Malondialdehyde, an Oxidative Stress Marker in Oral Squamous Cell Carcinoma—A Systematic Review and Meta-Analysis

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Abstract: Objective: To qualitative and quantitatively review published literature assessing the oxidative stress marker malondialdehyde (MDA) in oral squamous cell carcinoma (OSCC). Methodology: PubMed (MeSH), Science Direct, Scopus, Web of Science, Wiley Online Library, Cochrane, and Cross Reference were searched for studies assessing MDA levels in OSCC samples. Results: From the 1008 articles identified, 849 were excluded based on title and abstract screening due to duplication and irrelevance to the topic of interest. Full-text assessment of the remaining 159 articles led to the inclusion of only 46 articles that satisfied the selection criteria. Of these, only 26 studies had data compatible for quantitative analysis. The MDA levels in OSCC groups are significantly increased ($p < 0.00001$) in plasma, serum, and saliva samples in the majority of the studies evaluated. In contrast, MDA levels in OSCC tissue samples are significantly attenuated ($p < 0.00001$) compared to healthy controls, supported by fewer studies. Conclusions: The augmented MDA levels in plasma, serum, and saliva samples of the OSCC reflect the heightened oxidative stress level accurately. Further studies are required to understand the attenuated MDA levels in the tissue samples of OSCC. Correlation analysis between MDA levels with established clinicopathological prognostic markers could aid in formulating oxidative stress-based prognostication and treatment planning.

Keywords: oral squamous cell carcinoma; oral cancer; oxidative stress

1. Introduction

Squamous cell carcinoma (SCC) is one of the most common oral malignancies. The incidence of oral cancer varies greatly. The annual worldwide report states the incidence
of more than 400,000 new cases of OSCC [1]. Brazil, Central, Eastern Europe, France, and India have the highest reported oral cancer rates worldwide [2].

Various factors are known to play in the etiopathogenesis of oral squamous cell carcinoma. Carcinogenesis may be the interplay of socioeconomic factors and etiological factors such as habitual use of smoking or chewing tobacco, alcohol, oncogenic viral infections, oncogenes, and mutation of tumor suppressor genes. Recent literature showed that young patients who developed oral cancer were non-smokers and not addicted to tobacco/betel nut chewing. An epidemiological study of oral cavity cancers in Iran showed that tongue cancer is the oral cavity’s predominant cancer in non-smokers [3]. Thus, other factors may also be involved in etiopathogenesis. Factors such as phenols, radiation, trauma or sharp teeth, iron deficiency, vitamin A deficiency, syphilis, candidiasis, and a compromised immune status are the suggested other possible causes [4].

The continuous and direct exposure of the oral mucosal cells to the chemical carcinogens of tobacco products such as Polynuclear Aromatic Hydrocarbons (PAH) and nitrosamines tend to induce free radicals/reactive oxygen species (ROS) production [5].

Free radicals are molecules that show an unpaired electron in their external orbit and are therefore highly reactive [6]. Some of the free radicals (ROS) are such as superoxide anion radicals (O$_2^-$), hydroxyl radicals (HO), Hydroperoxyl (HO$_2$), peroxyl (ROO.), alkoxyl (RO.), and hydrogen peroxide (H$_2$O$_2$) [7]. ROS and reactive nitrogen species (RNS) exert beneficial effects on cellular responses and immune function at low or moderate levels. However, at higher levels, ROS produces various pathologies.

Anti-oxidants are cytoprotective chemicals that prevent oxidative damage caused by free radicals [8]. Due to harmful habits, ROS attain higher concentrations which overwhelm the anti-oxidant protective mechanisms provided by anti-oxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRx), carotenes, and vitamins of cells and tissues. It results in the depletion of anti-oxidants, which causes the accumulation of ROS and leads to the condition called oxidative stress (OS) [9]. OS induces cell metabolism impairment, including rising intracellular free Ca$^{2+}$ levels and damage of the membrane ion transporters. ROS also facilitates punctual mutations, DNA base oxidations and strand breakage, mutation of tumor suppressor genes, and activation of proto-oncogenes [6,10].

Furthermore, the decomposition of these peroxidized lipids are disintegrated quickly and forms reactive carbon compounds, including lipid hydroperoxides (LHP) and malondialdehyde (MDA). These by-products serve as an indicator of lipid peroxidation [11]. These lipid peroxidation products can modulate cell growth and promote tumor progression by activating the signal transduction pathway. In addition, they act as co-carcinogenic agents by expressing their high cytotoxicity [12].

There is a need for quantitation of biomarker expression to assess bio-molecular damage. The measurement of free radicals directly is not reliable due to the concise life of free radicals. Hence, the proposed method of OS evaluation includes the estimation of secondary lipid peroxidation products, such as MDA. MDA assessment expresses the extent of lipid peroxidation and free radical-mediated oxidative damage. MDA is a three-carbon dialdehyde compound that appears in blood, saliva, serum, tissue, and urine during lipid peroxidation [13]. Hence, the present review aimed to analyze oxidative stress using MDA as a biomarker of lipid peroxidation (LPx) in OSCC patients and compare them with the healthy control group with the help of the available literature.

2. Materials and Methods
2.1. Protocol and Registration

PRISMA guidelines had been strictly adhered to study selection. The review protocol was registered in the PROSPERO database (CRD42021249182).
2.2. Focused Question

Is there any significant difference in the MDA level of biological samples between oral squamous cell carcinoma patients and the control group?

Based on the objective of the present meta-analysis and the research question, the following components were focused:

(i) Population: patients with OSCC
(ii) Exposure or Diagnostic marker: mean and standard deviation value of MDA
(iii) Comparison: between patients with oral squamous cell carcinoma and healthy subjects
(iv) Outcome: assessment of MDA in various biological samples of patients with OSCC
(v) Study: identify related cross-sectional and case-controlled studies investigating the status of MDA in OSCC and control from 1999 to 2020.

2.3. Electronic Search Identification

Electronic databases, including PubMed (MeSH), Science Direct, Scopus, Web of Science, Willey Online Library, Cochrane, and Cross Reference, were searched for published articles addressing oxidative stress in oral squamous cell carcinoma using MDA assay between the years 1999–2020. The following keywords, ‘oral squamous cell carcinoma,’ ‘oxidative stress,’ and ‘Malondialdehyde was employed.’

2.4. Screening for Relevance

Articles discussing oxidative stress in OSCC were identified and shortlisted based on the titles and abstracts screening for relevance and duplication.

2.5. Inclusion Criteria

(a) Studies discussed the oxidative status of OSCC using lipid peroxidation marker-Malondialdehyde (MDA);
(b) Studies involving various biological samples and expressed the MDA data in mean, standard deviation along with p-value;
(c) Papers provided sufficient data to allow comparison of OSCC and control groups.

2.6. Exclusion Criteria

1. Articles with the unmatched objective and abstract;
2. Being literature reviews and systematic reviews;
3. Studies used other oxidative stress markers as a marker of evaluation;
4. The works provided inadequate data for the comparison between control and OSCC groups;
5. Studies related to head and neck squamous cell carcinoma

2.7. Retrieval of Full-Text Articles and Evaluation

K.M., U.S., and T.B. screened the titles/abstracts of all the studies and excluded studies at high risk of bias from the evidence synthesis based on pre-specified criteria. K.M., S.P., and A.T.R., have independently screened each included study’s full texts. K.M., M.M.A.A, M.A.A, H.S.A.D, Z.K., and A.T.R., have checked and discussed the relevant factors considered in each included study. After assessing all the particulars, the authors have considered the articles for eligibility criteria. The authors resolved disagreements by consensus. Finally, K.M., U.S., and S.P., have performed the data collection procedure.

2.8. Data Extraction

The extracted data from full-text articles were author, publication year, age groups, sample size, MDA measurements in OSCC, and control group expressed as the mean and standard deviation along with specific units. Collected data were tabulated separately in a specified format.
2.9. Statistical Analysis

The Forest plot was derived using the mean difference, and standard mean difference method to carry out a meta-analysis using comprehensive meta-analysis software version 3 (Biostat Inc. Englewood, NJ, USA). The overall mean difference or standardized mean difference value of MDA in OSCC was analyzed at a 95% confidence interval (CI). A random-effects model was used in the analysis due to the presence of significant heterogeneity. The articles, which expressed the MDA levels in similar units in each sample, only were included in the meta-analysis.

3. Results

Pubmed search yielded 517 papers; Science direct search yielded 292 papers; Scopus search yielded 141 papers; Web of Science yielded seven papers; Willey online library yielded 26 papers, and Cross-reference search yielded 25 papers. After search refinement, 849 articles were excluded due to unmatched titles and abstracts, including four duplicated data reports and one animal study. After extraction of these articles, 159 articles had their titles relevant to the present work. Full-text was retrieved for the screened articles. Articles with un-matched objectives (n = 84), systematic reviews (n = 1), critical reviews (n = 2), reviews (n = 25) and letter to the editor (n = 1) were excluded. Forty-six articles with matched objectives were included in the systematic review. Only 26 articles had data compatible for a meta-analysis (Figure 1).

![Figure 1. Prisma Flow Chart—Study Selection.](image)

Newcastle-Ottawa quality assessment scale was employed to grade the quality of included studies in the systematic review (Table 1). Collected MDA assessment data along with other findings of included articles in various biological samples were tabulated (Table 2). Few studies compared the MDA level concerning clinical stages of OSCC in various samples (Table 3) and changes in varying histopathological grades (Table 4). The analysis of MDA levels according to different clinical stages and histopathological grades could not be performed due to the scarcity of the reported studies.
Table 1. New Castle Ottawa Scale for studies included in the Systematic Review.

| Study (Reference Number)          | Selection | Comparability | Exposure |
|-----------------------------------|-----------|---------------|----------|
|                                   | Case Definition | Case Representativeness | Control Selection | Control Definition | Matching Known Confounding Factor | Matching Potential Confounding Factor | Secure Patient Records | Interviewer Blinded to Cases and Control | Similarity in Case and Control Ascertainment | Non-Response Rate | Total Stars |
| Saroja et al. 1999 [14]          | *          | *             | *         | +               | -               | *                               | - | *                               | *                               | *                               | 8        |
| Sabitha et al. 1999 [15]         | *          | *             | *         | -               | -               | -                               | * | -                               | *                               | *                               | 7        |
| Balasenthil et al. 2000 [16]     | *          | +             | *         | +               | -               | *                               | - | -                               | *                               | -                               | 7        |
| Subapriya et al. 2002 [5]        | *          | *             | *         | -               | -               | *                               | - | *                               | -                               | -                               | 8        |
| Subapriya et al. 2003 [17]       | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | *                               | 7        |
| Kolanjippan et al. 2003 [18]     | *          | *             | *         | -               | +               | -                               | - | *                               | -                               | -                               | 7        |
| Beevi et al. 2004 [19]           | *          | *             | *         | -               | -               | *                               | - | *                               | -                               | -                               | 8        |
| Manoharan et al. 2005 [20]       | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | *                               | 9        |
| Khanna et al. 2005 [21]          | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | *                               | 9        |
| Rasheed et al. 2007 [22]         | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | -                               | 8        |
| Rai B et al. 2008 [23]           | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | -                               | 7        |
| Bathi et al. 2009 [24]           | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | -                               | 7        |
| Chole et al. 2010 [25]           | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | *                               | 8        |
| Raghavendra et al. 2010 [26]     | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | *                               | 8        |
| Gokul et al. 2010 [27]           | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | -                               | 8        |
| Burlakova et al. 2010 [28]       | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | -                               | 8        |
| Arathi et al. 2010 [29]          | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | -                               | 8        |
| Barut et al. 2011 [30]           | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | -                               | 8        |
| Ramya et al. 2011 [31]           | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | -                               | 8        |
| Srivastava K et al. 2012 [32]    | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | -                               | 7        |
| Sree et al. 2013 [33]            | *          | *             | *         | -               | *               | -                               | - | *                               | -                               | -                               | 7        |
| Nath et al. 2014 [34]            | *          | *             | *         | -               | -               | -                               | - | *                               | -                               | -                               | 6        |
| Metgud et al. 2014 [12]          | *          | *             | *         | -               | *               | -                               | - | -                               | -                               | *                               | 8        |
| Rasool et al. 2014 [35]          | *          | *             | *         | -               | -               | -                               | - | *                               | -                               | -                               | 7        |
| Ganesan et al. 2014 [36]         | *          | *             | *         | -               | -               | -                               | - | *                               | -                               | *                               | 8        |
Table 1. Cont.

| Study (Reference Number) | Selection | Comparability | Exposure |
|--------------------------|-----------|---------------|----------|
|                          | Case Definition | Case Representativeness | Control Selection | Control Definition | Matching Known Confounding Factor | Matching Potential Confounding Factor | Secure Patient Records | Interviewer Blinded to Cases and Control | Similarity in Case and Control Ascertainment | Non-Response Rate | Total Stars |
| Malik et al. 2014 [37]    | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 8          |
| Huo et al. 2014 [38]      | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 7          |
| Shetty et al. 2014 [33]   | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 8          |
| Bhat et al. 2015 [39]     | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 7          |
| Rai S et al. 2015 [40]    | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 7          |
| Thomas et al. 2015 [38]   | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 7          |
| Kaur et al. 2015 [41]     | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 8          |
| Shankarram et al. 2015 [42]| *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 6          |
| Mishra et al. 2016 [43]   | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 7          |
| Nyamathi et al. 2016 [44] | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 7          |
| Srivastava K et al. 2016 [45]| *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 9          |
| Verma et al. 2017 [46]    | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 8          |
| Madhulatha et al. 2017 [47]| *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 7          |
| Banerjee et al. 2017 [48] | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 9          |
| Basu et al. 2018 [49]     | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 6          |
| Arya et al. 2019 [8]      | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 8          |
| Sabarathnam et al. 2019 [50]| *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 6          |
| Babiuch et al. 2019 [51]  | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 9          |
| Shahi et al. 2020 [52]    | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 9          |
| Oswal et al. 2020 [53]    | *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 7          |
| Abdelkawy et al. 2020 [54]| *          | *             | *          | *          | *          | *          | *          | *          | *          | *          | *          | 8          |
### Table 2. The levels of MDA in various biological samples between healthy controls and patients with OSCC of studies included in the qualitative synthesis.

| Author           | Sample       | Unit            | Mean   | Std. Dev | Sample Size | Mean   | Std. Dev | Sample Size |
|------------------|--------------|-----------------|--------|----------|-------------|--------|----------|-------------|
| Saroja 1999 [14] | Ti           | nmol/100 mg protein | 86.56  | 8.03     | 33          | 124.3  | 7.86     | 33          |
| Sabitha 1999 [15]| Se           | nmol/mL         | 0.598  | 0.169    | 12          |        |          |             |
| Balasenthil 2000 [16] | Ti     | nmol/100 mg protein | 85.5   | 4.4      | 10          | 125.3  | 4.8      | 10          |
| Subapriya 2002 [5] | Ti           | nmol/100 mg protein | 97.84  | 9.32     | 24          |        |          |             |
| Subapriya 2002 [5] | Pl           | nmol/mL         | 6.37   | 1.12     | 24          | 4.38   | 1.8      | 24          |
| Subapriya 2002 [5] | Er           | pm/mg Hg        | 1.98   | 0.21     | 24          | 1.11   | 0.13     | 24          |
| Subapriya 2003 [17] | Pl           | nmol/mL         | 6.27   | 0.72     | 6           | 3.81   | 0.35     | 12          |
| Subapriya 2003 [17] | Er           | mg/dL           | 39.44  | 3.6      | 6           | 34.61  | 3.3      | 12          |
| Kolanjiappan 2003 [18] | Ti       | nmol/100 mg protein | 93.4   | 10.5     | 48          | 123.9  | 14.5     | 16          |
| Beevi 2004 [19] | Pl           | nmol/mL         | 5.57   | 0.97     | 15          | 2.02   | 0.23     | 15          |
| Manoharan 2005 [20] | Pl           | nmol/mL         | 3.75   | 0.87     | 48          | 2.09   | 0.17     | 16          |
| Manoharan 2005 [20] | Er           | pm/mg Hb        | 3.35   | 0.43     | 48          | 2.43   | 0.17     | 16          |
| Manoharan 2005 [20] | Er memb      | nmol/mg protein | 0.62   | 0.2      | 48          | 0.34   | 0.06     | 16          |
| Khanna 2005 [21] | Se           | nmol/L          | 0.67   | 0.57     | 20          | 0.321  | 0.06     | 20          |
| Rasheed 2007 [22] | Pl           | nmol/mL         | 4.16   | 0.47     | 24          | 2.26   | 0.24     | 24          |
| Rai B 2008 [23] | Sa           | ng/mL           | 5.23   | 0.41     | 12          | 3.415  | 0.44     | 30          |
| Bathi 2009 [24] | Pl           |                  | 3.543  |          | 30          | 2.517  |          | 30          |
| Chole 2010 [25] | Se           | nmol/mL         | 14.34  | 1.43     | 25          | 5.107  | 2.32     | 30          |
| Raghavendra 2010 [26] | Er          | nmol/mL         | 7.22   | 1.52     | 30          | 4.379  | 0.97     | 25          |
| Gokul 2010 [27] | Er           | nmol/g Hg       | 159.8  | 36.4     | 18          | 139.4  | 22.3     | 25          |
| Gokul 2010 [27] | Ti           | nmol/mg protein | 1.12   | 0.76     | 18          | 0.68   | 0.33     | 18          |
| Burlakova 2010 [28] | Er          | µmol/10€ Er     | 3.5    | 0.52     | 50          | 3.92   | 1.06     | 54          |
| Arathi 2010 [29] | Sa           | nmol/L          | 0.017  | 0.01     | 25          | 0.002  | 0        | 25          |
| Barut 2011 [30] | Pl           | nmol/mL         | 7.4    | 2.55     | 29          | 4.9    | 1.25     | 29          |
| Ramya 2011 [31] | Se           | nmol/mL         | 1.79   | 0.29     | 40          | 1.16   | 0.31     | 40          |
| Srivastava K 2012 [32] | Pl         | nmol/mL         | 5.5    | 1.7      | 20          | 2.05   | 0.94     | 20          |
| Sree 2013 [33] | Se           | nmol/mL         | 5.32   | 1.12     | 30          | 3.18   | 0.23     | 30          |

* Method: Ohkawa et al. [55], Suematsu et al. [56], Yagi et al. [57], Donnan et al. [58], Buege et al. [59], Bergmeyer et al. [61], Draper et al. [60], Stocks et al. [63], Yagi et al. [57], Buege et al. [59].
Table 2. Cont.

| Author               | Sample | Unit    | Mean   | Std. Dev | Sample Size | Mean   | Std. Dev | Sample Size | Method                  |
|----------------------|--------|---------|--------|----------|-------------|--------|----------|-------------|-------------------------|
| Nath 2014 [34] *     | Se     | nmol/mL | 55.04  | 13.7     | 120         | 27.43  | 2.62     | 45           | Okhawa et al. [55]      |
| Metgud 2014 [12] *   | Se     | nmol/mL | 6.02   | 0.43     | 40          | 2.93   | 0.79     | 30           | Okhawa et al. [55]      |
| Metgud 2014 [12] *   | Sa     | nmol/mL | 0.32   | 0.03     | 40          | 0.2    | 0.01     | 30           | Okhawa et al. [55]      |
| Rasool 2014 [35]     | PI     | µmol/mL | 4.55   | 1.48     | 30          | 3.15   | 0.58     | 10           | Spectrophotometry       |
| rasool 2014 [35]     | Sa     | µmol/mL | 0.54   | 0.25     | 30          | 0.19   | 0.02     | 10           | Spectrophotometry       |
| Ganesan 2014 [36] *  | Se     | nmol/mL | 1.824  | 0.55     | 20          | 0.712  | 0.13     | 20           | Okhawa et al. [55]      |
| Ganesan 2014 [36] *  | Sa     | µmol/mL | 0.32   | 0.03     | 20          | 0.59   | 0.13     | 20           | Okhawa et al. [55]      |
| Rasool 2014 [35]     | PI     | µmol/mL | 18.72  | 5.56     | 45          | 8.5    | 2.83     | 30           | Okhawa et al. [55]      |
| Huo 2014 [38]        | Er     | nmol/g Hg | 164   |          | 25          | 144    |           | 25           | Okhawa et al. [55]      |
| Huo 2014 [38]        | Ti     | nmol/mg protein | 3   |          | 15          | 0.8    |           | 15           | Okhawa et al. [55]      |
| Shetty 2014 [33] *   | Sa     | nmol/mL | 0.931  | 0.03     | 50          | 0.181  | 0.03     | 65           | TBA-TCA                 |
| Bhat 2015 [39] *     | PI     | nmol/mL | 5.58   | 0.98     | 30          | 2.12   | 0.23     | 30           | Draper et al. [60]      |
| Rai S 2015 [40]      | PI     | nmol/mL | 13.16  | 0.55     | 20          | 2.92   | 0.36     | 20           | Satoh et al. [66]       |
| Thomas 2015 [67] *   | PI     | nmol/mL | 5.2    | 0.49     | 20          | 2.9    | 0.49     | 20           | Mahfouz et al. [68]     |
| Kaur 2015 [41] *     | Sa     | nmol/mL | 1.02   | 0.21     | 40          | 0.08   | 0.07     | 40           | Buege et al. [59]       |
| Shankaram 2015 [42] *| Sa     | nmol/mL | 5.94   | 0.9      | 25          | 4.43   | 0.81     | 25           | NWLSS NWK               |
| Mishra 2016 [43]     | Se     | nmol/mL | 14.15  | 0.47     | 20          | 2.92   | 0.36     | 20           | Satoh et al. [66]       |
| Nyamathi 2016 [44] * | Se     | nmol/mL | 13.22  | 2.4      | 10          | 3.4    | 0.56     | 10           | Satoh et al. [66]       |
| Srivastava K 2016 [45]| Ti   | nmol/mL | 87.53  | 2.65     | 20          | 127.9  | 2.97     | 20           | Okhawa et al. [55]      |
| Verma 2017 [46]      | PI     | µmol/mL | 3.38   | 0.14     | 20          | 2.45   | 0.13     | 20           | Sinnhuber et al. [69]   |
| Madhulatha 2017 [47] | Se     | nmol/mL | 4.34   | 1.69     | 25          | 2.97   | 1.09     | 25           | Gavino et al. [70]      |
| Benerjee 2017 [48]   | MI     | nmol/mg protein | 6.093 | 0.76     | 60          | 1.49   | 0.19     | 20           | Ogura et al. [71]       |
| Basu 2018 [49]       | PI     | nmol/mL | 20.35  | 4.15     | 30          | 13.94  | 2.51     | 50           | Yagi et al. [57]        |
| Arya 2019 [50]       | Se     | nmol/mL | 57     | 26.8     | 50          | 10.5   | 8.43     | 50           | Oxitek Assay kit        |
Table 2. Cont.

| Author                  | Sample | Unit     | OSCC       | Control       | Method                  |
|-------------------------|--------|----------|------------|---------------|-------------------------|
|                         | Sample | Unit     | Mean       | Std. Dev      | Sample Size            | Mean       | Std. Dev      | Sample Size |
| Sabarathinam 2019 [50]  | Sa     | µg/mg    | 2.7        | 0.15          | 10                      | 0.9        | 0.05          | 15          | Spectrophotometry |
| Babiuch 2019 [51]       | Sa     | nmol/L   | 8.58       | 6.23          | 20                      | 2.32       | 5.36          | 20          | Kit-My BioSource (USA) |
| Shahi 2020 [52]         | Pl     | µmol/mL  | 0.82       | 0.7           | 25                      | 0.39       | 0.2           | 45          | Nair et al. [72] |
| Oswal 2020 [53]         | Se     |          | 13.4       | 25            | 2.91                    | 30          |               |             | ELISA kit Sun Long Biotech |
| Abdelkawy 2020 [54] *   | Sa     | nmol/mL  | 3.62       | 0.61          | 20                      | 1.03       | 0.19          | 20          |               |

Abbreviations: Ti—Tissue, Se—Serum, Pl—Plasma, Er—Erythrocyte, Er memb—Erythrocyte Membrane, Mi—Mitochondria, Sa—Saliva, Std. Dev—Standard Deviation *—Studies used for Meta-analysis.

Table 3. The levels of MDA in various samples of patients with different clinical stages of OSCC.

| Author                  | Sample | Sample Size | Unit        | OSCC Stage II Mean | OSCC Stage III Mean | OSCC Stage IV Mean | Stat Sig | Clinical Stage Criteria |
|-------------------------|--------|-------------|-------------|-------------------|--------------------|-------------------|----------|------------------------|
| Manoharan 2005 [20]     | Pl     | 48          | nmol/mL     | 2.88              | 3.54               | 4.83              | <0.01    | Sobin et al. (UICC) [73] |
| Srivastava K 2012 [32]  | Pl     | 20          | nmol/mL     | 3.2               | 5.42               | 7.12              | <0.001   | TNM                    |
| Manoharan 2005 [20]     | Er     | 48          | pm/mg Hb    | 2.67              | 3.35               | 4.02              | <0.01    | Sobin et al. (UICC) [73] |
| Manoharan 2005 [20]     | Er memb| 48          | nmol/mg protein | 0.41 | 0.6               | 0.87              | <0.01    | Sobin et al. (UICC) [73] |
| Kolanjiappan 2003 [18]  | Ti     | 48          | nmol/100 mg protein | 105.4 | 94.3              | 80.51             | <0.01    | AJCC 1992 [74] |
| Srivastava K 2016 [32]  | Ti     | 20          | nmol/mL     | 89.64             | 88.1               | 85.72             | > 0.05   | TNM                    |
| Banerjee 2017 [48]      | Mi     | 60          | nmol/mg protein | 8.25 | 3.3               | 5.33              | 0.986    | TNM                    |
| Babiuch 2019 [51]       | Sa     | 20          | 10.5        | 8.22              | 8.7                | 8.59              | 7.57     | 4.16                   | 0.73      | T Stage |

Abbreviations: Ti—Tissue, Pl—Plasma, Er—Erythrocyte, Er memb—Erythrocyte Membrane, Mi—Mitochondria, Sa—Saliva, Std. Dev—Standard Deviation, Stat Sig—Statistical Significance.
Table 4. The levels of MDA in various samples of patients with different histopathological grades of OSCC.

| Author         | OSCC (WD) | OSCC (MD) | OSCC (PD) |
|----------------|-----------|-----------|-----------|
| Sample SIZE   | Unit      | Mean      | Std Dev   | Mean      | Std Dev   | Mean      | Std Dev   | Stat Sig | Histological Grade Criteria |
| Rai S 2015 [40] | Pl 20     | 12.98     | 0.67      | 13.34     | 0.42      | -         | -         | <0.001   | Akhter et al. [75]. |
| Chole 2010 [25] | Se 30     | 14.81     | 1.54      | 14.68     | 1.8       | 13.2      | 0.54      | >0.05    | |
| Nath 2014 [34] | Se 120    | 39.11     | 9.031     | 49.6      | 6.53      | 76.4      | 25.68     | <0.01    | Anneroth et al. [76]. |
| Metgud 2014 [12] | Se 40     | 6.12      | 0.36      | 5.92      | 0.49      | -         | -         | >0.05    | |
| Arya 2019 [8]  | Se 50     | 14.81     | 1.54      | 14.68     | 1.8       | 13.2      | 0.54      | >0.05    | Bryne et al. [74]. |
| Metgud 2014 [12] | Sa 40     | 0.33      | 0.035     | 0.325     | 0.024     | -         | -         | >0.05    | |

Abbreviations: Se—Serum, Pl—Plasma, Sa—Saliva, WD—Well Differentiation, MD—Moderate Differentiation, PD—Poor Differentiation, Std. Dev—Standard Deviation, Stat Sig—Statistical Significance.

MDA levels are significantly increased ($p < 0.00001$) in OSCC in the plasma, serum, and saliva samples of most of the studies evaluated. On the contrary, MDA levels of tissue samples are significantly decreased ($p < 0.00001$) in OSCC compared to healthy tissues, supported only by fewer studies. The plasma samples showed an overall mean difference of 2.81 with 95% CI (2.280–3.362) [Figure 2]. The serum samples showed an overall standard mean difference of 3.112 with 95% CI (2.478–3.746) [Figure 3]. The saliva samples showed an overall standard mean difference of 7.383 with 95% CI (4.354–10.413) [Figure 4]. The tissue samples showed an overall mean difference of $-36.671$ with 95% CI ($-41.197$ to $-32.145$) [Figure 5].

The levels of MDA in plasma samples between healthy controls and patients with OSCC

| Study name   | Difference in means | Standard error | Variance | Lower limit | Upper limit | Z Value | p Value | Relative weight |
|--------------|---------------------|----------------|----------|-------------|-------------|---------|---------|----------------|
| Su et al. 2002 [5] | 1.999               | 0.433          | 0.167    | 1.142       | 2.838       | 4.599   | 0.000  | 5.15            |
| Su et al. 2002 [4]  | 2.169               | 0.240          | 0.662    | 1.974       | 2.946       | 9.915   | 0.000  | 10.62           |
| Ikeda 2004 [17]     | 3.559               | 0.257          | 0.666    | 3.046       | 4.054       | 13.792  | 0.000  | 10.76           |
| Mihelcic 2005 [18]  | 1.680               | 0.220          | 0.648    | 1.213       | 2.091       | 7.546   | 0.000  | 11.63           |
| Ratner 2007 [19]     | 1.990               | 0.208          | 0.612    | 1.609       | 2.111       | 7.638   | 0.000  | 11.66           |
| Barut 2011 [21]       | 2.550               | 0.527          | 0.370    | 1.946       | 3.554       | 6.741   | 0.000  | 0.20            |
| Silvestri 2012 [22]  | 2.459               | 0.425          | 0.269    | 2.138       | 3.782       | 7.935   | 0.000  | 9.47            |
| Bhat 2015 [23]       | 3.460               | 0.184          | 0.634    | 3.100       | 3.820       | 18.826  | 0.000  | 11.27           |
| Thomas 2015 [30]     | 2.301               | 0.355          | 0.634    | 1.996       | 2.604       | 14.643  | 0.000  | 11.44           |
| Roca 2018 [25]       | 6.410               | 0.743          | 0.533    | 4.933       | 7.807       | 8.023   | 0.000  | 0.38            |
| 2.532               | 0.276          | 0.676    | 2.280       | 3.302       | 10.218  | 0.000  |                  |

Figure 2. Forest plot shows mean difference estimates with 95% confidence intervals representing differences in plasma levels of MDA between the oral squamous cell carcinoma group and healthy controls.
The levels of MDA in serum samples between healthy controls and patients with OSCC

| Study name | Statistics for each study | Std diff in means | Standard error | Variance | Lower limit | Upper limit | Z-Value | p-Value | Relative weight |
|------------|---------------------------|-------------------|---------------|----------|-------------|-------------|---------|---------|----------------|
| Chula 2010 [33] | 4.791 0.508 0.258 3.796 5.787 9.433 0.000 | 4.791 0.508 0.258 3.796 5.787 9.433 0.000 |
| Rauroya 2011 [36] | 2.117 0.279 0.078 1.570 2.664 7.500 0.000 | 2.117 0.279 0.078 1.570 2.664 7.500 0.000 |
| Sow 2013 [29] | 2.647 0.354 0.125 1.954 3.340 7.405 0.000 | 2.647 0.354 0.125 1.954 3.340 7.405 0.000 |
| Nath 2014 [37] | 2.356 0.217 0.047 1.911 2.751 10.764 0.000 | 2.356 0.217 0.047 1.911 2.751 10.764 0.000 |
| Ganesan 2013 [30] | 2.783 0.444 0.197 1.913 3.652 6.273 0.000 | 2.783 0.444 0.197 1.913 3.652 6.273 0.000 |
| Mergud 2014 [12] | 5.065 0.491 0.242 4.102 6.028 10.305 0.000 | 5.065 0.491 0.242 4.102 6.028 10.305 0.000 |
| Mali 2014 [31] | 2.188 0.296 0.087 1.608 2.768 7.398 0.000 | 2.188 0.296 0.087 1.608 2.768 7.398 0.000 |
| Nymathi 2016 [39] | 5.635 0.997 0.994 3.681 7.589 5.652 0.000 | 5.635 0.997 0.994 3.681 7.589 5.652 0.000 |
| Arya 2019 [8] | 3.112 0.323 0.105 2.478 3.746 9.619 0.000 | 3.112 0.323 0.105 2.478 3.746 9.619 0.000 |

Figure 3. Forest plot shows mean difference estimates with 95% confidence intervals representing differences in serum levels of MDA between oral squamous cell carcinoma group and healthy controls.

The levels of MDA in saliva samples between healthy controls and patients with OSCC

| Study name | Statistics for each study | Std diff in means | Standard error | Variance | Lower limit | Upper limit | Z-Value | p-Value | Relative weight |
|------------|---------------------------|-------------------|---------------|----------|-------------|-------------|---------|---------|----------------|
| Ganesan 2014 [30] | 5.069 0.649 0.421 3.797 6.341 7.811 0.000 | 5.069 0.649 0.421 3.797 6.341 7.811 0.000 |
| Netgud 2014 [12] | 5.156 0.498 0.248 4.180 6.133 10.349 0.000 | 5.156 0.498 0.248 4.180 6.133 10.349 0.000 |
| Shetty 2014 [47] | 2.260 3.150 2.258 19.663 25.553 15.946 0.000 | 2.260 3.150 2.258 19.663 25.553 15.946 0.000 |
| Kazir 2015 [50] | 5.070 0.516 0.266 4.067 6.060 11.390 0.000 | 5.070 0.516 0.266 4.067 6.060 11.390 0.000 |
| Shankararam 2015 [51] | 1.764 0.333 0.131 1.110 2.417 5.291 0.000 | 1.764 0.333 0.131 1.110 2.417 5.291 0.000 |
| Abdekewey 2020 [40] | 5.733 0.715 0.511 4.332 7.134 8.921 0.000 | 5.733 0.715 0.511 4.332 7.134 8.921 0.000 |
| 7.383 1.546 2.389 4.254 10.413 14.777 0.000 | 7.383 1.546 2.389 4.254 10.413 14.777 0.000 |

Figure 4. Forest plot shows mean difference estimates with 95% confidence intervals representing differences in salivary levels of MDA between oral squamous cell carcinoma group and healthy controls.
Figure 4. Forest plot shows mean difference estimates with 95% confidence intervals representing differences in salivary levels of MDA between oral squamous cell carcinoma group and healthy controls.

Figure 5. Forest plot shows mean difference estimates with 95% confidence intervals representing differences in tissue levels of MDA between oral squamous cell carcinoma group and healthy controls.

The meta-analysis presented high heterogeneity, reflected by the $I^2$ values 92.648, 86.785, 97.769, and 64.792 of Figures 2–4, respectively. The different methodologies utilized to measure MDA levels could be the reason for the high heterogeneity.

4. Discussion

Lipid peroxidation is a sequential reaction providing a constant supply of free radicals that initiate further peroxidation and free radicals accumulation, resulting in OS [77]. The endogenous formation of MDA during lipid peroxidation serves as a suitable biomarker of endogenous DNA damage [12]. MDA interacts with cellular DNA and forms MDA deoxyguanosine (M1-dG), a DNA-MDA covalently bonded adduct, resulting in DNA damage that causes interference in repair [78]. This mutagenic transformation within the DNA alters their chemical behavior and possibly contributing to carcinogenesis. These reactive aldehydes (MDA) also bind to membrane proteins. They cause profound changes in their function, tonicity, permeability, rigidity, structural integrity, and enhancing neoplastic transformation of the affected tissues. Thus, the developed OS affects the cell membrane’s essential constituents, which ultimately increases cell proliferation and actively influences cancer initiation, promotion, and progression [79].

The present systematic review included the research articles that involve 1307 patients diagnosed with OSCC and 1217 healthy volunteers for MDA analysis in various biological samples.

Previous studies demonstrated enhanced lipid peroxidation and malondialdehyde in patients with OSCC. The included studies had found a statistically significant increase in plasma or serum MDA levels in OSCC patients compared with controls ($p < 0.001$) [8,12,19–22,24,30,32,35–37,39,40,43,46,47,49,65]. Similarly, other studies also observed a significant rise compared with the control group ($p < 0.05$) [8,17,25,31,34,44,52,53,67]. Other studies also reported MDA rise in erythrocytes with statistical significance ($p < 0.001$) [20,26], ($p < 0.01$) [38] and ($p < 0.05$) [5,17,27]. On the contrary, one report did not show any change in blood MDA level in OSCC patients than in control [28]. In the present meta-analysis, the plasma samples showed an overall mean difference of 2.79 with a 95% CI (2.26–3.32). The serum samples showed an overall mean difference of 7.43 with 95% CI (5.99–8.87). The serological changes are consistent even though they are secondary to the tissue changes taking place anywhere in the body. A few studies had also reported higher salivary MDA levels in OSCC compared with healthy subjects with statistical
significance \( (p < 0.001) \) \[ 12,29,33,35,36,54 \] and \( (p < 0.05) \) \[ 23,41,42,50 \]. However, three included studies expressed that the increase in the MDA level in saliva and mitochondria was insignificant \( (p > 0.05) \) \[ 48,51 \]. In the present work, the saliva samples showed an overall mean difference of 0.91 with a 95\% CI \((0.63–1.18)\). The increased levels could be due to the disintegration of polyunsaturated fatty acids of bio-membranes due to oxidative lipid damage \[ 19 \]. The evaluation of tissue MDA level also showed a rise in OSCC patients than the control group with statistical significance \( (p < 0.001) \) \[ 36 \], \( (p < 0.01) \) \[ 38 \], and \( (p < 0.05) \) \[ 27 \]. On the contrary, few authors differently reported the tissue MDA levels of the OSCC group \[5,14,16,18,45\]. Their studies in tissue displayed a decrease in mean MDA level in OSCC patients compared to the control group with statistical significance. \( (p < 0.001) \) \[ 55–58 \] and \( (p < 0.05) \) \[ 5 \]. In the present analysis, the tissue samples showed an overall mean difference of \(-37.08\) with 95\% CI \((-41.25 \text{ to } -32.92)\). The decrease in MDA levels observed in the tumor tissues of oral cancer patients reflects a decreased susceptibility of oral tumor tissue to lipid peroxidation. Srivastava 2016 et al. hypothesized that serum biology compared to tissue poses a considerable threat and produces free radicals in excess amounts \[ 45 \]. They are readily diffused inside the cell to cause various mutations, favoring carcinogenesis. On the other hand, the tissue produces a relatively lesser amount of free radicals and, at the same time, is capable of counteracting them with the available enzymes. Therefore, Srivastava et al. stated that the external environment and the internal factors influence the selective growth of the tumor cells \[ 45 \].

There is a gradual increase in the MDA level in plasma and erythrocyte when the clinical stage of OSCC advances on further analysis. According to severity, the difference in the rise of plasma MDA levels between the advancing stages was statistically significant within all the clinical grades \( (p < 0.01) \) \[ 20 \] and \( (p < 0.001) \) \[ 32 \]. Arya et al. observed a significant increase in serum MDA value from T1 to T3 group, and the \( p \)-value was <0.05 \[ 8 \]. Therefore, a positive relationship between serum MDA level and tumor size was found. The authors stated that lipid peroxidation increases with the disease severity. Therefore, serological levels are reflecting the extent of tissue injury \[ 24 \].

In contrast, Babiuch et al. observed decreasing salivary MDA value when the tumor progresses from T1 to T4 in size, statistically insignificant \[ 51 \]. Two reported studies in tissue displayed a decreasing mean MDA level when the clinical stage of OSCC advances, which is statistically significant in one study \( (p < 0.01) \) \[ 18 \] and insignificant in another report \( (p > 0.05) \) \[ 45 \]. Few studies reported an increase in plasma and serum MDA level when histological grades of the disease advance with statistical significance \( (p < 0.001) \) \[ 40 \] and \( (p < 0.01) \) \[ 34 \]. On the contrary, three studies stated that lipid peroxidation level was inversely proportional to the degree of differentiation of OSCC as the grade advances. However, the change was statistically non-significant \( (p > 0.05) \) \[ 8,12,25 \]. These results correlated with Salzman et al. 2009, who showed a negative correlation of MDA and tumor grade \[ 80 \]. Thus, there was no definitive correlation pattern in lipid peroxidation between degrees of differentiation of malignant oral lesions. The expression of serum MDA levels in different histopathological grades exhibits a complex relationship. The present meta-analysis showed the MDA levels are significantly increased \( (p < 0.00001) \) in OSCC in all the samples of plasma, serum, and saliva except the tissue samples where MDA levels are significantly decreased \( (p < 0.00001) \) in OSCC compared to healthy tissues. The tissue-level changes with advancing clinical stages of the tumors were also very poorly explored. The authors used different methodologies to assess MDA levels in various biological samples \[ 55,57–64,66,68–72 \]. The reported studies utilized different clinical staging systems \[73,81\] and histopathological grading systems \[74–76\] to categorize the OSCC group patients. It will be worthwhile if future studies consider these facts in the MDA assessment of the OSCC group to evaluate the effect of oxidative stress on tumors. Although various treatments have been proposed to manage this type of cancer, its aggressiveness and ability to metastasize make this cancer one of the most difficult to treat, so early diagnosis is crucial when facing this condition \[ 82,83 \].
Therefore, the studies evaluating the OS will improve the understanding of the anti-oxidant enzyme activity in the early diagnosis and treatment of oral cancer [15].

5. Conclusions

The oxidant/anti-oxidant equilibrium is a critical step toward developing more effective strategies for prevention, early detection, and treatment of oral cancer. Estimating lipid peroxidation by-products in the OSCC group could assess the degree of oxidative stress-related tissue injury. Therefore, the assay of malondialdehyde level in oral cancer may be helpful to evaluate the disease severity for both preventive and clinical intervention. Most studies revealed the significant elevation of malondialdehyde levels in oral squamous cell carcinoma patients than healthy controls. Therefore, there is a requirement of large-scale studies with better-matched controls and equal distribution of samples among different clinical stages and histological grades of OSCC to conclude MDA as a potential biomarker for oxidative stress and valid prognostic marker of OSCC.

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