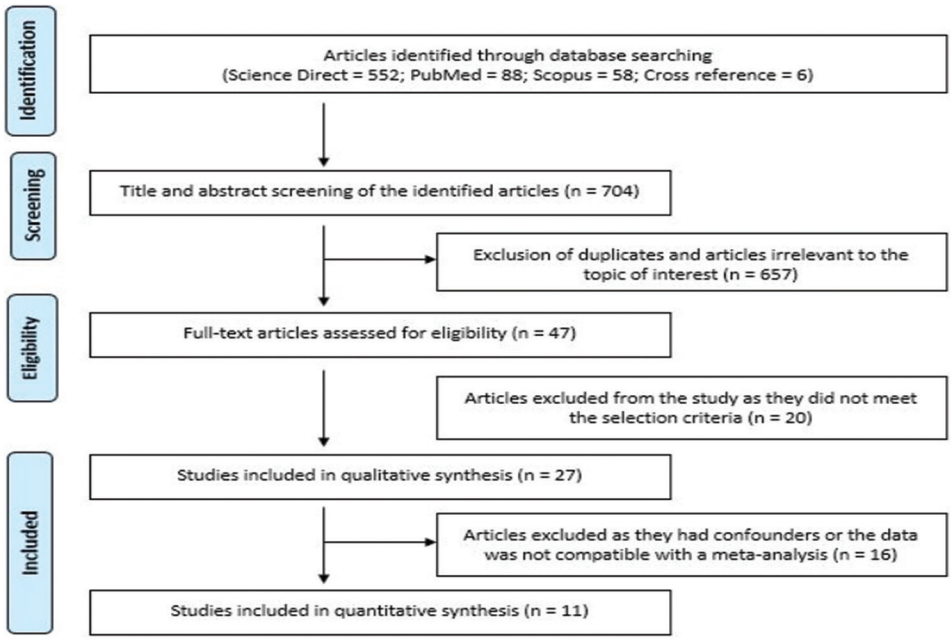

Figure 1: Prisma flow chart – study selection

have decreased serum levels of antioxidant-GSH than healthy individuals.[46] Earlier studies have reported a strong correlation between decreased risks of oral cancer with increasing blood glutathione levels.[10]

Glutathione is an essential water-soluble antioxidant synthesized from the amino acids glycine, g-glutamate and cysteine. GSH has a total electron-donating capacity. Due to its high redox potential, GSH functions as a potent

Table 2: The levels of reduced glutathione in various biological samples between healthy controls and patients with oral squamous cell carcinoma

Study	Sample type	Measurement unit	Mean	SD	Sample size	Mean	SD	Sample size	*P*	Method of assessment
			OSCC			Control				
Nagini *et al*.[11]	Ti	moles/mg protein	17.406	4.27	24	6.326	1.82	24	<0.001	Beutler *et al*.[35]
Saroja *et al*.[12]	Ti	mg/100 g tissue	29.56	3.87	33	21.36	3.46	33	<0.001	Beutler *et al*.[35]
Balasenthil *et al*.[13]	Ti	mg/100 g tissue	26.5	2.6	10	20.4	2.3	10	<0.001	Beutler *et al*.[35]
Subapriya *et al*.[14]	Pl	mg/dl	26.4	2.72	24	35.32	3.75	24	<0.05	Ellman[36]
Subapriya *et al*.[14]	Ti	mg/100 g tissue	29.51	2.93	24	20.5		24	<0.05	Ellman[36]
Subapriya *et al*.[15]	Pl	mg/dl	24.72	2.45	6	36.6	0.35	12	<0.05	Anderson[37]
Kolanjiappan *et al*.[16]	Ti	nmol/mg protein	15.87	2.27	16	6.8	0.91	16	<0.001	Beutler *et al*.[35]
Beevi *et al*.[17]	Pl	mg/dl	3.09	0.53	15	10.02	0.55	15	<0.001	Thomas and Skrinska[38]
Manoharan *et al*.[18]	Pl	mg/dl	37.71	5.15	48	50.64	5.17	16	<0.001	Beutler *et al*.[35]
Manoharan *et al*.[18]	Er	mg/dl	41.1	3.32	48	50.15	4.12	16	<0.001	Lowry *et al*.[39]
Fiaschi *et al*.[19]	Ti	nmol/mg protein	25.71	4.976	18	4.39	0.899	20	<0.001	Tietze[40]
Fiaschi *et al*.[19]	Bl	µmol/ml	0.69	0.109	18	2.54	0.331	20	<0.001	Tietze[40]
Elango *et al*.[20]	Bl	nmol/dl	26.4	3.1	63	44.3	4.8	45	<0.001	Moron *et al*.[41]
Rasheed *et al*.[21]	Pl	mg/dl	3.01	0.57	24	8.28	1.2	24	<0.001	Thomas and Skrinska[38]
Sharma *et al*.[22]	Er	mg/dl	7.8401	0.7048	30	9.0873	0.51078	15	<0.001	Beutler *et al*.[35]
Sharma *et al*.[22]	Er	ug/ml	78.401	7.0478	30	90.873	5.1078	15	<0.001	Beutler *et al*.[35]
Bathi *et al*.[23]	Pl		8.8149		30	2.2367		30	<0.001	Beutler *et al*.[35]
Raghavendra *et al*.[24]	Er	mg/dl	40.69	7.04	25	53.06	7.79	25	<0.001	Beutler *et al*.[35]
Srivastava *et al*.[8]	Pl	mg/dl	32.43	2.8	20	48.93	0.893	20	<0.001	Ellman[36]
Rasool *et al*.[25]	Sa	mg/dl	0.88	0.25	30	2.09	0.24	10	<0.001	Spectrophotometric method
Rasool *et al*.[25]	Bl	mg/dl	2.4	0.77	30	9.82	1.32	10	<0.001	Spectrophotometric method
Shetty *et al*.[26]	Se	mg/dl	0.09479	0.02706	50	0.18804	0.03656	65	<0.001	DTNB method
Shetty *et al*.[26]	Sa	mg/dl	0.04787	0.02317	35	0.094.67	0.03659	35	<0.001	DTNB method
Shetty *et al*.[26]	Se	µg/dl	94.79	27.06	50	188.04	36.56	65	<0.001	DTNB method
Shetty *et al*.[26]	Sa	µg/dl	47.87	23.17	35	94.67	36.59	35	<0.001	DTNB method
Metgud and Bajaj[9]	Sa	nmol/dl	7.04	0.67	30	9.74	0.53	30	<0.001	Beutler *et al*.[35]
Metgud and Bajaj[9]	Se	nmol/dl	17.31	1.55	30	32.18	5.53	30	<0.001	Beutler *et al*.[35]
Bhat *et al*.[27]	Pl	mg/dl	2.89	0.65	30	10.5	0.55	30	<0.001	Thomas and Skrinska[38]
Thomas and Sethupathy[28]	Pl	mg/dl	26.3	2.4	20	41.15	1.89	20	<0.05	Ellman[36]
Srivastava *et al*.[3]	Ti	mg/dl	33.6	4.65	20	22.9	1.1	20	<0.001	Ellman[36]
Banerjee *et al*.[29]	Mi	mM	15.225	3.02	60	11.1	2.38	20	<0.001	Akerboom and Sies[42]
Madhulatha *et al*.[30]	Se		5.55	0.91	25	4.46	0.39	25	<0.001	
Khan *et al*.[31]	Se	mg/dl	2.58	0.031	50	9.77	1.37	20	<0.05	Tietze[40] and Moron *et al*.[41]
Basu *et al*.[32]	Ly	nmol/mg of protein	35.52	7.65	30	56.7	8.85	50	<0.001	Beutler[43]
Babiuch *et al*.[33]	Sa	mmol/l	0.01	0.01	20	0.02	0.01	20	<0.001	Beutler *et al*.[35]
Shahi *et al*.[34]	Pl	µmol/l	11.7	5.4	25	6.2	1.5	45	0.004	Moron *et al*.[41]

Ti: Tissue, Pl: Plasma, Er: Erythrocyte, Bl: Blood, Sa: Saliva, Se: Serum, Mi: Mitochondria, Ly: Lymphocyte, SD: Standard deviation, OSCC: Oral squamous cell carcinoma , DTNB: Ellman's Reagent (5,5-dithio-bis-2-nitrobenzoic acid)

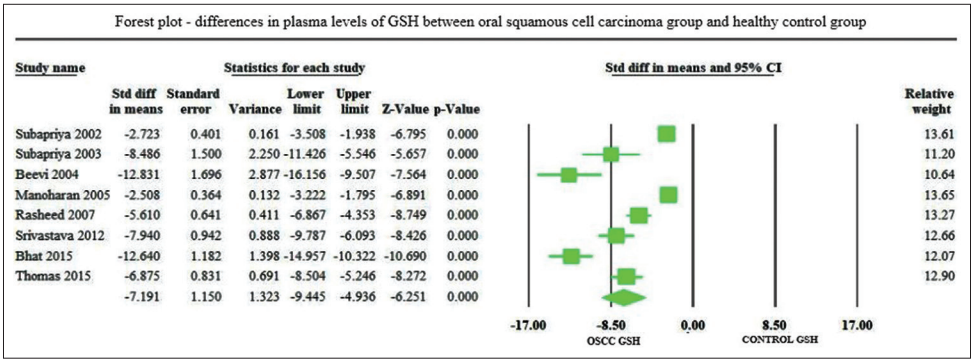

Figure 2: Forest plot shows standardized mean difference estimates with 95% confidence intervals representing differences in plasma levels of reduced glutathione between oral squamous cell carcinoma group and healthy controls

antioxidant and a convenient cofactor for the enzymatic reaction. It also maintains the redox state of protein sulfhydryls necessary for DNA repair.[47] This molecule is also involved in conjugation with electrophilic carcinogens

Table 3: Test for overall effect size and Heterogeneity values for studies included in meta-analysis - plasma samples.

Model	Effect size and 95% confidence interval				Test of null (2-Tail)		Heterogeneity				Tau-squared			
Model (no of studies)	Point Estimate	SE	Variance	CI Values Lower/ Upper	Z - value	P-value	Q-value	Df (Q)	P-value	I-Squared	Tau Squared	SE	Variance	Tau
Fixed (8)	-4.224	0.222	0.049	-4.659/-3.789	-19.026	0.000	151.118	7	0.000	95.368	9.563	7.411	54.926	3.092
Random (8)	-7.191	1.150	1.323	-9.445/-4.936	- 6.251	0.000								

Table 4: Test for overall effect size and Heterogeneity values for studies included in meta-analysis - erythrocyte samples.

Model	Effect size and 95% confidence interval				Test of null (2-Tail)		Heterogeneity				Tau-squared			
Model (no of studies)	Point Estimate	SE	Variance	CI Values Lower/ Upper	Z - value	P-value	Q-value	Df (Q)	P-value	I-Squared	Tau Squared	SE	Variance	Tau
Fixed (3)	-2.023	0.205	0.042	-2.425/-1.621	-9.868	0.000	3.420	2	0.181	41.528	0.090	0.217	0.047	0.300
Random (3)	-2.036	0.269	0.072	-2.563/-1.508	-7.568	0.000								

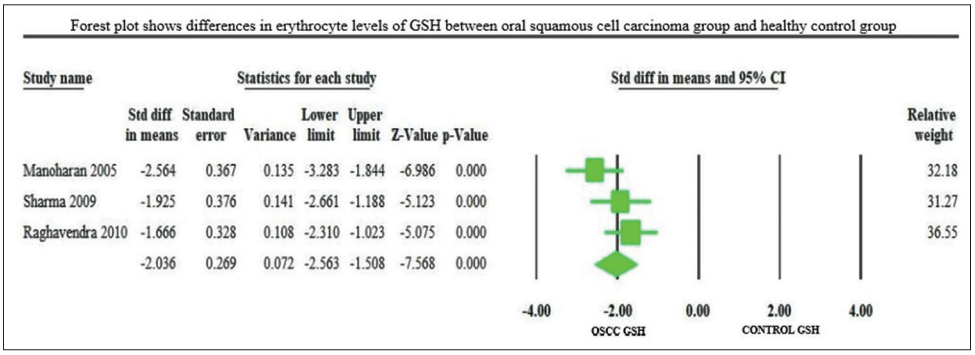

Figure 3: Forest plot shows standardized mean difference estimates with 95% confidence intervals representing differences in erythrocyte levels of reduced glutathione between oral squamous cell carcinoma group and healthy controls

and catalyzes the decomposition and detoxification of ROS. Thus, it prevents accumulation, protects the cells against oxidative damage and limits cell injury, preventing carcinogenesis.[9] It regenerates the essential antioxidants, Vitamins C and E, back to their active forms. Glutathione includes a reduced form (or GSH) and an oxidized form (or glutathione disulfide [GSSG]). The regenerating capacity of Glutathione is linked with the Redox state of the GSSG-glutathione couple (GSSG/2GSH). Therefore, the intracellular "Redox homeostasis" or "Redox buffering" capacity is substantiated primarily by GSH.[48] GSH and its precursors prevent the arecoline-induced cytotoxicity of tobacco and betel quid chewers. When exposed to excessive xenobiotics, including carcinogens, more Glutathione is utilized for conjugation (detoxification). It decreases the GSH/GSSG ratio, making it less available, decreasing body defense against free radicals.[18] GSH depletion is sufficient to sensitize cancer cells to oxidative and nitrative stress, which leads to DNA damage.[49] DNA degeneration that may result in the activation of carcinogens leads to cancer initiation and progression.[8] Thus, assessing antioxidant thiol levels is a valuable biomarker to predict oral cancer patients' risk of progression of carcinogenesis and overall survival.[50]

There has been a report of decreased antioxidants and increased protein-and DNA oxidation products in the saliva of OSCC patients.[51]

The present systematic review included 1052 patients diagnosed with OSCC and 910 healthy volunteers for GSH analysis. The present work evaluated the literature to analyze antioxidant enzyme GSH in various biological samples of patients diagnosed with OSCC and healthy controls. The authors used various laboratory methods to assess GSH levels.[35-42]

The present systematic review displayed a significant decrease of mean GSH level in various biological samples of OSCC patients as compared with the control ($P < 0.001$).[8,9,17-27,32,33] A similar diminishing trend was reported in plasma level of GSH in oral cancer patients compared to controls with statistical significance $P < 0.05$.[14,15,28,31] Antioxidant depletion in the blood may be due to increased utilization in lipid peroxides scavenging

Table 5: The levels of reduced glutathione in various samples of patients with different clinical stages of oral squamous cell carcinoma

Study	Sample type	Measurement unit	Clinical stages								Statistical significance	Criteria
			Stage I		Stage II		Stage III		Stage IV		*P*	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Manoharan *et al*.[18]	Pl	mg/dl	-	-	44.09	5.62	37.56	4.98	31.48	4.86	<0.01	UICC
Manoharan *et al*. [18]	Er	mg/dl	-	-	45.24	3.86	40.92	2.93	37.14	3.16	<0.01	UICC
Srivastava *et al*.[8]	Pl	mg/dl	-	-	34.58	2.01	32.8	2.5	30.76	2.64	Nonsignificant	TNM
Srivastava *et al*.[3]	Ti	mg/dl	-	-	28.3	0.91	32.8	2.83	37.56	3.65	<0.01	TNM
Kolanjiappan *et al*.[16]	Ti	nmol/mg protein	-	-	10.9	1.3	15.8	1.3	20.9	4.2	<0.001	AJCC
Banerjee *et al*.[29]	Mi	mM	15.2	3.87	13.2	2.36	15.3	2.63	17.2	3.25	<0.001	TNM

Pl: Plasma, Er: Erythrocyte, Ti: Tissue, Mi: Mitochondria, SD: Standard deviation, UICC: Union for International Cancer Control, TNM: Tumor Node Metastasis, AJCC: American Joint Committee on Cancer

Table 6: The levels of reduced glutathione in various samples of patients with different histopathological grades of oral squamous cell carcinoma

Study	Sample	Unit	WD		MD		PD		Statistical significance
			Mean	SD	Mean	SD	Mean	SD	*P*
Sharma *et al*.[22]	Er	ug/ml	80.554		77.63072		76.55867		>0.05
Metgud and Bajaj[9]	Sa	nmol/dl	6.9	0.75	7.18	0.59	-		>0.05
Metgud and Bajaj[9]	Se	nmol/dl	16.84	1.48	17.78	1.62	-		>0.05

Er: Erythrocyte, Sa: Saliva, Se: Serum, SD: Standard Deviation, WD: Well-differentiated, MD: Moderately differentiated, PD: Poorly differentiated

and the counter-reaction of oxidative conditions induced by pro-oxidants.[9] The decrease in GSH levels is from the repression of synthesizing enzymes and increased conjugation with arecoline,[52] and increased sequestration by the tumor cells to meet a growing tumor's demands.[14] Depletion of GSH may sensitize tumors to chemotherapy and radiotherapy.[53] Thus concerning these facts, assessing antioxidant status both in tumor tissue and adjacent normal tissue might prove beneficial for cancer patients before radiotherapy and chemotherapy.

On the contrary, another reported study in plasma and serum samples displayed an increase in GSH level with statistical significance ($P < 0.001$)[23,30] and ($P < 0.01$) in OSCC patients than in the control group.[34] Glutathione levels were significantly increased in all groups compared to controls. The increased levels of GSH in erythrocytes may be in response to the toxins released by cytologically altered cells. It is believed that these erythrocytes are resistant to oxidative hemolysis. They were adequately protected against any free radical damage even if serum levels of other antioxidants were suppressed.[23] The increased levels of GSH in these patients reflect the increased detoxification capacity and resistance in response to the cytotoxic substances released by carcinogen-altered cells.[23]

Similarly, few authors reported significantly increased tissue levels of GSH in the OSCC group ($P < 0.001$).[3,11-13,16,19] and ($P < 0.05$).[14] Another study also expressed a statistically significant GSH level increase in mitochondria samples of OSCC patients compared to the control group ($P < 0.001$).[29]

Prolonged direct contact of the quid with the oral mucosa leads to the seepage of the carcinogens. Finally, it gets concentrated in high volumes in the local environment of the tissue. These carcinogens from tobacco smoke or quid are predominantly detoxified by enhanced glutathione-dependent enzymes in the tumor tissue. GSH offers cell protection against ROS's cytotoxic effect and makes them more resistant to OS.[3] It can also be suggested that tumor tissue and plasma are two different compartments regarding behavior toward OS.[3]

Some of the studies compared the levels of GSH in each of the clinical stages of OSCC. The mean GSH level of one study in plasma and erythrocyte samples showed a significant gradual reduction ($P < 0.01$) when the clinical grade of OSCC advances.[18] One study in plasma displayed that the reduction of GSH levels was insignificant when the clinical-stage OSCC advanced.[8] Moreover, the decrease in antioxidant enzyme levels showed no significant relation with the tumor stage in the patients.[8] The GSH levels were gradually decreased from stage II to stage IV of oral cancer patients.[8,18] Few studies documented that the mean GSH level showed a significant gradual rise ($P < 0.001$)[16,29] and ($P < 0.01$) when the clinical grade of OSCC advances.[3] In Sharma *et al*.'s study, a significantly lower level of GSH was observed in patients with tongue SCC (Stage III/IV) compared to control individuals. Early stages of tongue SCC were not evaluated in their study since all of their patients were in advanced stages.[22]

Sharma *et al*. had observed that GSH levels were mainly reduced in poorly differentiated tumors than in well and moderately differentiated tumors. On statistical analysis, the comparison is found to be insignificant.[22] On the contrary, Metgud and Bajaj reported a statistically insignificant rise in serum and salivary GSH levels in moderately differentiated tumors compared to well-differentiated tumors ($P > 0.05$).[9]

Limitation

The apparent discrepancies could be partly due to different laboratory techniques used to measure the GSH level. Only a few studies had expressed the clinical stage-wise and histopathological grade-wise analysis. Hence, there is no definitive GSH level change prediction according to varying clinical stages and histopathological grades.

CONCLUSION

The ROS in the blood is supposed to play a vital role in mutations in the cell; thus, normalization of oxidant-antioxidant status might improve the prognosis of patients.

Hence, antioxidants play a vital role as valuable markers in the prognosis of oral cancer. Further elaborative studies with a larger sample size could ratify the value of GSH as a prognostic marker of OS to determine the progression of various stages of cancer.

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Conflicts of interest

There are no conflicts of interest.

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