UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
FACULDADE DE ODONTOLOGIA DE ARAÇATUBA

JORDANA RESENDE MARTINS

BIOMARCADORES SALIVARES DO ESTRESSE OXIDATIVO EM CRIANÇAS COM CÁRIE DENTÁRIA: REVISÃO SISTEMÁTICA E META-ANÁLISES

ARAÇATUBA - SP
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Dissertação apresentada à Faculdade de Odontologia de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP para obtenção do título de Mestre em Ciência Odontológica, área de concentração em Saúde Bucal da Criança.

Orientadora: Profa. Assoc. Cristina Antoniali Silva

Coorientador: Prof. Assoc. Juliano Pelim Pessan

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2021

Dedico a Deus que esteve comigo em todos os meus momentos de tristeza, aflito e desespero, sempre sendo a minha força, foi um importante guia em minha trajetória.

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"Aqueles que passam por nós, não vão sós, não nos deixam sós. Deixam um pouco de si, e levam um pouco de nós."

- Antoine de Saint-Exupéry.

"*Sem sonhos a vida é uma manhã sem orvalhos, um céu sem estrelas, um oceano sem ondas, uma vida sem aventura, uma existência sem sentido.*"

- Augusto Cury

RESUMO

Objetivo. Avaliar a relação entre biomarcadores salivares de estresse oxidativo e cárie dentária em crianças. **Métodos.** Estudos realizados em crianças de até 12 anos comparando biomarcadores salivares de estresse oxidativo como malondialdeído (MDA), superóxido dismutase (SOD), ácido úrico, capacidade antioxidante total (TAC) e proteína total, considerando crianças com lesões de cárie dentária e sem cárie foram selecionados. Além disso, parâmetros salivares como fluxo salivar, pH, capacidade tampão e níveis de cálcio foram avaliados. Uma revisão sistemática da literatura foi realizada em 8 bases de dados. A diferença média padronizada (SMD) foi medida usando variância inversa como método estatístico e efeitos aleatórios como modelo de análise, correspondendo a um intervalo de confiança (IC) de 95%. **Resultados.** Os níveis de TAC foram maiores em crianças afetadas por cárie dentária em comparação com as sem cárie (grupo controle), independentemente da idade (SMD 2,66; IC 1,33; 3,98) ou sexo (SMD 0,98; IC 0,56; 1,39). Quando ajustados para proteína normalizada, os níveis de MDA foram menores no grupo de cárie dentária do que no grupo controle (SMD -16,5; IC -29,02; -4,00), e os níveis de SOD foram maiores no grupo de cárie dentária (SMD 5,09; IC 0,01; 10,18). A concentração de proteína total na saliva de crianças com cárie dentária foi maior do que no grupo controle, independentemente da idade (SMD 0,98; IC 0,27; 1,69) ou sexo (SMD 0,77; IC 0,45; 1,10). Os parâmetros salivares avaliados apresentaram níveis mais baixos em crianças com cárie dentária (p<0,05). **Conclusões.** Os níveis de biomarcadores de estresse oxidativo e parâmetros salivares estão alterados na saliva de crianças com cárie dentária.

Palavras-chave: Biomarcadores. Cárie Dentária. Estresse Oxidativo. Saliva. Proteínas e Peptídeos Salivares.

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ABSTRACT

Objective. To assess the relationship between salivary biomarkers of oxidative stress and dental caries in children. **Methods.** Studies conducted in children up to 12 years old comparing salivary biomarkers of oxidative stress such as malondialdehyde (MDA), superoxide dismutase (SOD), uric acid, total antioxidant capacity (TAC), and total protein, considering children with dental caries lesions and caries-free ones were selected. In addition, salivary parameters such as salivary flow, pH, buffering capacity, and calcium levels were evaluated. A systematic literature review was carried out in 8 databases. The standardized mean difference (SMD) was measured using inverse variance as a statistical method and random effects as an analysis model, corresponding to a 95% confidence interval (CI). **Results.** The TAC levels were higher in children affected by dental caries compared to caries-free ones (control group), regardless of age (SMD 2.66; CI 1.33; 3.98), or gender (SMD 0.98; CI 0.56; 1.39). When adjusted for normalized protein, MDA levels were lower in the dental caries group than in the control group (SMD -16.51; CI -29.02; -4.00), and SOD levels were higher in the dental caries group (SMD 5.09; CI 0.01; 10.18). The total protein concentration in saliva of children with dental caries was higher than in the control group, regardless of age (SMD 0.98; CI 0.27; 1.69), or gender (SMD 0.77; CI 0.45; 1.10). The salivary parameters assessed had lower levels in children affected by dental caries ($p<0.05$). **Conclusions.** The levels of oxidative stress biomarkers and salivary parameters are altered in saliva of children with dental caries.

Keywords: Biomarkers. Dental Caries. Oxidative Stress. Saliva. Salivary Proteins and Peptides.

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LISTA DE ABREVIATURAS

CI   Confidence Interval

ICCMS™   "*International Caries Classification and Management System",* Sistema Internacional de Classificação e Gerenciamento de Cárie

ICDAS   "*International Caries Detection and Assessment System",* Sistema Internacional de Detecção e Avaliação da Cárie

MDA   *"Malondialdehyde"*, Malonaldeído

NOS   Newcastle Ottawa Scale

ROS   *"Reactive Oxygen Species"*, Espécies Reativas de Oxigênio

SMD   Standardized Mean Difference

SOD   *"Superoxide Dismutase"*, Superóxido Dismutase

TAC   "*Total Antioxidant Capacity*", Capacidade Antioxidante Total

SUMÁRIO

1 INTRODUÇÃO GERAL[*]

A cárie dentária é uma doença de alta prevalência e que acomete pacientes de várias faixas etárias, sendo um problema de saúde pública global (Kawashita; Kitamura; Saito, 2011; Colak *et al.*, 2013; Albino; Tiwari, 2015; Anil; Anand, 2017; Manton, 2018). Esta doença é mediada por biofilme, modulada pela dieta, de origem multifatorial, não transmissível e dinâmica, a qual resulta na perda de minerais dos tecidos dentais (Machiulskiene *et al.*, 2020).

O diagnóstico de cárie é o julgamento clínico integrado das informações disponíveis, incluindo a detecção e avaliação de sintomas e sinais relacionadas ao desenvolvimento das lesões, determinando-se assim a presença da doença (Machiulskiene *et al.*, 2020). Dado que os métodos de diagnóstico da cárie devem capturar com precisão as manifestações em qualquer momento do processo de desenvolvimento, os métodos convencionais como visual, táctil e radiográfico ainda apresentan limitações (Hoskin; Keenan, 2016). Os métodos de diagnóstico apesar dos avances tecnológicos na área, continuam sendo subjetivos e dependentes da experiência do pesquisador (Brouwer *et al.,* 2016; Hoskin; Keenan, 2016). Com intuito de colaborar com a prática clínica, outros métodos de detecção da cárie, como a avaliação de biomarcadores salivares tem sido estudada e estabelecida como possíveis ferramentas para auxiliar/complementar o diagnóstico.

A saliva humana é um fluido biológico que contém uma mistura de secreções de glândulas salivares maiores e menores, além de substâncias de fontes não glandulares (Cunha-Cruz *et al.,* 2013). A saliva também contém hormônios, anticorpos, fatores de crescimento, enzimas e microrganismos, podendo, dessa maneira, ser vista, em muitos casos, como um reflexo da função fisiológica/patológica do corpo (Javaid *et al.,* 2016). Este fluido executa múltiplas funções como a limpeza e lubrificação dos tecidos bucais, redução da solubilidade, efeito tampão, além de atividade antibacteriana (Senthil Eagappan *et al.,* 2016).

Além disso, a saliva é constituída por diversos componentes, incluindo sódio, potássio, cálcio, magnésio, bicarbonato e fosfatos. O cálcio, o fosfato e as proteínas

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[*] Referências da Introdução Geral - ANEXO E

atuam em conjunto, sendo um fator de anti-solubilidade e responsáveis por modular a desmineralização e a remineralização (Humphrey; Williamson, 2001). A remineralização do esmalte é ocasionada pelas altas concentrações salivares do cálcio e do fosfato, sendo estas mantidas pelas proteínas salivares (Roth; Calmes, 1981). As proteínas salivares podem ter efeitos inibitórios contra o desenvolvimento do processo de cárie, principalmente devido à sua atividade sequestradora de radicais livres. Assim, a avaliação da proteína salivar total pode ser representativa na defesa contra doenças bucais (Dodwad, Betigeri, Preeti, 2011).

Com a presença de carboidratos na cavidade bucal, ocorre o processo de fermentação por bactérias cariogênicas, levando à redução do pH da placa bacteriana, resultando na desmineralização do dente. Por outro lado, a ação mecânica do fluxo salivar atua de forma preventiva neste processo (Lenander-Lumikari; Loimaranta, 2000), pois a capacidade tampão da saliva é capaz de restituir o pH salivar e favorecendo a remineralização dos tecidos dentários (Fenoll-Palomares et al., 2004).

A saliva tem sido empregada na detecção de doenças sistêmicas e orais, pois contém biomarcadores que podem ser analisados e quantificados (Spielmann; Wong, 2011; Malamud et al., 2011; Senthil Eagappan et al., 2016; Hassaneen; Maron, 2017). Além disso, a coleta da saliva é um método não invasivo, de fácil execução, seguro, de baixo custo. Entre todos os pacientes de clínicas odontológicas, os que mais se beneficiam com a praticidade desse método de coleta são os pacientes da clínica de odontopediatria, por ser rápido, de fácil coleta e indolor (Hassaneen; Maron, 2017).

A avaliação de marcadores salivares do dano oxidativo tem sido utilizada para o diagnóstico de doenças que afetam a cavidade oral (Buczko; Zalewska; Szarmach, 2015; Darczuk et al., 2016; Silva et al., 2016; Arana et al., 2017; Araujo et al., 2020), uma vez que estaria envolvido com o aparecimento e/ou desenvolvimento de doenças mediadas por biofilme como a cárie dentária (Mahjoub et al., 2014). O dano oxidativo é consequente do estresse oxidativo definido pelo aumento da concentração de espécies reativas de oxigênio ou nitrogênio, associada ou não com a redução dos sistemas antioxidantes (Betteridge, 2000). Entre os principais biomarcadores de dano oxidativo se encontram o malonaldeído (MDA) como produto

final estável da peroxidação dos lipídios da membrana, o 8-hydroxy-desoxguanosine (8-Hodgkins) e a proteína carbonilada (Jurczak *et al.,* 2017; Tartaglia *et al.,* 2017).

Os sistemas de defesa antioxidante são de alta complexidade, tendo como função mais importante controlar as bactérias orais que formam a placa dentária e levam ao desenvolvimento de cárie dentária e doenças periodontais inflamatórias crônicas (Tulunoglu; Demirtas; Tulunoglu*,* 2006). Os sistemas antioxidante salivares podem se classificar como enzimáticos e não enzimáticos (Jurczak *et al.,* 2017; Tartaglia *et al.,* 2017).

Entre os enzimáticos encontram-se glutationa peroxidase, catalase e superóxido dismutase (SOD), sendo esta última a principal enzima no efeito antioxidante (Jurczak *et al.,* 2017; Tartaglia *et al.,* 2017). A atividade da SOD catalisa a dismutação do ânion- radical superóxido ($O2^{\bullet-}$) em oxigênio e peróxido de hidrogênio, convertendo-o assim, em uma espécie menos reativa (Halliwell, 1999), e adicionalmente a atividade aumentada da SOD na saliva acrescentaria a biodisponibilidade do óxido nítrico favorecendo a sua atividade anticariogênica.

Por outro lado, temos os biomarcadores do sistema antioxidante não enzimáticos que incluem o ácido úrico, a glutationa, entre outros (da Silva *et al.*, 2016). A capacidade antioxidante total (TAC) se dá através da ação e atividade de todos os sistemas antioxidantes não enzimáticos (Battino *et al.*, 2002).

Estudos tem mostrado um comportamento singular na resposta antioxidante ao estresse oxidativo em crianças com cárie dentária, apresentando valores maiores do TAC e SOD neste grupo quando comparado com crianças saudáveis num ambiente onde o MDA está diminuído (Araújo *et al.*, 2020; Silva *et al.*, 2016). Esses dados sugerem a existência de um mecanismo compensatório entre o sistema antioxidante na redução do dano oxidativo (Silva *et al.*, 2016). Por outro lado, existem estudos apontando que não tem diferenças entre os biomarcadores salivares comparando crianças com e sem a doença (Subramanyam *et al.,* 2018; Tulunoglu; Demirtas; Tulunoglu*,* 2006). Com o objetivo de abordar a complexa dinâmica entre os biomarcadores salivares de estresses oxidativo e a cárie dentária, a presente revisão sistemática será conduzida.

2 MANUSCRITO†

SALIVARY BIOMARKERS OF OXIDATIVE STRESS IN CHILDREN WITH DENTAL CARIES: SYSTEMATIC REVIEW AND META-ANALYSIS

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2.1 Introduction

The evaluation of salivary biomarkers of oxidative stress has been used for the diagnosis of several diseases in the oral cavity of children (Arana et al., 2017; Araujo, Nakamune, Garcia, Pessan, & Antoniali, 2020; Buczko, Zalewska, & Szarmach, 2015; Darczuk et al., 2016; Silva, Troiano, Nakamune, Pessan, & Antoniali, 2016), since they may be involved in the onset and/or development of biofilm-mediated diseases, such as dental caries (Mahjoub, Ghasempour, Gharage, Bijani, & Masrourroudsari, 2014). In addition, saliva collection is characterized as a non-invasive, straightforward, safe, inexpensive, fast and painless method (Hassaneen & Maron, 2017).

Oxidative damage is a consequence of oxidative stress, which is defined by the increased concentration of reactive oxygen and nitrogen species, associated or not with the reduced activity of antioxidant systems (Betteridge, 2000). Among main markers of oxidative damage are malondialdehyde (MDA), as a stable end product of membrane lipid peroxidation, and 8-hydroxy-desoxguanosine (8-Hodgkins) (Jurczak et al., 2017). In fact, antioxidant systems are highly complex, having as an important function the protection against the effects of reactive oxygen species (ROS) (Pyati, Naveen Kumar, Kumar, Praveen Kumar, & Parveen Reddy, 2018; Tulunoglui, Demirtas, & Tulunoglu, 2006). Salivary antioxidant systems can be classified as enzymatic and non-enzymatic (Jurczak et al., 2017). Glutathione peroxidase, catalase and superoxide dismutase (SOD) are examples of enzymatic systems, with SOD being the main antioxidant enzyme (Jurczak et al., 2017). In contrast, the non-enzymatic antioxidant system includes the uric acid and the glutathione, which together make up the total antioxidant capacity (TAC) (Battino, Ferreiro, Gallardo, Newman, & Bullon, 2002).

Studies have shown a unique behavior regarding salivary antioxidant response to oxidative stress in children with dental caries, with increased TAC and SOD levels in this group when compared to caries-free children, in an environment where MDA is decreased (Araujo et al., 2020; Silva et al., 2016). On the other hand, other studies suggest no differences in biomarker levels between children with and without the disease (Subramanyam, Gurunathan, Gaayathri, & Priya, 2018; Tulunoglui et al., 2006). In view of the conflicting evidence described above, the present systematic

review with meta-analysis aimed to assess the relationship between salivary biomarker levels related to oxidative stress, as well as salivary parameters related to dental caries in children with or without dental caries. The question review was structured as follows: Could salivary biomarkers associated with oxidative stress be altered in saliva of children with dental caries?

2.2 Materials and Methods

Search

The systematic literature search was carried out by two independent researchers (JRM and BDF) according to the eligibility criteria in PubMed, Scopus, Web of Sciences, Embase, Cochrane Library, Lilacs, Google Scholar (first hundred results), and Open Grey databases. In addition, the researchers (JRM and WRC) performed a search through the reference lists of included studies. Mendeley Desktop 1.19.8 was used as reference manager. Any disagreement was resolved by consensus and with the help of a third researcher (CA). Search terms included were "Child", "Child, Preschool", "Dental caries", "Biomarkers", "Saliva", and "Oxidative Stress" (Supplement 1: Search strategy).

Eligibility criteria and studies selection

Studies were selected in the first search phase, according to the following criteria: children up to 12 years old as study population; dental caries as exposure; caries-free condition as control; and salivary biomarkers of oxidative stress as main outcome. Furthermore, salivary flow rate, pH, buffer capacity, and calcium concentration were assessed. Also, the eligibility criteria included observational studies written in English, Spanish and Portuguese, and published before March 10th 2021 (last update). In the second selection phase, mentally and physically compromised children, who use medication or who had systemic or local diseases, that may alter biomarkers of oxidative stress were considered as exclusion criteria.

Data collection process

The data collection process was carried out by two independent researchers in duplicate (JRM and BDF). Any doubts or disagreements were resolved by consensus. In the case of any incomplete or missing information in the studies included, contact with the author of the articles was done by e-mail. The data collected were: authors, year and country of publication, study design, ages, gender, data from both the exposure and control groups, main results on the biomarkers/salivary parameters, study limitations, conflicts of interest and funding source.

Risk of bias in studies

The risk of bias was performed by the version modified of Newcastle Ottawa Scale (NOS) for cross-sectional studies (Modesti et al., 2016). Those tool assessed selection, comparability and outcome process according to bias of cross-sectional studies. This process was carried out by two researchers in duplicate and individually (JRM and WRC). Doubts or disagreements were resolved by consensus. Regarding the risk of bias, individual studies were assessed as low risk (≥ 7 stars) or high risk (< 7 stars) (Islam et al., 2016). Studies with high risk of bias were excluded from meta-analysis.

Data analysis

For data analysis, the mean and standard deviation of the salivary biomarkers and salivary parameters assessed were collected from the articles studied, as well as the total number of participants in both control and caries groups, and they were pooling according to the biomarker and the parameter. Data with different units of measure were converted to compatible units of measure for the analysis. Standardized Mean Difference (SMD) was measured using Inverse Variance as statistical method and the Random-Effects as analysis model, with 95% of confidence interval (CI). The chi-square ($p<0.10$) test and I^2 statistic were used to assess the heterogeneity in the studies. The overall effect was assessed using the Z statistic at a 5% significance level. The meta-analysis was performed using the software Review Manager 5.4. Publication bias and small study effects were

assessed by funnel plot graphical and Egger' regression test for any analyses that included at least 10 studies (p<0.10). The certainty of evidence for each outcome was assessed by the software GRADEpro (GRADEpro; https://gradepro.org/).

2.3 Results

Study selection

For the selection of studies, the electronic databases mentioned above were used. A total of 6,632 articles were recovered, out of which 1,032 were duplicates, and only 22 were selected following the eligibility criteria. After evaluating the titles, abstracts and full texts, two articles were excluded due to the assessment of a different exposure (dental abscess), and dichotomization of groups in a different standard. In addition, two articles had duplicate data (Supplement 2: Studies excluded and their causes). Thus, 18 studies were included in the systematic review, and 14 studies included in the meta-analysis. The reasons for exclusion of 4 studies from the meta-analysis include a high variability in the evaluation methods of salivary biomarkers (Banda, Singh, & Markam, 2016), unmatched biomarkers (Syed, Sachdev, & Chopra, 2016), and missing data (Jurczak et al., 2017; Shaki, Arab-Nozari, Maleki, Charati, & Nahvi, 2020) (Figure 1).

Study characteristics

From 18 studies selected, all were classified as cross-sectional studies, out of which eleven were from India (Banda et al., 2016; Geethika, Mathew, Priya, & Gayathri, 2019; Hegde, Neekhra, & Shetty, 2008; Hegde, Rai, & Padmanabhan, 2009; Kumar, Hedge, & Dixit, 2011; Muchandi, Walimbe, Bijle, Nankar, Chaturvedi, & Karekar, 2015; Pandey, Reddy, Rao, Saxena, & Chaudhary, 2015; Prabhakar, Dodawad, & Os, 2009; Pyati et al., 2018; Subramanyam et al., 2018; Syed et al., 2016), three from Brazil (Araujo et al., 2020; Farghaly, Fachin, Otton, Guaré, & Leite, 2013; Silva et al., 2016), two from Iran (Mahjoub et al., 2014; Shaki et al., 2020), one from Turkey (Tulunoglui et al., 2006) and one from Poland (Jurczak et al., 2017). The total number of children assessed with dental caries was 702, and the total number of children in the control group were 625. The studies included children up to 12

years old, comprising different age groups: up to 5 years (12 studies), 6 to 12 years (8 studies), and 4 to 6 years (one study). The World Health Organization, ICDAS and ICCMS^{TM} caries indices were assessed in the studies. For the meta-analyses, the caries-free group did not include teeth with white spot lesions (ICDAS scores 1 and 2), and the caries group only included dental caries with dentin cavitation (ICDAS scores 5 and 6), in order to normalize the indices. The salivary biomarkers of oxidative stress analysed were: TAC (14 studies), MDA (4 studies), SOD (3 studies), uric acid (2 studies), nitric oxide (3 studies), and total protein (10 studies). In addition, the salivary parameters evaluated were salivary flow unstimulated (6 studies), pH (6 studies), buffer capacity (4 studies) and calcium concentration (3 studies). Different analytical methods were used for the evaluation of salivary biomarkers were detected among studies, which are shown in details in supplement (Supplement 3: Characteristics of included studies).

Risk of bias in studies

The analysis of risk of bias resulted in studies with low risk of bias according to the NOS. This analysis also found deficiencies in the population samples, as the lack of representativeness (n= 9) and the non-justification for the size sample (n= 16) (Supplement 4: Risk of bias in individual studies).

Meta-analysis

Higher TAC levels were observed for children affected by dental caries compared to caries-free (control group) ones, regardless of age (up to 5 years old versus 6 to 12 years old; SMD 2.66, CI 1.33, 3.98, I^2 97%, p<0.01) (Figure 2A), or gender (female versus male; SMD 0.98, CI 0.56, 1.39, I^2 38%, p<0.01) (Figure 2B). MDA, SOD and UA levels were not significantly different between the groups assessed (Figure 3A, Figure 4A, and Figure 5). However, when adjusted or normalized by protein concentration, the caries group had significantly higher MDA levels (SMD -16.51, CI -29.02, -4.00, I^2 96%, p=0.01) (Figure 3B), and lower SOD levels compared to the control group (SMD 5.09, CI 0.01,10.18, I^2 92%, p=0.05) (Figure 4B). Furthermore, the total protein concentration in saliva of children with

dental caries were higher than caries-free group, regardless of age (up to 6 years old versus 6 to 12 years old; SMD 0.98, CI 0.27, 1.69, I^2 89%, p<0.01) (Figure 6A), or gender (female versus male; SMD 0.77, CI 0.45, 1.10, I^2 0%, p<0.01) (Figure 6B).

The subgroup analysis showed a high homogeneity for gender (Figure 2B and Figure 6B), but not for children up to 6 years old (p<0.01) comparing to those between 6 to 12 years old (Figure 2A and Figure 6A). On the other hand, when salivary parameters as salivary flow rate (unstimulated), pH, buffer capacity and calcium concentration were assessed, the results showed that all these parameters had significantly lower levels in the caries group (p < 0.05), without subgroups differences (Table 1) (Supplement 5: Forest plot of salivary parameters).

Publication bias and certainty of evidence

It was not possible to evaluate publication bias due to the number of studies for analysis (less than 10). The certainty of evidence was moderate and low for the primary and the secondary outcomes (Supplement 6: Summary of findings and certainty of evidence).

2.4 Discussion

Among the biomarkers of the antioxidant system analyzed in this study, TAC and SOD showed higher levels in the group of children with dental caries compared with caries-free ones, regardless of the assessed age range and gender. A similar trend was observed for the concentrations of total proteins in saliva. On the other hand, for the oxidative damage biomarker MDA, salivary flow, pH, buffering capacity and calcium concentrations, significantly lower values were observed for the caries group.

Caries disease alters the balance between ROS production and antioxidant systems. A shift in balance in favor of oxidative damage has been associated with the development of several oral infectious diseases (Battino, Bullon, Wilson, & Newman, 1999). However, salivary antioxidant response varies according to different oral diseases, as the total antioxidant capacity was shown to be reduced in

periodontal diseases (Diab-Ladki, Pellat, & Chahine, 2003), but increased in dental caries (Ahmadi-Motamayel, Goodarzi, Hendi, Kasraei, & Moghimbeigi, 2013). Furthermore, changes in salivary concentration of ROS could impair the antibacterial action of saliva (Kamodyová, Červenka, & Celec, 2015), thus preventing the control of bacteria and therefore, favoring tooth decay.

A strong positive correlation between the increased antioxidant systems activity (TAC, uric acid and SOD) and different stages of dental caries progression was described in a recent study in toddlers (Araujo et al., 2020), suggesting that the higher caries severity, the higher salivary antioxidant system activity, with a consequent reduction in salivary oxidative damage or MDA. These data seems to support the hypothesis that the organism may develop an adaptive response to the disease and, following this rationale, the decrease in oxidative damage in saliva of children with caries could be a consequence of the increased activity of antioxidant systems, both enzymatic (SOD) and non-enzymatic (TAC, uric acid) (AlAnazi, Pani, & AlKabbaz, 2018; Silva et al., 2016).

The existence of a possible association between dental caries, age and salivary proteins concentration has been assessed in the literature (Farghaly et al., 2013). Although the age has been shown to determine variations in salivary protein concentrations in subjects spanning a wide age range (Tappuni, & Challacombe, 1994), the results in the present review did not find such a trend, both for children up to 6 years old or 6 to 12 years old. Based on the above, it may be advised that salivary biomarkers levels should be normalized by the protein concentration. This could help to overcome issues related to the influence of age (especially for study groups with wide age range, or when comparing different age groups) and related to the variability of analytical methods for assessment of salivary biomarkers.

Another aspect that deserves comment is that the concentration of salivary proteins is increased in children affected by dental caries (Araujo et al., 2020; Mahjoub et al., 2014; Pandey et al., 2015; Prabhakar et al., 2009; Pyati et al., 2018; Silva et al., 2016; Tulunoglui et al., 2006). Increased protein concentrations are eminent in children with a higher prevalence of *Streptococcus mutans*, which could suggest a response to the infectious nature of severe dental caries in contrast to the presence of the disease in its early stages (Koga-Ito, Martins, Balducci, & Jorge,

2004).

In caries disease, salivary parameters are involved in the development of the process, in which pH drops in saliva lead to increases in its acidity, allowing a favorable environment for cariogenic oral biofilms formation (Hurlbutt, & Young, 2014), and promoting demineralization of tooth enamel (Shetty, Hegde, & Darshana, 2013). On the other hand, saliva's ability to remineralize tooth enamel depends on several factors, including salivary proteins, buffering capacity, enzymes, as well as fluoride, phosphorus and calcium ions (Farooq, & Bugshan, 2020). However, calcium precipitation in tooth enamel is impaired after sugar intake and, consequently, a drop in biofilm/salivary pH (<5.5) is maintained for long periods. In this situation, saliva cannot fully replenish enamel calcium, causing demineralization (Neel et al., 2016). Consequently, decreased calcium values are found in the saliva of children with dental caries (Machiulskiene et al., 2020), what is in line with the results from the present review. The buffering capacity of saliva neutralizes acids and increases salivary pH, as salivary flow increases, what changes salivary composition (Buzalaf et al., 2012). Buffering capacity tends to be lower in children with carious lesions compared to those without the disease, as shown in the results of the meta-analysis.

This is the first systematic review that addresses changes in salivary oxidative stress biomarkers in children with dental caries, compared with caries-free ones. The results obtained in the meta-analyses involving different salivary biomarkers/parameters may be useful to guide future studies in the fields of cariology and oxidative stress. Specifically, the results showing that salivary oxidative stress biomarkers are altered by caries could be used for the development of new tools to assist the diagnosis.

Although the studies evaluated do not present a considerable risk of bias, their level of evidence is not high. Considering that they are cross-sectional studies, they have limitations inherent to the methodology, especially due to the fact that data were collected a single time in the timeline (Sedgwick, 2015). With respect to this limitation, we cannot determine with certainty that oxidative damage decreases as a direct consequence of the increased action of the antioxidant system in a cause-effect relationship, but it is possible to theorize the existence of a possible association between both processes.

In conclusion, considering moderate and low certainty of the evidence, the levels of oxidative stress biomarkers and salivary parameters are altered in saliva of children with dental caries. Antioxidant system biomarkers (TAC and SOD) and total protein concentration were shown to be higher in children affected by the disease. On the other hand, the salivary oxidative damage biomarker (MDA), and the salivary parameters of flow rate, pH, buffering capacity and calcium concentration showed reduced values in children with caries lesions. Thus, it might be suggested that there is an influence of caries disease on the levels of oxidative stress biomarkers.

Other information

The systematic review was carried out according to the Preferred Reporting Items for Systematic Review and Meta-analyses PRISMA guidelines (Page et al., 2021). It was registered in PROSPERO (March 10th, 2021; No CRD42021241894).

2.5 References

Ahmadi-Motamayel, F., Goodarzi, M. T., Hendi, S. S., Kasraei, S., & Moghimbeigi, A. (2013). Total antioxidant capacity of saliva and dental caries. Medicina oral, patologia oral y cirugia bucal, 18(4), e553–e556. https://doi.org/10.4317/medoral.18762.

AlAnazi, G. S., Pani, S. C., & AlKabbaz, H. J. (2018). Salivary antioxidant capacity of children with severe early childhood caries before and after complete dental rehabilitation. Archives of oral biology, 95, 165–169. https://doi.org/10.1016/j.archoralbio.2018.08.002.

Arana, C., Moreno-Fernández, A. M., Gómez-Moreno, G., Morales-Portillo, C., Serrano-Olmedo, I., ... Martín Hernández, T. (2017). Increased salivary oxidative stress parameters in patients with type 2 diabetes: relation with periodontal disease. *Endocrinologia, Diabetes y Nutricion,* 64(5), 258–264. https://doi.org/10.1016/j.endinu.2017.03.005.

Araujo, H. C., Nakamune, A. C. M. S., Garcia, W. G., Pessan, J. P., & Antoniali, C. (2020). Carious lesion severity induces higher antioxidant system activity and consequently reduces oxidative damage in children's saliva. *Oxidative Medicine and Cellular Longevity*, 2020, 3695683. https://doi.org/10.1155/2020/3695683.

Banda, N. R., Singh, G., & Markam, V. (2016). Evaluation of total antioxidant level of saliva in modulation of caries occurrence and progression in children. *Journal of the Indian Society of Pedodontics and Preventive Dentistry*, 34(3), 227–232. https://doi.org/10.4103/0970-4388.186747.

Battino, M., Bullon, P., Wilson, M., & Newman, H. (1999). Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. *Critical Reviews in Oral Biology and Medicine,* 10(4), 458–476. https://doi.org/10.1177/10454411990100040301.

Battino, M., Ferreiro, M. S., Gallardo, I., Newman, H. N., & Bullon, P. (2002). The antioxidant capacity of saliva. *Journal of Clinical Periodontology*, 29(3), 189–194. https://doi.org/10.1034/j.1600-051x.2002.290301x.x.

Betteridge, D. J. (2000). What is oxidative stress? *Metabolism*, 49(2 Suppl 1), 3–8.

https://doi.org/10.1016/s0026-0495(00)80077-3.

Buczko, P., Zalewska, A., & Szarmach, I. (2015). Saliva and oxidative stress in oral cavity and in some systemic disorders. *Journal of Physiology and Pharmacology*, 66(1), 3–9.

Buzalaf, M. A. R., Massaro, C. S., Rodrigues, M. H. C., Fukushima, R., Pessan, J. P., ... Sampaio, F. C. (2012). Validation of fingernail fluoride concentration as a predictor of risk for dental fluorosis. *Caries Research*, 46(4), 394–400. https://doi.org/10.1159/000339088.

Darczuk, D., Krzysciak, W., Vyhouskaya, P., Kesek, B., Galecka-Wanatowicz, D., Lipska, W., … Chomyszyn-Gajewska, M. (2016). Salivary oxidative status in patients with oral lichen planus. *Journal of Physiology and Pharmacology*, 67(6), 885–894.

Diab-Ladki, R., Pellat, B., & Chahine, R. (2003). Decrease in the total antioxidant activity of saliva in patients with periodontal diseases. *Clinical Oral Investigations*, 7(2), 103–107. https://doi.org/10.1007/s00784-003-0208-5.

Farghaly, J. G., Fachin, L. V, Otton, R., Guaré, R. O., & Leite, M. F. (2013). Effect of gender on dental caries and salivary parameters of children. *Pesquisa Brasileira Em Odontopediatria e Clinica Integrada*, 13(1), 11–15. https://doi.org/10.4034/PBOCI.2013.131.02.

Farooq, I., & Bugshan, A. (2020). The role of salivary contents and modern technologies in the remineralization of dental enamel: a narrative review. *F1000Research*, 9, 171. https://doi.org/10.12688/f1000research.22499.3.

Geethika, B., Mathew, M. G., Priya, V., & Gayathri, R. (2019). Evaluation and comparison of salivary superoxide dismutase and zinc levels in children with early childhood caries and caries-free children. *Drug Invention Today*, *11*, 189–191.

Hassaneen, M., & Maron, J. L. (2017). Salivary diagnostics in pediatrics: applicability, translatability, and limitations. *Frontiers in Public Health*, 5, 83. https://doi.org/10.3389/fpubh.2017.00083.

Hegde, A. M., Neekhra, V., & Shetty, S. (2008). Evaluation of levels of nitric oxide in

saliva of children with rampant caries and early childhood caries: A comparative study. *Journal of Clinical Pediatric Dentistry*, 32(4), 283–286. https://doi.org/10.17796/jcpd.32.4.4010kl5262687528.

Hegde, A., Rai, K., & Padmanabhan, V. (2009). Total antioxidant capacity of saliva and its relation with early childhood caries and rampant caries. *Journal of Clinical Pediatric Dentistry*, *33*(3), 231–234. https://doi.org/10.17796/jcpd.33.3.c730518021m56077.

Hurlbutt, M., & Young, D. A. (2014). A best practices approach to caries management. *The Journal of Evidence-Based Dental Practice*, *14 Suppl*, 77–86. https://doi.org/10.1016/j.jebdp.2014.03.006.

Islam, M. M., Iqbal, U., Walther, B., Atique, S., Dubey, N. K., Nguyen, P.-A., … Shabbir, S.-A. (2016). Benzodiazepine use and risk of dementia in the elderly population: a systematic review and meta-analysis. *Neuroepidemiology*, *47*(3–4), 181–191. https://doi.org/10.1159/000454881.

Jurczak, A., Kościelniak, D., Skalniak, A., Papież, M., Vyhouskaya, P., & Krzyściak, W. (2017). The role of the saliva antioxidant barrier to reactive oxygen species with regard to caries development. *Redox Report : Communications in Free Radical Research*, 22(6), 524–533. https://doi.org/10.1080/13510002.2017.1301625.

Kamodyová, N., Červenka, T., & Celec, P. (2015). Salivary markers of oxidative stress in oral diseases. *Frontiers in Cellular and Infection Microbiology*, *5*, 73. https://doi.org/10.3389/fcimb.2015.00073.

Koga-Ito, C. Y., Martins, C. A. P., Balducci, I., & Jorge, A. O. C. (2004). Correlation among mutans streptococci counts, dental caries, and IgA to Streptococcus mutans in saliva. *Brazilian Oral Research*, *18*(4), 350–355. https://doi.org/10.1590/s1806-83242004000400014.

Kumar, A., Hedge, R., & Dixit, U. (2011). Role of plaque in the clearance of salivary sucrose and its influence on salivary ph. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, *29*(4), 310–314. https://doi.org/10.4103/0970-4388.86377.

Machiulskiene, V., Campus, G., Carvalho, J. C., Dige, I., Ekstrand, K. R., Jablonski-Momeni, A., … Nyvad, B. (2020). Terminology of dental caries and dental caries management: consensus report of a workshop organized by ORCA and Cariology Research Group of IADR. *Caries Research*, *54*(1), 7–14. https://doi.org/10.1159/000503309.

Mahjoub, S., Ghasempour, M., Gharage, A., Bijani, A., & Masrourroudsari, J. (2014). Comparison of total antioxidant capacity in saliva of children with severe early childhood caries and caries-free children. *Caries Research*, *48*(4), 271–275. https://doi.org/10.1159/000355581.

Modesti, P. A., Reboldi, G., Cappuccio, F. P., Agyemang, C., Remuzzi, G., Rapi, S., … Parati, G. (2016). Panethnic differences in blood pressure in europe: a systematic review and meta-analysis. *PloS One*, *11*(1), e0147601. https://doi.org/10.1371/journal.pone.0147601.

Muchandi, S., Walimbe, H., Bijle, M. N., Nankar, M., Chaturvedi, S., & Karekar, P. (2015). Comparative evaluation and correlation of salivary total antioxidant capacity and salivary pH in caries-free and severe early childhood caries children. *The Journal of Contemporary Dental Practice*, *16*(3), 234–237. https://doi.org/10.5005/jp-journals-10024-1667.

Neel, E. A. A., Aljabo, A., Strange, A., Ibrahim, S., Coathup, M., Young, A. M., … Mudera, V. (2016). Demineralization–remineralization dynamics in teeth and bone. *International Journal of Nanomedicine*, *11*, 4743–4763. https://doi.org/10.2147/IJN.S107624.

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., … Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*, *372*, 71. https://doi.org/10.1136/bmj.n71.

Pandey, P., Reddy, N. V., Rao, V. A. P., Saxena, A., & Chaudhary, C. P. (2015). Estimation of salivary flow rate, pH, buffer capacity, calcium, total protein content and total antioxidant capacity in relation to dental caries severity, age and gender. *Contemporary Clinical Dentistry*, *6*(Suppl 1), S65-71. https://doi.org/10.4103/0976-237X.152943.

Prabhakar, A., Dodawad, R., & Os, R. (2009). Evaluation of flow rate, pH, buffering

capacity, calcium, total protein and total antioxidant levels of saliva in caries free and caries active children-an in vivo study. *International Journal of Clinical Pediatric Dentistry*, *2*(1), 9–12. https://doi.org/10.5005/jp-journals-10005-1034.

Pyati, S. A., Naveen Kumar, R., Kumar, V., Praveen Kumar, N. H., & Parveen Reddy, K. M. (2018). Salivary flow rate, pH, buffering capacity, total protein, oxidative stress and antioxidant capacity in children with and without dental caries. *Journal of Clinical Pediatric Dentistry*, 42(6), 445–449. https://doi.org/10.17796/1053-4625-42.6.7.

Sedgwick, P. (2015). Bias in observational study designs: cross sectional studies. *BMJ*, *350*, h1286. https://doi.org/10.1136/bmj.h1286.

Shaki, F., Arab-Nozari, M., Maleki, F., Charati, J. Y., & Nahvi, A. (2020). Evaluation of some caries-related factors in the saliva of 3-5 year old children in Sari, Northern Iran. *International Journal of Pediatrics*, *8*(4), 11115–11123. https://doi.org/10.22038/ijp.2019.42952.3598.

Shetty, C., Hegde, M., & Darshana, D. (2013). Correlation between dental caries with salivary flow, pH, and buffering capacity in adult south Indian population: an in-vivo study. *International Journal of Research in Ayurveda and Pharmacy*, *4*, 219–223. https://doi.org/10.7897/2277-4343.04226.

Silva, P. V., Troiano, J. A., Nakamune, A. C. M. S., Pessan, J. P., & Antoniali, C. (2016). Increased activity of the antioxidants systems modulate the oxidative stress in saliva of toddlers with early childhood caries. *Archives of Oral Biology*, *70*, 62–66. https://doi.org/10.1016/j.archoralbio.2016.06.003.

Subramanyam, D., Gurunathan, D., Gaayathri, R., & Priya, V. V. (2018). Comparative evaluation of salivary malondialdehyde levels as a marker of lipid peroxidation in early childhood caries. *European Journal of Dentistry*, *12*(1), 67-70. https://doi.org/10.4103/ejd.ejd_266_17.

Syed, M., Sachdev, V., & Chopra, R. (2016). Intercomparison of salivary nitric oxide as a biomarker of dental caries risk between caries-active and caries-free children. *European Archives of Paediatric Dentistry*, *17*(4), 239–243. https://doi.org/10.1007/s40368-016-0234-z.

Tappuni, A. R., & Challacombe, S. J. (1994). A comparison of salivary immunoglobulin A (IgA) and IgA subclass concentrations in predentate and dentate children and adults. *Oral Microbiology and Immunology*, *9*(3), 142–145. https://doi.org/10.1111/j.1399-302x.1994.tb00050.x.

Tulunoglui, O., Demirtas, S., & Tulunoglu, I. (2006). Total antioxidant levels of saliva in children related to caries, age, and gender. *International Journal of Paediatric Dentistry*, *16*(3), 186–191. https://doi.org/10.1111/j.1365-263X.2006.00733.x.

Figure 1: Flow-Diagram of the systematic review and meta-analysis.

Identification of studies via databases

| Identification | Records identified from databases searching (PubMed n = 1,263; Scopus n = 1,916; Web of Science n = 980; Embase n = 2,073; Cochrane Library n = 166; Lilacs n = 134; Google Scholar n = 100; and Open Grey n = 0. Total n = 6,632). | → | Records removed *before screening*: Duplicate records removed (n = 1,032) |

Screening	Records screened (n = 5,600)	→	Records excluded by eligibility criteria (n = 5,577)
	Reports sought for retrieval* (n = 4)	→	Reports not retrieved¶ (n = 4)
	Reports assessed for eligibility (n = 22)	→	Reports excluded due to the exposure evaluated was dental abscess (n = 1), and the groups were dichotomized different to dental caries/caries-free (n = 1). In addition, two articles had data duplicated (n = 2). Searched the reference lists of included studies (n = 0).

| Included | Studies included in qualitative synthesis (n = 18) Studies included in meta-analysis (n = 14) |

¶Authors of four studies with data of interest measured but not reported in the manuscript were contacted by email. None of them responded, even so 3 were eligible, containing other data of interest for our review.

Figure 2: Forest plot of meta-analysis investigating total antioxidant capacity (mmol/l) as a salivary biomarker in children with dental caries versus caries-free ones, according to age (Figure 2. A) and gender (Figure 2. B) subgroups.

Figure 2. A: Forest plot of meta-analysis investigating total antioxidant capacity (TAC, mmol/l) as a salivary biomarker in children with dental caries versus caries-free ones, according to age. CI= Confidence Interval, and IV= Inverse Variance method. The forest plot shows increased TAC levels in the dental caries group. The subgroups were statistically different, but with a similar behavior in relation to the increase in TAC levels in the dental caries groups.

Figure 2. B: Forest plot of meta-analysis investigating total antioxidant capacity (TAC, mmol/l) as a salivary biomarker in children with dental caries versus caries-free ones, according to gender. CI= Confidence Interval, and IV= Inverse Variance method. The forest plot shows increased TAC levels in the dental caries group. The subgroups were not statistically different.

Figure 3: Forest plot of meta-analysis investigating malondialdehyde (nmol/l x10^3) (Figure 3. A) and malondialdehyde (nmol/l/mg/protein) (Figure 3. B) as salivary biomarkers in children with dental caries versus caries-free ones.

Study or Subgroup	Dental caries Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Araujo et al., 2020	0.0014	0.0006	30	0.0031	0.0001	30	31.9%	-3.90 [-4.78, -3.02]
Pyati et al., 2018	4.89	2.03	50	3.95	1.31	50	34.0%	0.55 [0.15, 0.95]
Subramanyam et al., 2018	0.26	0.24	75	0.17	0.14	75	34.2%	0.46 [0.13, 0.78]
Total (95% CI)			**155**			**155**	**100.0%**	**-0.90 [-2.69, 0.88]**

Heterogeneity: Tau² = 2.40; Chi² = 88.27, df = 2 (P < 0.00001); I² = 98%
Test for overall effect: Z = 0.99 (P = 0.32)
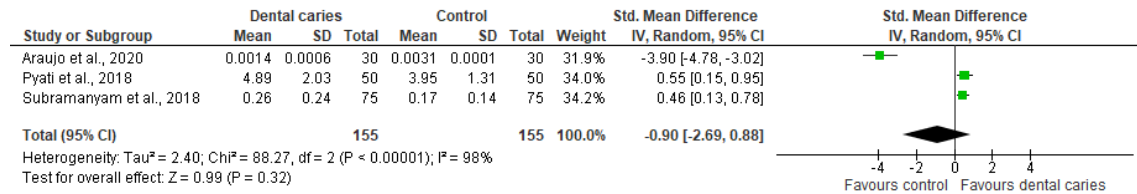
Figure 3. A: Forest plot of meta-analysis investigating malondialdehyde (MDA, nmol/l x10^3) as salivary biomarkers in children with dental caries versus caries-free ones. CI= Confidence Interval, and IV= Inverse Variance method. The forest diagram did not show differences between the groups.

Study or Subgroup	Dental caries Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Araujo et al., 2020	0.148	0.01	30	0.385	0.0306	30	51.2%	-10.28 [-12.24, -8.31]
Silva et al., 2016	0.0019	0.0008	30	0.039	0.0021	30	48.8%	-23.04 [-27.34, -18.75]
Total (95% CI)			**60**			**60**	**100.0%**	**-16.51 [-29.02, -4.00]**

Heterogeneity: Tau² = 78.61; Chi² = 28.05, df = 1 (P < 0.00001); I² = 96%
Test for overall effect: Z = 2.59 (P = 0.010)
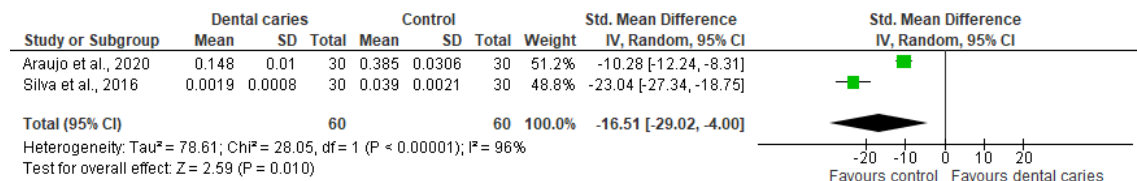
Figure 3. B: Forest plot of meta-analysis investigating malondialdehyde (MDA, nmol/l/mg/protein) as salivary biomarkers in children with dental caries versus caries-free ones. CI= Confidence Interval, and IV= Inverse Variance method. The forest plot shows reduced MDA levels when normalized by protein in the dental caries group.

Figure 4: Forest plot of meta-analysis investigating superoxide dismutase (UE/ml) (Figure 4. A) and superoxide dismutase (U/mg protein) (Figure 4. B) as salivary biomarkers in children with dental caries versus caries-free ones.
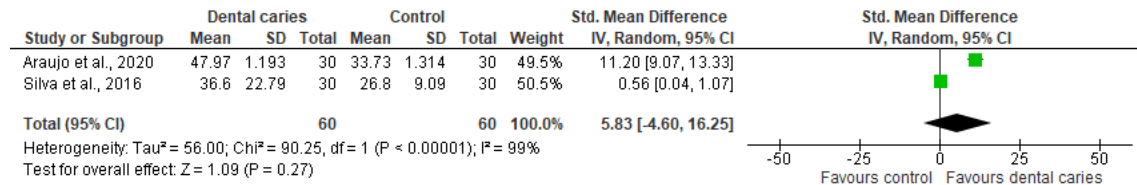
Figure 4. A: Forest plot of meta-analysis investigating superoxide dismutase (SOD, UE/ml) as salivary biomarkers in children with dental caries versus caries-free ones. CI= Confidence Interval, and IV= Inverse Variance method. The forest diagram did not show differences between the groups.
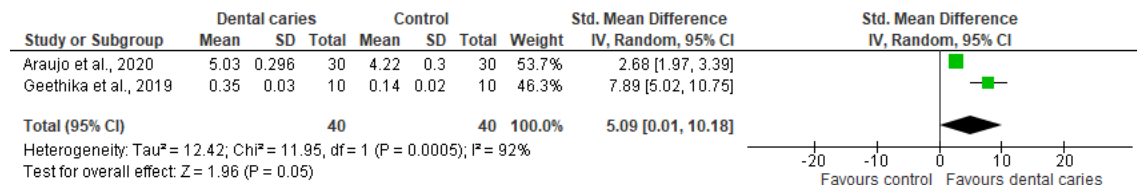
Figure 4. B: Forest plot of meta-analysis investigating superoxide dismutase (SOD, U/mg protein) as salivary biomarkers in children with dental caries versus caries-free ones. CI= Confidence Interval, and IV= Inverse Variance method. The forest plot shows increased SOD levels when normalized by protein in the dental caries group.

Figure 5: Forest plot of meta-analysis investigating uric acid (mg/ml) as salivary biomarker in children with dental caries versus caries-free ones.

Study or Subgroup	Dental caries Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Araujo et al., 2020	7.558	0.1782	30	5.853	0.2047	30	49.4%	8.77 [7.07, 10.47]
Silva et al., 2016	7.05	1.4	30	5.02	1.61	30	50.6%	1.33 [0.77, 1.89]
Total (95% CI)			60			60	100.0%	5.00 [-2.29, 12.30]

Heterogeneity: Tau² = 27.27; Chi² = 66.32, df = 1 (P < 0.00001); I² = 98%
Test for overall effect: Z = 1.34 (P = 0.18)
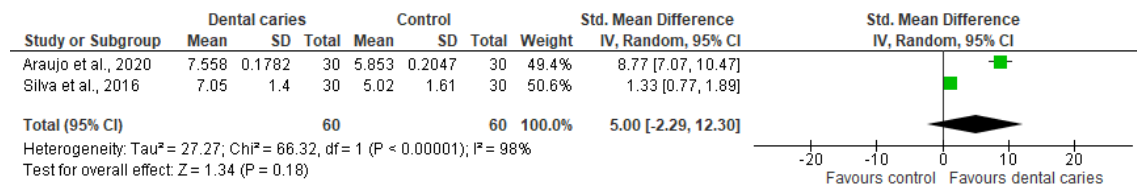
Figure 5: Forest plot of meta-analysis investigating uric acid (mg/ml) as salivary biomarkers in children with dental caries versus caries-free ones. CI= Confidence Interval, and IV= Inverse Variance method. The forest diagram did not show differences between the groups.

Figure 6: Forest plot of meta-analysis investigating total protein concentration (mg/dl) as salivary biomarker in children with dental caries versus caries-free ones, according to age (Figure 6. A) and gender (Figure 6. B) subgroups.
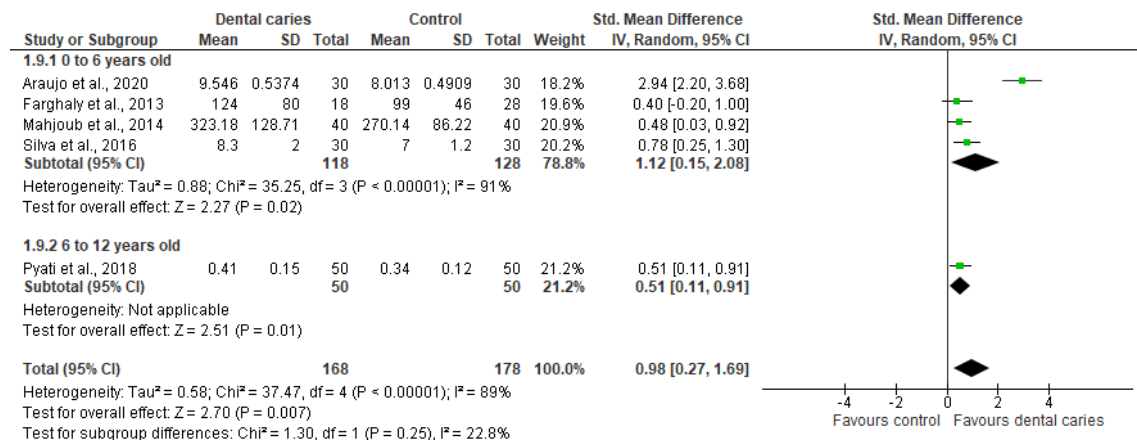
Figure 6. A: Forest plot of meta-analysis investigating total protein concentration (mg/dl) as salivary biomarkers in children with dental caries versus caries-free ones, according to age. CI= Confidence Interval, and IV= Inverse Variance method. The forest plot shows increased total protein concentration levels in the dental caries group. The subgroups were not statistically different.
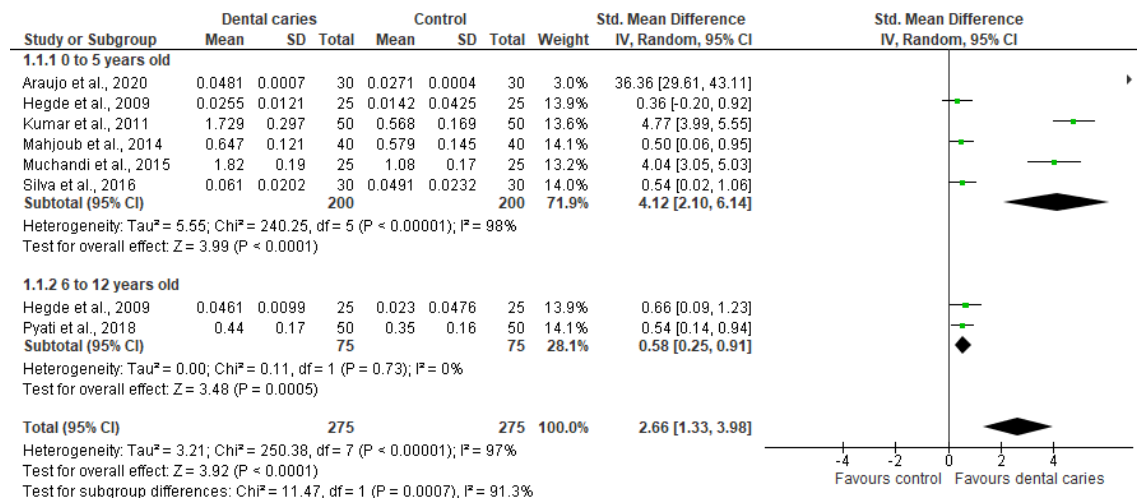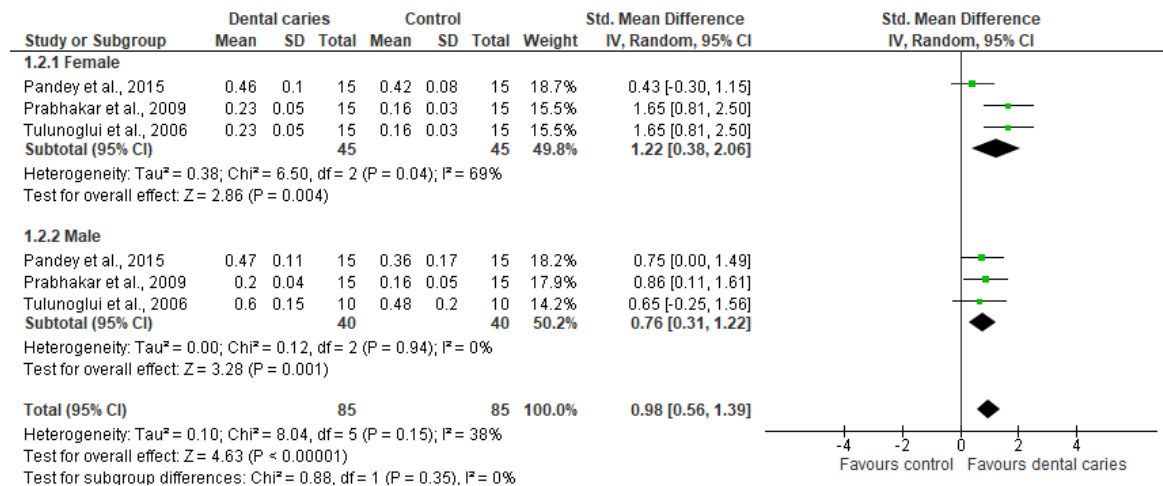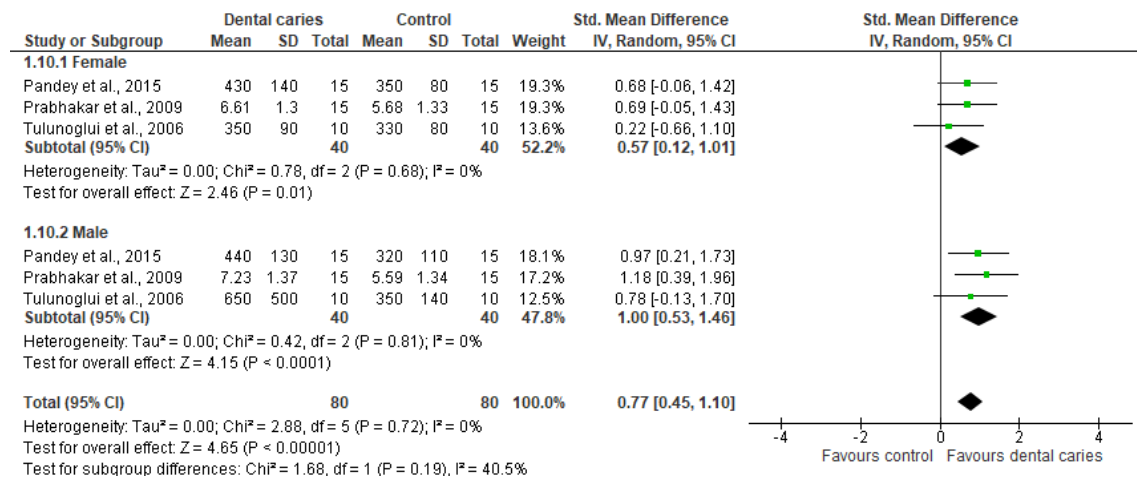

Figure 6. B: Forest plot of meta-analysis investigating total protein concentration (mg/dl) as salivary biomarkers in children with dental caries versus caries-free ones, according to gender. CI= Confidence Interval, and IV= Inverse Variance method. The forest plot shows increased total protein concentration levels in the dental caries group. The subgroups were not statistically different.

Table 1: Salivary parameters in children with dental caries versus caries-free ones, according to gender subgroups.

Salivary parameters	Gender	Dental caries	Control	SMD (CI 95%)	I^2	p value
Salivary flow rate unstimulated (ml/min)	Female	40	40	-0.43 (-0.87, 0.02)	0%	0.06
	Male	40	40	-0.22 (-0.66, 0.23)	0%	0.34
	Overall	80	80	-0.32 (-0.63, -0.01)	0%	0.05[a]
	Subgroups differences					0.50
pH	Female	40	40	-0.69 (-1.60, 0.22)	73%	0.13
	Male	40	40	-0.57 (-2.00, 0.87)	89%	0.44
	Both gender	75	75	-2.22 (-3.42, -1.03)	85%	<0.01[a]
	Overall	155	155	-1.05 (-1.82, -0.28)	88%	<0.01[a]
	Subgroups differences					0.10
Buffer capacity (mg/dl)	Female	40	40	-0.78 (-1.36, -0.19)	37%	<0.01[a]
	Male	40	40	-0.37 (-1.37, 0.64)	79%	0.48
	Overall	80	80	-0.58 (-1.13, -0.03)	64%	0.04[a]
	Subgroups differences					0.49
Calcium (mg/dl)	Female	40	40	-0.69 (-1.21, -0.18)	20%	<0.01[a]
	Male	40	40	-1.04 (-1.53, -0.55)	6%	<0.01[a]
	Overall	80	80	-0.86 (-1.21, -0.51)	13%	<0.01[a]
	Subgroups differences					0.34

Meta-analysis of salivary parameters by gender subgroups. Standardized Mean Difference (SMD) was measured using Inverse Variance as statistical method and the Random-Effects model, corresponding 95% of confidence interval (CI). The I^2 statistic was used to assess the heterogeneity in the studies. The overall effect and test for subgroups differences were assessed at a 5% significance level. Superscript letters represent difference statistically significant, [a] The results show reduced levels of salivary parameters in the dental caries group.

Materiais Suplementares

Supplement 1: Search strategy.

PubMed		
#1	(((((Child, Preschool[MeSH Terms]) OR (Child[MeSH Terms])) OR (Preschool Child[Title/Abstract])) OR (Children, Preschool[Title/Abstract])) OR (Preschool Children[Title/Abstract])) OR (Children[Title/Abstract])	2,272,182 results
#2	((((((((((((((Dental Caries[MeSH Terms]) OR (Dental Decay[Title/Abstract])) OR (Decay, Dental[Title/Abstract])) OR (Carious Lesions[Title/Abstract])) OR (Carious Lesion[Title/Abstract])) OR (Lesion, Carious[Title/Abstract])) OR (Lesions, Carious[Title/Abstract])) OR (Caries, Dental[Title/Abstract])) OR (Carious Dentin[Title/Abstract])) OR (Carious Dentins[Title/Abstract])) OR (Dentin, Carious[Title/Abstract])) OR (Dentins, Carious[Title/Abstract])) OR (Dental White Spot[Title/Abstract])) OR (Spot, Dental White[Title/Abstract])) OR (Spots, Dental White[Title/Abstract])) OR (White Spot, Dental[Title/Abstract])) OR (White Spots, Dental[Title/Abstract])) OR (Dental White Spots[Title/Abstract])	49,149 results
#3	(((Saliva[MeSH Terms]) OR (Salivas[Title/Abstract])) OR (((((((((((((((((((((((((((((((((((((Biomarkers[MeSH Terms])) OR (Marker, Biological[Title/Abstract])) OR (Biological Marker[Title/Abstract])) OR (Biologic Marker[Title/Abstract])) OR (Marker, Biologic[Title/Abstract])) OR (Biological Markers[Title/Abstract])) OR (Biologic Markers[Title/Abstract])) OR (Markers, Biologic[Title/Abstract])) OR (Biomarker[Title/Abstract])) OR (Markers, Biological[Title/Abstract])) OR (Markers, Immunologic[Title/Abstract])) OR (Immune Markers[Title/Abstract])) OR (Markers, Immune[Title/Abstract])) OR (Marker, Immunologic[Title/Abstract])) OR (Immunologic Markers[Title/Abstract])) OR (Immune Marker[Title/Abstract])) OR (Marker, Immune[Title/Abstract])) OR (Immunologic Marker[Title/Abstract])) OR (Serum Markers[Title/Abstract])) OR (Markers, Serum[Title/Abstract])) OR (Marker, Serum[Title/Abstract])) OR (Serum Marker[Title/Abstract])) OR (Surrogate Endpoints[Title/Abstract])) OR (Endpoints, Surrogate[Title/Abstract])) OR (Surrogate End Point[Title/Abstract])) OR (End Point, Surrogate[Title/Abstract])) OR (Surrogate End Points[Title/Abstract])) OR (End Points, Surrogate[Title/Abstract])) OR (Surrogate Endpoint[Title/Abstract])) OR (Endpoint, Surrogate[Title/Abstract])) OR (Markers, Clinical[Title/Abstract])) OR (Clinical Markers[Title/Abstract])) OR (Clinical Marker[Title/Abstract])) OR (Marker, Clinical[Title/Abstract])) OR (Viral Markers[Title/Abstract])) OR (Markers, Viral[Title/Abstract])) OR (Viral Marker[Title/Abstract])) OR (Marker, Viral[Title/Abstract])) OR (Biochemical Marker[Title/Abstract])) OR (Markers, Biochemical[Title/Abstract])) OR (Marker, Biochemical[Title/Abstract])) OR (Biochemical Markers[Title/Abstract])) OR (Markers, Laboratory[Title/Abstract])) OR (Laboratory Markers[Title/Abstract])) OR (Laboratory Marker[Title/Abstract])) OR (Marker, Laboratory[Title/Abstract])) OR (Surrogate Markers[Title/Abstract])) OR (Markers, Surrogate[Title/Abstract])) OR (Marker, Surrogate[Title/Abstract])) OR (Surrogate Marker[Title/Abstract])) OR (TBARS[Title/Abstract])) OR (MDA[Title/Abstract])) OR (TAC[Title/Abstract])) OR (SOD[Title/Abstract])) OR (Uric acid[Title/Abstract]))) OR ((((((((((((((((((((((((((Oxidative Stress[MeSH Terms]) OR (Oxidative Stresses[Title/Abstract])) OR (Stress, Oxidative[Title/Abstract])) OR (Antioxidative Stress[Title/Abstract])) OR (Antioxidative Stresses[Title/Abstract])) OR (Stress, Antioxidative[Title/Abstract])) OR (Anti-oxidative Stress[Title/Abstract])) OR (Anti oxidative Stress[Title/Abstract])) OR (Anti-oxidative Stresses[Title/Abstract])) OR (Stress, Anti-oxidative[Title/Abstract])) OR (Oxidative Damage[Title/Abstract])) OR (Damage, Oxidative[Title/Abstract])) OR (Oxidative Damages[Title/Abstract])) OR (Oxidative Stress Injury[Title/Abstract])) OR (Injury, Oxidative Stress[Title/Abstract])) OR (Oxidative Stress Injuries[Title/Abstract])) OR (Stress Injury, Oxidative[Title/Abstract])) OR (Oxidative Injury[Title/Abstract])) OR (Injury, Oxidative[Title/Abstract])) OR (Oxidative Injuries[Title/Abstract])) OR (Oxidative Cleavage[Title/Abstract])) OR (Cleavage, Oxidative[Title/Abstract])) OR (Oxidative Cleavages[Title/Abstract])) OR (Oxidative DNA Damage[Title/Abstract])) OR (DNA Damage, Oxidative[Title/Abstract])) OR (Damage, Oxidative DNA[Title/Abstract])) OR (Oxidative DNA Damages[Title/Abstract])) OR (DNA Oxidative Damage[Title/Abstract])) OR (DNA Oxidative Damages[Title/Abstract])) OR (Damage, DNA Oxidative[Title/Abstract])) OR (Oxidative Damage, DNA[Title/Abstract])) OR (Oxidative[Title/Abstract] AND Nitrosative Stress[Title/Abstract])) OR (Oxidative Nitrative Stress[Title/Abstract])) OR (Nitrative Stress, Oxidative[Title/Abstract])) OR (Oxidative Nitrative Stresses[Title/Abstract])) OR (Stress, Oxidative Nitrative[Title/Abstract])) OR	1,190,637 results

	(Nitro-Oxidative Stress[Title/Abstract])) OR (Nitro Oxidative Stress[Title/Abstract])) OR (Nitro-Oxidative Stresses[Title/Abstract])) OR (Stress, Nitro-Oxidative[Title/Abstract])) OR (Stresses, Nitro-Oxidative[Title/Abstract]))	
#1 AND #2	((((((Child, Preschool[MeSH Terms]) OR (Child[MeSH Terms])) OR (Preschool Child[Title/Abstract])) OR (Children, Preschool[Title/Abstract])) OR (Preschool Children[Title/Abstract])) OR (Children[Title/Abstract])) AND ((((((((((((((((Dental Caries[MeSH Terms]) OR (Dental Decay[Title/Abstract])) OR (Decay, Dental[Title/Abstract])) OR (Carious Lesions[Title/Abstract])) OR (Carious Lesion[Title/Abstract])) OR (Lesion, Carious[Title/Abstract])) OR (Lesions, Carious[Title/Abstract])) OR (Caries, Dental[Title/Abstract])) OR (Carious Dentin[Title/Abstract])) OR (Carious Dentins[Title/Abstract])) OR (Dentin, Carious[Title/Abstract])) OR (Dentins, Carious[Title/Abstract])) OR (Dental White Spot[Title/Abstract])) OR (Spot, Dental White[Title/Abstract])) OR (Spots, Dental White[Title/Abstract])) OR (White Spot, Dental[Title/Abstract])) OR (White Spots, Dental[Title/Abstract])) OR (Dental White Spots[Title/Abstract]))	19,472 results
#1 AND #2 AND #3	((((((Child, Preschool[MeSH Terms]) OR (Child[MeSH Terms])) OR (Preschool Child[Title/Abstract])) OR (Children, Preschool[Title/Abstract])) OR (Preschool Children[Title/Abstract])) OR (Children[Title/Abstract])) AND ((((((((((((((((Dental Caries[MeSH Terms]) OR (Dental Decay[Title/Abstract])) OR (Decay, Dental[Title/Abstract])) OR (Carious Lesions[Title/Abstract])) OR (Carious Lesion[Title/Abstract])) OR (Lesion, Carious[Title/Abstract])) OR (Lesions, Carious[Title/Abstract])) OR (Caries, Dental[Title/Abstract])) OR (Carious Dentin[Title/Abstract])) OR (Carious Dentins[Title/Abstract])) OR (Dentin, Carious[Title/Abstract])) OR (Dentins, Carious[Title/Abstract])) OR (Dental White Spot[Title/Abstract])) OR (Spot, Dental White[Title/Abstract])) OR (Spots, Dental White[Title/Abstract])) OR (White Spot, Dental[Title/Abstract])) OR (White Spots, Dental[Title/Abstract])) OR (Dental White Spots[Title/Abstract]))) AND ((((Saliva[MeSH Terms]) OR (Salivas[Title/Abstract])) OR ((Biomarkers[MeSH Terms])) OR (Marker, Biological[Title/Abstract])) OR (Biological Marker[Title/Abstract])) OR (Biologic Marker[Title/Abstract])) OR (Marker, Biologic[Title/Abstract])) OR (Biological Markers[Title/Abstract])) OR (Biologic Markers[Title/Abstract])) OR (Markers, Biologic[Title/Abstract])) OR (Biomarker[Title/Abstract])) OR (Markers, Biological[Title/Abstract])) OR (Markers, Immunologic[Title/Abstract])) OR (Immune Markers[Title/Abstract])) OR (Markers, Immune[Title/Abstract])) OR (Marker, Immunologic[Title/Abstract])) OR (Immunologic Markers[Title/Abstract])) OR (Immune Marker[Title/Abstract])) OR (Marker, Immune[Title/Abstract])) OR (Immunologic Marker[Title/Abstract])) OR (Serum Markers[Title/Abstract])) OR (Markers, Serum[Title/Abstract])) OR (Marker, Serum[Title/Abstract])) OR (Serum Marker[Title/Abstract])) OR (Surrogate Endpoints[Title/Abstract])) OR (Endpoints, Surrogate[Title/Abstract])) OR (Surrogate End Point[Title/Abstract])) OR (End Point, Surrogate[Title/Abstract])) OR (Surrogate End Points[Title/Abstract])) OR (End Points, Surrogate[Title/Abstract])) OR (Surrogate Endpoint[Title/Abstract])) OR (Endpoint, Surrogate[Title/Abstract])) OR (Markers, Clinical[Title/Abstract])) OR (Clinical Markers[Title/Abstract])) OR (Clinical Marker[Title/Abstract])) OR (Marker, Clinical[Title/Abstract])) OR (Viral Markers[Title/Abstract])) OR (Markers, Viral[Title/Abstract])) OR (Viral Marker[Title/Abstract])) OR (Marker, Viral[Title/Abstract])) OR (Biochemical Marker[Title/Abstract])) OR (Markers, Biochemical[Title/Abstract])) OR (Marker, Biochemical[Title/Abstract])) OR (Biochemical Markers[Title/Abstract])) OR (Markers, Laboratory[Title/Abstract])) OR (Laboratory Markers[Title/Abstract])) OR (Laboratory Marker[Title/Abstract])) OR (Marker, Laboratory[Title/Abstract])) OR (Surrogate Markers[Title/Abstract])) OR (Markers, Surrogate[Title/Abstract])) OR (Marker, Surrogate[Title/Abstract])) OR (Surrogate Marker[Title/Abstract])) OR (TBARS[Title/Abstract])) OR (MDA[Title/Abstract])) OR (TAC[Title/Abstract])) OR (SOD[Title/Abstract])) OR (Uric acid[Title/Abstract]))) OR (((((((((((((((((((((((((Oxidative Stress[MeSH Terms]) OR (Oxidative Stresses[Title/Abstract])) OR (Stress, Oxidative[Title/Abstract])) OR (Antioxidative Stress[Title/Abstract])) OR (Antioxidative Stresses[Title/Abstract])) OR (Stress, Antioxidative[Title/Abstract])) OR (Anti-oxidative Stress[Title/Abstract])) OR (Anti oxidative Stress[Title/Abstract])) OR (Anti-oxidative Stresses[Title/Abstract])) OR (Stress, Anti-oxidative[Title/Abstract])) OR (Oxidative Damage[Title/Abstract])) OR (Damage, Oxidative[Title/Abstract])) OR (Oxidative Damages[Title/Abstract])) OR (Oxidative Stress Injury[Title/Abstract])) OR (Injury, Oxidative Stress[Title/Abstract])) OR (Oxidative Stress Injuries[Title/Abstract])) OR (Stress Injury, Oxidative[Title/Abstract])) OR (Oxidative Injury[Title/Abstract])) OR	1,263 results

	(Injury, Oxidative[Title/Abstract])) OR (Oxidative Injuries[Title/Abstract])) OR (Oxidative Cleavage[Title/Abstract])) OR (Cleavage, Oxidative[Title/Abstract])) OR (Oxidative Cleavages[Title/Abstract])) OR (Oxidative DNA Damage[Title/Abstract])) OR (DNA Damage, Oxidative[Title/Abstract])) OR (Damage, Oxidative DNA[Title/Abstract])) OR (Oxidative DNA Damages[Title/Abstract])) OR (DNA Oxidative Damage[Title/Abstract])) OR (DNA Oxidative Damages[Title/Abstract])) OR (Damage, DNA Oxidative[Title/Abstract])) OR (Oxidative Damage, DNA[Title/Abstract])) OR (Oxidative[Title/Abstract] AND Nitrosative Stress[Title/Abstract])) OR (Oxidative Nitrative Stress[Title/Abstract])) OR (Nitrative Stress, Oxidative[Title/Abstract])) OR (Oxidative Nitrative Stresses[Title/Abstract])) OR (Stress, Oxidative Nitrative[Title/Abstract])) OR (Nitro-Oxidative Stress[Title/Abstract])) OR (Nitro Oxidative Stress[Title/Abstract])) OR (Nitro-Oxidative Stresses[Title/Abstract])) OR (Stress, Nitro-Oxidative[Title/Abstract])) OR (Stresses, Nitro-Oxidative[Title/Abstract])))	
	Scopus	
#1	TITLE-ABS-KEY ("Child, Preschool" OR child OR "Preschool Child" OR "Children, Preschool" OR "Preschool Children" OR children)	3,194,992 results
#2	TITLE-ABS-KEY ("Dental Caries" OR "Dental Decay" OR "Decay, Dental" OR "Carious Lesions" OR "Carious Lesion" OR "Lesion, Carious" OR "Lesions, Carious" OR "Caries, Dental" OR "Carious Dentin" OR "Carious Dentins" OR "Dentin, Carious" OR "Dentins, Carious" OR "Dental White Spot" OR "Spot, Dental White" OR "Spots, Dental White" OR "White Spot, Dental" OR "White Spots, Dental" OR "Dental White Spots")	64,671 results
#3	TITLE-ABS-KEY (saliva OR salivas OR biomarkers OR "Marker, Biological" OR "Biological Marker" OR "Biologic Marker" OR "Marker, Biologic" OR "Biological Markers" OR "Biologic Markers" OR "Markers, Biologic" OR biomarker OR "Markers, Biological" OR "Markers, Immunologic" OR "Immune Markers" OR "Markers, Immune" OR "Marker, Immunologic" OR "Immunologic Markers" OR "Immune Marker" OR "Marker, Immune" OR "Immunologic Marker" OR "Serum Markers" OR "Markers, Serum" OR "Marker, Serum" OR "Serum Marker" OR "Surrogate Endpoints" OR "Endpoints, Surrogate" OR "Surrogate End Point" OR "End Point, Surrogate" OR "Surrogate End Points" OR "End Points, Surrogate" OR "Surrogate Endpoint" OR "Endpoint, Surrogate" OR "Markers, Clinical" OR "Clinical Markers" OR "Clinical Marker" OR "Marker, Clinical" OR "Viral Markers" OR "Markers, Viral" OR "Viral Marker" OR "Marker, Viral" OR "Biochemical Marker" OR "Markers, Biochemical" OR "Marker, Biochemical" OR "Biochemical Markers" OR "Markers, Laboratory" OR "Laboratory Markers" OR "Laboratory Marker" OR "Marker, Laboratory" OR "Surrogate Markers" OR "Markers, Surrogate" OR "Marker, Surrogate" OR "Surrogate Marker" OR "TBARS" OR "MDA" OR "TAC" OR "uric acid" OR "SOD" OR "Oxidative Stress" OR "Oxidative Stresses" OR "Stress, Oxidative" OR "Antioxidative Stress" OR "Antioxidative Stresses" OR "Stress, Antioxidative" OR "Anti-oxidative Stress" OR "Anti oxidative Stress" OR "Anti-oxidative Stresses" OR "Stress, Anti-oxidative" OR "Oxidative Damage" OR "Damage, Oxidative" OR "Oxidative Damages" OR "Oxidative Stress Injury" OR "Injury, Oxidative Stress" OR "Oxidative Stress Injuries" OR "Stress Injury, Oxidative" OR "Oxidative Injury" OR "Injury, Oxidative" OR "Oxidative Injuries" OR "Oxidative Cleavage" OR "Cleavage, Oxidative" OR "Oxidative Cleavages" OR "Oxidative DNA Damage" OR "DNA Damage, Oxidative" OR "Damage, Oxidative DNA" OR "Oxidative DNA Damages" OR "DNA Oxidative Damage" OR "DNA Oxidative Damages" OR "Damage, DNA Oxidative" OR "Oxidative Damage, DNA" OR "Oxidative and Nitrosative Stress" OR "Oxidative Nitrative Stress" OR "Nitrative Stress, Oxidative" OR "Oxidative Nitrative Stresses" OR "Stress, Oxidative Nitrative" OR "Nitro-Oxidative Stress" OR "Nitro Oxidative Stress" OR "Nitro-Oxidative Stresses" OR "Stress, Nitro-Oxidative" OR "Stresses, Nitro-Oxidative")	1,307,053 results
#1 AND #2	#1 AND #2	24,253 results
#1 AND #2 AND #3	#1 AND #2 AND #3	1,916 results
	Web of Science	
#1	TÓPICO: ("Child, Preschool") OR TÓPICO: ("Child") OR TÓPICO: ("Preschool Child ") OR TÓPICO: ("Children, Preschool ") OR TÓPICO: ("Preschool Children ") OR TÓPICO: ("Children")	1,739,979 results
#2	TÓPICO: ("Dental Caries") OR TÓPICO: ("Dental Decay") OR TÓPICO: ("Decay,	21.822 results

	Dental") OR TÓPICO: ("Carious Lesions ") OR TÓPICO: ("Carious Lesion") OR TÓPICO: ("Lesion, Carious") OR TÓPICO: ("Lesions, Carious") OR TÓPICO: ("Caries, Dental") OR TÓPICO: ("Carious Dentin") OR TÓPICO: ("Carious Dentins") OR TÓPICO: ("Dentin, Carious") OR TÓPICO: ("Dentins, Carious") OR TÓPICO: ("Dental White Spot") OR TÓPICO: ("Spots, Dental White") OR TÓPICO: ("White Spot, Dental") OR TÓPICO: ("White Spots, Dental") OR TÓPICO: ("Dental White Spots")	
#3	TÓPICO: ("Saliva") OR TÓPICO: ("Biomarkers") OR TÓPICO: ("Salivas" OR "Marker, Biological" OR "Biological Marker" OR "Biologic Marker" OR "Marker, Biologic" OR "Biological Markers" OR "Biologic Markers" OR "Markers, Biologic" OR "Biomarker" OR "Markers, Biological" OR "Markers, Immunologic" OR "Immune Markers" OR "Markers, Immune" OR "Marker, Immunologic" OR "Immunologic Markers" OR "Immune Marker" OR "Marker, Immune" OR "Immunologic Marker" OR "Serum Markers" OR "Markers, Serum" OR "Marker, Serum" OR "Serum Marker" OR "Surrogate Endpoints" OR "Endpoints, Surrogate" OR "Surrogate End Point" OR "End Point, Surrogate" OR "Surrogate End Points" OR "End Points, Surrogate" OR "Surrogate Endpoint" OR "Endpoint, Surrogate" OR "Markers, Clinical" OR "Clinical Markers" OR "Clinical Marker" OR "Marker, Clinical" OR "Viral Markers" OR "Markers, Viral" OR "Viral Marker" OR "Marker, Viral" OR "Biochemical Marker" OR "Markers, Biochemical" OR "Marker, Biochemical" OR "Biochemical Markers" OR "Markers, Laboratory" OR "Laboratory Markers" OR "Laboratory Marker" OR "Marker, Laboratory" OR "Surrogate Markers" OR "Markers, Surrogate" OR "Marker, Surrogate" OR "Surrogate Marker" OR "TBARS" OR "MDA" OR "TAC" OR "uric acid" OR "SOD") OR TÓPICO: ("Oxidative Stress") OR TÓPICO: ("Oxidative Stresses" OR "Stress, Oxidative" OR "Antioxidative Stress" OR "Antioxidative Stresses" OR "Stress, Antioxidative" OR "Anti-oxidative Stress" OR "Anti oxidative Stress" OR "Anti-oxidative Stresses" OR "Stress, Anti-oxidative" OR "Oxidative Damage" OR "Damage, Oxidative" OR "Oxidative Damages" OR "Oxidative Stress Injury" OR "Injury, Oxidative Stress" OR "Oxidative Stress Injuries" OR "Stress Injury, Oxidative" OR "Oxidative Injury" OR "Injury, Oxidative" OR "Oxidative Injuries" OR "Oxidative Cleavage" OR "Cleavage, Oxidative" OR "Oxidative Cleavages" OR "Oxidative DNA Damage" OR "DNA Damage, Oxidative" OR "Damage, Oxidative DNA" OR "Oxidative DNA Damages" OR "DNA Oxidative Damage" OR "DNA Oxidative Damages" OR "Damage, DNA Oxidative" OR "Oxidative Damage, DNA" OR "Oxidative and Nitrosative Stress" OR "Oxidative Nitrative Stress" OR "Nitrative Stress, Oxidative" OR "Oxidative Nitrative Stresses" OR "Stress, Oxidative Nitrative" OR "Nitro-Oxidative Stress" OR "Nitro Oxidative Stress" OR "Nitro-Oxidative Stresses" OR "Stress, Nitro-Oxidative" OR "Stresses, Nitro-Oxidative")	986,719 results
#1 AND #2	#1 AND #2 Índices=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Tempo estipulado=Todos os anos	8,342 results
#1 AND #2 AND #3	#1 AND #2 AND #3 Índices=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Tempo estipulado=Todos os anos	980 results
Embase		
#1	#1 'preschool child'/exp #2 'child'/exp #3 'preschool child' OR 'children, preschool' OR 'preschool children' OR 'children' #4 #1 OR #2 OR #3	4,012,714 results
#2	#5 'dental caries'/exp #6 'dental decay' OR 'decay, dental' OR 'carious lesions' OR 'carious lesion' OR 'lesion, carious' OR 'lesions, carious' OR 'caries, dental' OR 'carious dentin' OR 'carious dentins' OR 'dentin, carious' OR 'dentins, carious' OR 'dental white spot' OR 'spot, dental white' OR 'spots, dental white' OR 'white spot, dental' OR 'white spots, dental' OR 'dental white spots' #7 #5 OR #6	70,265 results
#3	#8 'saliva'/exp #9 'biological marker'/exp #10 'oxidative stress'/exp #11 'salivas' OR 'marker, biological' OR 'biological marker' OR 'biologic marker' OR 'marker, biologic' OR 'biological markers' OR 'biologic markers' OR 'markers, biologic' OR 'biomarker' OR 'markers, biological' OR 'markers, immunologic' OR 'immune markers' OR 'markers, immune' OR 'marker, immunologic' OR 'immunologic markers' OR 'immune marker' OR 'marker, immune' OR 'immunologic	1,162,305 results

	marker' OR 'serum markers' OR 'markers, serum' OR 'marker, serum' OR 'serum marker' OR 'surrogate endpoints' OR 'endpoints, surrogate' OR 'surrogate end point' OR 'end point, surrogate' OR 'surrogate end points' OR 'end points, surrogate' OR 'surrogate endpoint' OR 'endpoint, surrogate' OR 'markers, clinical' OR 'clinical markers' OR 'clinical marker' OR 'marker, clinical' OR 'viral markers' OR 'markers, viral' OR 'viral marker' OR 'marker, viral' OR 'biochemical marker' OR 'markers, biochemical' OR 'marker, biochemical' OR 'biochemical markers' OR 'markers, laboratory' OR 'laboratory markers' OR 'laboratory marker' OR 'marker, laboratory' OR 'surrogate markers' OR 'markers, surrogate' OR 'marker, surrogate' OR 'surrogate marker' OR 'tbars' OR 'mda' OR 'tac' OR 'sod' OR 'uric acid' OR 'oxidative stresses' OR 'stress, oxidative' OR 'antioxidative stress' OR 'antioxidative stresses' OR 'stress, antioxidative' OR 'anti-oxidative stress' OR 'anti oxidative stress' OR 'anti-oxidative stresses' OR 'stress, anti-oxidative' OR 'oxidative damage' OR 'damage, oxidative' OR 'oxidative damages' OR 'oxidative stress injury' OR 'injury, oxidative stress' OR 'oxidative stress injuries' OR 'stress injury, oxidative' OR 'oxidative injury' OR 'injury, oxidative' OR 'oxidative injuries' OR 'oxidative cleavage' OR 'cleavage, oxidative' OR 'oxidative cleavages' OR 'oxidative dna damage' OR 'dna damage, oxidative' OR 'damage, oxidative dna' OR 'oxidative dna damages' OR 'dna oxidative damage' OR 'dna oxidative damages' OR 'damage, dna oxidative' OR 'oxidative damage, dna' OR 'oxidative and nitrosative stress' OR 'oxidative nitrative stress' OR 'nitrative stress, oxidative' OR 'oxidative nitrative stresses' OR 'stress, oxidative nitrative' OR 'nitro-oxidative stress' OR 'nitro oxidative stress' OR 'nitro-oxidative stresses' OR 'stress, nitro-oxidative' OR 'stresses, nitro-oxidative' #12 #8 OR #9 OR #10 OR #11	
#1 AND #2	#13 #4 AND #7	25,939 results
#1 AND #2 AND #3	#14 #4 AND #7 AND #12	2,073 results
Cochrane Library		
#1	#1 MeSH descriptor: [Child, Preschool] explode all trees #2 MeSH descriptor: [Child] explode all trees #3 ((Preschool Child) OR (Children, Preschool) OR (Preschool Children) OR (Children)):ti,ab,kw #4 #1 OR #2 OR #3	149,520 results
#2	#5 MeSH descriptor: [Dental Caries] explode all trees #6 ((Dental Decay) OR (Decay, Dental) OR (Carious Lesions) OR (Carious Lesion) OR (Lesion, Carious) OR (Lesions, Carious) OR (Caries, Dental) OR (Carious Dentin) OR (Carious Dentins) OR (Dentin, Carious) OR (Dentins, Carious) OR (Dental White Spot) OR (Spot, Dental White) OR (Spots, Dental White) OR (White Spot, Dental) OR (White Spots, Dental) OR (Dental White Spots)):ti,ab,kw #7 #5 OR #6	5,831 results
#3	#8 MeSH descriptor: [Saliva] explode all trees #9 MeSH descriptor: [Biomarkers] explode all trees #10 MeSH descriptor: [Oxidative Stress] explode all trees #11 ((Salivas)):ti,ab,kw #12 ((Marker, Biological) OR (Biological Marker) OR (Biologic Marker) OR (Marker, Biologic) OR (Biological Markers) OR (Biologic Markers) OR (Markers, Biologic) OR (Biomarker) OR (Markers, Biological) OR (Markers, Immunologic) OR (Immune Markers) OR (Markers, Immune) OR (Marker, Immunologic) OR (Immunologic Markers) OR (Immune Marker) OR (Marker, Immune) OR (Immunologic Marker) OR (Serum Markers) OR (Markers, Serum) OR (Marker, Serum) OR (Serum Marker) OR (Surrogate Endpoints) OR (Endpoints, Surrogate) OR (Surrogate End Point) OR (End Point, Surrogate) OR (Surrogate End Points) OR (End Points, Surrogate) OR (Surrogate Endpoint) OR (Endpoint, Surrogate) OR	80,830 results

	(Markers, Clinical) OR (Clinical Markers) OR (Clinical Marker) OR (Marker, Clinical) OR (Viral Markers) OR (Markers, Viral) OR (Viral Marker) OR (Marker, Viral) OR (Biochemical Marker) OR (Markers, Biochemical) OR (Marker, Biochemical) OR (Biochemical Markers) OR (Markers, Laboratory) OR (Laboratory Markers) OR (Laboratory Marker) OR (Marker, Laboratory) OR (Surrogate Markers) OR (Markers, Surrogate) OR (Marker, Surrogate) OR (Surrogate Marker) OR (TBARS) OR (MDA) OR (TAC) OR (uric acid) OR (SOD)):ti,ab,kw #13 ((Oxidative Stresses) OR (Stress, Oxidative) OR (Antioxidative Stress) OR (Antioxidative Stresses) OR (Stress, Antioxidative) OR (Anti-oxidative Stress) OR (Anti oxidative Stress) OR (Anti-oxidative Stresses) OR (Stress, Anti-oxidative) OR (Oxidative Damage) OR (Damage, Oxidative) OR (Oxidative Damages) OR (Oxidative Stress Injury) OR (Injury, Oxidative Stress) OR (Oxidative Stress Injuries) OR (Stress Injury, Oxidative) OR (Oxidative Injury) OR (Injury, Oxidative) OR (Oxidative Injuries) OR (Oxidative Cleavage) OR (Cleavage, Oxidative) OR (Oxidative Cleavages) OR (Oxidative DNA Damage) OR (DNA Damage, Oxidative) OR (Damage, Oxidative DNA) OR (Oxidative DNA Damages) OR (DNA Oxidative Damage) OR (DNA Oxidative Damages) OR (Damage, DNA Oxidative) OR (Oxidative Damage, DNA) OR (Oxidative and Nitrosative Stress) OR (Oxidative Nitrative Stress) OR (Nitrative Stress, Oxidative) OR (Oxidative Nitrative Stresses) OR (Stress, Oxidative Nitrative) OR (Nitro-Oxidative Stress) OR (Nitro Oxidative Stress) OR (Nitro-Oxidative Stresses) OR (Stress, Nitro-Oxidative) OR (Stresses, Nitro-Oxidative)):ti,ab,kw #14 #8 OR #9 OR #10 OR #11 OR #12 OR #13	
#1 AND #2	#4 AND #7	2,587 results
#1 AND #2 AND #3	#4 AND #7 AND #14	166 results
Lilacs		
#1	(mh:(Child, Preschool)) OR (mh:(Child)) OR ((tw: Preschool Child OR Children, Preschool OR Preschool Children OR Children))	103,292 results
#2	(mh:(Dental Caries)) OR ((tw: Dental Decay OR Decay, Dental OR Carious Lesions OR Carious Lesion OR Lesion, Carious OR Lesions, Carious OR Caries, Dental OR Carious Dentin OR Carious Dentins OR Dentin, Carious OR Dentins, Carious OR Dental White Spot OR Spot, Dental White OR Spots, Dental White OR White Spot, Dental OR White Spots, Dental OR Dental White Spots))	6,638 results
#3	(mh:(Saliva)) OR (mh:(Biomarkers)) OR (mh:(Oxidative Stress)) OR ((tw: Salivas OR Marker, Biological OR Biological Marker OR Biologic Marker OR Marker, Biologic OR Biological Markers OR Biologic Markers OR Markers, Biologic OR Biomarker OR Markers, Biological OR Markers, Immunologic OR Immune Markers OR Markers, Immune OR Marker, Immunologic OR Immunologic Markers OR Immune Marker OR Marker, Immune OR Immunologic Marker OR Serum Markers OR Markers, Serum OR Marker, Serum OR Serum Marker OR Surrogate Endpoints OR Endpoints, Surrogate OR Surrogate End Point OR End Point, Surrogate OR Surrogate End Points OR End Points, Surrogate OR Surrogate Endpoint OR Endpoint, Surrogate OR Markers, Clinical OR Clinical Markers OR Clinical Marker OR Marker, Clinical OR Viral Markers OR Markers, Viral OR Viral Marker OR Marker, Viral OR Biochemical Marker OR Markers, Biochemical OR Marker, Biochemical OR Biochemical Markers OR Markers, Laboratory OR Laboratory Markers OR Laboratory Marker OR Marker, Laboratory OR Surrogate Markers OR Markers, Surrogate OR Marker, Surrogate OR Surrogate Marker OR TBARS OR MDA OR TAC OR SOD OR Uric acid OR Oxidative Stresses OR Stress, Oxidative OR Antioxidative Stress OR Antioxidative Stresses OR Stress, Antioxidative OR Anti-oxidative Stress OR Anti oxidative Stress OR Anti-oxidative Stresses OR Stress, Anti-oxidative OR Oxidative Damage OR Damage, Oxidative OR Oxidative Damages OR Oxidative Stress Injury OR Injury, Oxidative Stress OR Oxidative Stress Injuries OR Stress Injury, Oxidative OR Oxidative Injury OR Injury, Oxidative OR Oxidative Injuries OR Oxidative Cleavage OR Cleavage, Oxidative OR Oxidative Cleavages OR Oxidative DNA Damage OR DNA Damage, Oxidative OR Damage, Oxidative DNA OR Oxidative DNA Damages OR DNA Oxidative Damage OR DNA Oxidative Damages OR Damage, DNA Oxidative OR Oxidative Damage, DNA OR Oxidative and Nitrosative Stress OR Oxidative Nitrative Stress OR Nitrative Stress, Oxidative OR Oxidative Nitrative Stresses OR Stress, Oxidative	2,293 results

	Nitrative OR Nitro-Oxidative Stress OR Nitro Oxidative Stress OR Nitro-Oxidative Stresses OR Stress, Nitro-Oxidative OR Stresses, Nitro-Oxidative))	
#1 AND #2	((mh:(Child, Preschool)) OR (mh:(Child)) OR ((tw: Preschool Child OR Children, Preschool OR Preschool Children OR Children))) AND ((mh:(Dental Caries)) OR ((tw: Dental Decay OR Decay, Dental OR Carious Lesions OR Carious Lesion OR Lesion, Carious OR Lesions, Carious OR Caries, Dental OR Carious Dentin OR Carious Dentins OR Dentin, Carious OR Dentins, Carious OR Dental White Spot OR Spot, Dental White OR Spots, Dental White OR White Spot, Dental OR White Spots, Dental OR Dental White Spots)))	2,710 results
#1 AND #2 AND #3	((mh:(Child, Preschool)) OR (mh:(Child)) OR ((tw: Preschool Child OR Children, Preschool OR Preschool Children OR Children))) AND ((mh:(Dental Caries)) OR ((tw: Dental Decay OR Decay, Dental OR Carious Lesions OR Carious Lesion OR Lesion, Carious OR Lesions, Carious OR Caries, Dental OR Carious Dentin OR Carious Dentins OR Dentin, Carious OR Dentins, Carious OR Dental White Spot OR Spot, Dental White OR Spots, Dental White OR White Spot, Dental OR White Spots, Dental OR Dental White Spots))) AND ((mh:(Saliva)) OR (mh:(Biomarkers)) OR (mh:(Oxidative Stress)) OR ((tw: Salivas OR Marker, Biological OR Biological Marker OR Biologic Marker OR Marker, Biologic OR Biological Markers OR Biologic Markers OR Markers, Biologic OR Biomarker OR Markers, Biological OR Markers, Immunologic OR Immune Markers OR Markers, Immune OR Marker, Immunologic OR Immunologic Markers OR Immune Marker OR Marker, Immune OR Immunologic Marker OR Serum Markers OR Markers, Serum OR Marker, Serum OR Serum Marker OR Surrogate Endpoints OR Endpoints, Surrogate OR Surrogate End Point OR End Point, Surrogate OR Surrogate End Points OR End Points, Surrogate OR Surrogate Endpoint OR Endpoint, Surrogate OR Markers, Clinical OR Clinical Markers OR Clinical Marker OR Marker, Clinical OR Viral Markers OR Markers, Viral OR Viral Marker OR Marker, Viral OR Biochemical Marker OR Markers, Biochemical OR Marker, Biochemical OR Biochemical Markers OR Markers, Laboratory OR Laboratory Markers OR Laboratory Marker OR Marker, Laboratory OR Surrogate Markers OR Markers, Surrogate OR Marker, Surrogate OR Surrogate Marker OR TBARS OR MDA OR TAC OR SOD OR Uric acid OR Oxidative Stresses OR Stress, Oxidative OR Antioxidative Stress OR Antioxidative Stresses OR Stress, Antioxidative OR Anti-oxidative Stress OR Anti oxidative Stress OR Anti-oxidative Stresses OR Stress, Anti-oxidative OR Oxidative Damage OR Damage, Oxidative OR Oxidative Damages OR Oxidative Stress Injury OR Injury, Oxidative Stress OR Oxidative Stress Injuries OR Stress Injury, Oxidative OR Oxidative Injury OR Injury, Oxidative OR Oxidative Injuries OR Oxidative Cleavage OR Cleavage, Oxidative OR Oxidative Cleavages OR Oxidative DNA Damage OR DNA Damage, Oxidative OR Damage, Oxidative DNA OR Oxidative DNA Damages OR DNA Oxidative Damage OR DNA Oxidative Damages OR Damage, DNA Oxidative OR Oxidative Damage, DNA OR Oxidative and Nitrosative Stress OR Oxidative Nitrative Stress OR Nitrative Stress, Oxidative OR Oxidative Nitrative Stresses OR Stress, Oxidative Nitrative OR Nitro-Oxidative Stress OR Nitro Oxidative Stress OR Nitro-Oxidative Stresses OR Stress, Nitro-Oxidative OR Stresses, Nitro-Oxidative)))	134 results
Google Scholar		
oxidative stress AND children AND dental caries		100 results
Open Grey		
oxidative stress AND children AND dental caries		0 results

Supplement 2: Studies excluded and reasons for exclusions.

Children with dental abscess as exposure were excluded, because it was not possible to determine that the cause of this condition was dental caries in the study *"Comparison of Salivary Antioxidants in Children with Primary Tooth Abscesses before and after Treatment in Comparison with Healthy Subjects"*. Also, the study *"Salivary enzymatic antioxidant activity and dental caries: A cross-sectional study"* was excluded because it involved a different dichotomization approach for grouping the children as with dental caries or caries-free. In addition, two articles had duplicate data (*"Evaluation of Flow Rate, pH, Buffering Capacity, Calcium, Total Proteins and Total Antioxidant Capacity Levels of Saliva in Caries Free and Caries Active Children: An In Vivo Study"* and *"Estimation of total antioxidant capacity levels in saliva of caries-free and caries-active children"*).

Dodwad R, Betigeri AV, Preeti BP. Estimation of total antioxidant capacity levels in saliva of caries-free and caries-active children. Contemp Clin Dent. 2011 Jan;2(1):17-20. doi: 10.4103/0976-237X.79296. PMID: 22114448; PMCID: PMC3220168.

Preethi BP, Reshma D, Anand P. Evaluation of Flow Rate, pH, Buffering Capacity, Calcium, Total Proteins and Total Antioxidant Capacity Levels of Saliva in Caries Free and Caries Active Children: An In Vivo Study. Indian J Clin Biochem. 2010 Oct;25(4):425-8. doi: 10.1007/s12291-010-0062-6. Epub 2010 Sep 14. PMID: 21966118; PMCID: PMC2994560.

Vahabzadeh Z, Hashemi ZM, Nouri B, Zamani F, Shafiee F. Salivary enzymatic antioxidant activity and dental caries: A cross-sectional study. Dent Med Probl. 2020 Oct-Dec;57(4):385-391. doi: 10.17219/dmp/126179. PMID: 33448164.

Zarban A, Ebrahimipour S, Sharifzadeh GR, Rashed-Mohassel A, Barkooi M. Comparison of Salivary Antioxidants in Children with Primary Tooth Abscesses before and after Treatment in Comparison with Healthy Subjects. Asian Pac J Cancer Prev. 2017;18(12):3315-3318.

Supplement 3: Characteristics of the included studies.

Study Identification	Country	Study Desing	No. children (ages)	Exposure/control	Results	Limitations	Conflict of interests	Funding
Araujo *et al.,* 2020	Brazil	Cross-sectional study	120 children (1 to 3 years old)	Early, moderate and advanced caries lesion according to the ICCMS™ criteria/caries-free ¶ ICCMS™: International Caries Classification and Management System methods for staging of the caries process and enabling dentists to manage caries	**Malondialdehyde (MDA):** Children with early caries lesion or caries-free showed lower MDA levels than others groups ($p < 0.001$). The oxidative damage was evaluated by the Substances Reactive to Thiobarbituric Acid (TBARS) method. TBARS was determined as described by Buege and Aust in 1978. **Uric Acid (UA):** Children with advanced caries lesion showed higher UA levels than others groups ($p < 0.001$). Early caries lesion showed similar results than caries-free ($p > 0.05$). A commercial kit (Labtest Diagnóstica SA, MG, Brazil) was used to determine UA in 20 μL of saliva by means of the enzymatic method of Trinder. **Superoxide Dismutase (SOD):** Children with advanced caries lesion showed higher SOD levels than others groups ($p < 0.001$). Early caries lesion showed similar results than caries-free ($p > 0.05$). SOD activity was determined	Not reported	None	Study was supported by *Fundaçãode Amparo à Pesquisa do Estado de São Paulo* (FAPESP, grant #2016/22180-9). Partially financed by *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, grant #302526/2016-1). *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil* (CAPES) - Finance Code 001, and UNESP-PROPG/PROPE (Edital 12/2019).

					in saliva by the Maklund method. **Total antioxidant capacity (TAC):** Children with advanced caries lesion showed higher TAC levels than others groups (p < 0.001). Early caries lesion showed similar results than caries-free (p > 0.05). Salivary TAC was evaluated by the antioxidant power of iron reduction (FRAP assay). *Positive correlation there was between TAC, SOD and UA, and severity of caries lesion. On the other hand, negative correlation there was between MDA and severity of caries lesion. **Salivary Flow** (unstimulated): Salivary flow rate was not significantly different among the groups. Salivary flow was determined by the total value of each sample collected (ml) during 5 minutes, and the results were expressed in ml/min. **Total Protein Concentration:** Children with advanced caries lesion showed higher levels than others groups (p < 0.001).		

					Early caries lesion showed similar results than caries-free (p > 0.05). Protein was quantified in 20 μl saliva aliquots using the by Lowry method.			
Banda *et al.,* 2016	India	Cross-sectional study	60 children (6 to 12 years old)	Caries-active (number of decayed teeth >4) /caries-free ¶ dmft (d = decayed, m = missing, f = filled, t = teeth) score	**Total antioxidant level (TAL):** Children with caries-active showed higher TAL levels than caries-free groups (p < 0.0001). Phosphomolybdenum method was used for estimation of salivary TAL. *Positive correlation between decayed missing filled teeth scores (dmft) and TAL.	Stricter standardization protocol including same dietary pattern would give an in depth knowledge of the vagaries in antioxidant variations. Unable to differentiate the source of antioxidants whether it is host or pathogen in origin. Stricter standardized protocol to pin point the exact reason of variation in antioxidant levels, e.g., Children having same diet would help. Small sample size.	None	None
Silva *et al.,* 2016	Brazil	Cross-sectional study	60 children (0 to 3 years old)	Severe early childhood caries (S-ECC)/ caries-free	**MDA:** Salivary MDA levels were significantly lower (p < 0.0001) in the S-ECC group than caries-free group. MDA was evaluated by the method thiobarbituric acid-reactive substances (TBARS), **AU:** Salivary uric acid values were significantly increased (p < 0.0001) in S-ECC group than caries-free groups. Uric acid was determined in saliva using a	Not reported	None	CAPES-PROAP and *Programa de Pós-Graduação em Ciência Odontológica*

					commercial kit (Labtest Diadgnóstica SA, MG, Brazil) based on enzymatic Trinder method.			
					SOD: SOD activity was significantly higher in S-ECC ($p < 0.05$) when compared to the caries-free group. SOD activity was determined in saliva by the method of Maklund based on the inhibition of the pyrogallol autoxidation.			
					TAC: TAC in saliva was significantly higher ($p < 0.05$) in S-ECC group compared to the caries-free group. Salivary total antioxidant capacity was assessed by ferric reducing antioxidant power (FRAP) assay.			
					Total Protein Concentration: Salivary total protein was significantly higher ($p < 0.01$) in the S-ECC group when compared to CF group. Protein was measured by the method of Lowry, Rosebrough, Farr, and Randall.			
Farghaly *et al.,* 2013	Brazil	Cross-sectional study	46 children (4 to 6 years old)	Dental caries/caries-free	**Peroxidase:** Not changes were observed in the peroxidase activity ($p = 0.425$) among the groups studied. Total salivary peroxidase activity was determined by the assay	The presence of lesions of white spot, as the saliva collection was not performed. Considering the data epidemiological factors of tooth decay, the number of children with	Not reported	Not reported

					proposed by Chandra *et al.,* 1977 later modified by Anderson, 1986 using the lactoperoxidase as standard. **Total Protein Concentration:** Not changes were observed in the concentration of total protein (p=0.427) between the groups. Total protein was assessed by Lowry method. **Salivary flow** (stimulated): Children with cavities of dental caries had lower salivary flow compared with children free of caries (p = 0.046). Total saliva collection was mechanically stimulated by parafilm chewing.	carious lesions assessed in the study is not adequate for conclusions about the effect of gender on development of dental caries. In addition, due to the multifactorial nature of tooth decay, elements such as lifestyle, habits hygiene, dietary patterns, exposure to fluorides and behavioral aspects should also be taken into consideration.		
Geethika *et al.,* 2019	India	Cross-sectional study	20 children (< 5 years old)	ECC/non-ECC	**SOD:** SOD activity was significantly higher in ECC (p < 0.05) when compared to the non-ECC group. The estimation of SOD enzyme activity was carried out by the Beauchamp and Fridovich methods.	Not reported	None	None
Hegde *et al.,* 2008	India	Cross-sectional study	60 children (< 5 years old) 60 children (6 to 12 years old)	ECC/control Rampant caries/control	**Nitric Oxide (NO):** The mean nitrite levels of both the control groups were higher when compared with the study groups, which was statistically significant. Estimation of salivary nitric oxide was measured by the concentration of its stable metabolite nitrite using Classical Griess Reaction.	Not reported	Not reported	Not reported

					Salivary Flow (unstimulated): The difference was not statistically significant between the groups.			
Hegde *et al.,* 2009	India	Cross-sectional study	50 children (< 5 years old) 50 children (6 to 12 years old)	ECC/control Rampant caries/control	**TAC:** TAC in saliva was significantly higher (p < 0.05) in both groups with dental caries compared to the control groups. The TAC of saliva was evaluated using the spectrophotometric assay.	Not reported	Not reported	Not reported
Jurczak *et al.,* 2017	Poland	Cross-sectional study	81 children (1 to 5 years old)	Initial stage decay, termed non-cavitated (ICDAS 1 and 2), and extensive decay, termed cavitated lesions (ICDAS 5 and 6)/ caries-free ¶ ICDAS: International Caries Detection and Assessment System	**TAC:** TAC in saliva was significantly higher (p < 0.001) in both groups with dental caries compared to the control groups. The ICDAS 1 and 2 was significantly higher (p < 0.001) than the ICDAS 5 and 6. Determination of salivary TAC as the reduction of Fe3+ ions was performed using Benzie and Strain's method. **Total Protein Concentration:** Inter-group differences in salivary protein concentrations were statistically significant. The protein concentrations of the samples were determined using a bicinchoninic acid assay kit (Sigma-Aldrich, USA), as described by Smith.	Not reported	None	This study was supported by the National Research and Development Center MNISW/2016/DIR/181/NN, Poland) within the framework of the project 'Best of the Best!' Operational Program Knowledge Education Development 2014–2020. The study was also supported by Jagiellonian University Programs No. K/ZDS/ 005485, /ZDS/005484, and K/DSC/001959.

Kumar *et al.,* 2011	India	Cross-sectional study	100 children (3 to 5 years old)	S-ECC/ caries-free	**TAC:** TAC in saliva was significantly higher (p < 0.05) in S-ECC group compared to the caries-free group. Total antioxidant capacity of saliva was measured using antioxidant assay kit. The antioxidant assay kit was provided by Cayman Chemical Company, Ann Arbor, USA. *A statistically significant linear regression was seen between the TAC and the dmft score (R2 = 0.93, F = 128.92, P < 0.001).	Not reported	Not reported	Not reported
Mahjoub *et al.,* 2014	Iran	Cross-sectional study	80 children (3 to 5 years old)	S-ECC with dmfs ≥ 4 (age 3), ≥ 5 (age 4) or ≥ 6 (age 5)/ caries-free (dmfs = 0) ¶ dmfs (d = decayed, m = missing, f = filled, s = surface) score	**TAC:** The mean TAC in saliva samples from children with S-ECC was significantly greater than in the group without caries (p = 0.025). TAC was measured by the FRAP (ferric-reducing antioxidant power) method and total protein in unstimulated whole saliva was evaluated spectrophotometrically. *There was a positive correlation between salivary TAC and dmfs scores in S-ECC children by Pearson's correlation test (r = 0.725, p < 0.001). **Total Protein Concentration**: The mean total protein in the saliva of children with S-ECC was significantly greater than in	The limitation of the study was that, ethically, we could not completely match the nutritional program the day before sampling and also the fasting period of more than 1 h before saliva sampling, because of the low age of the children in the two groups.	None	This project was financially supported by the Vice Chancellery of Research and Technology of Babol University of Medical Sciences (grant No. 6131228).

					the caries-free group (p = 0.033). Total protein was measured by the Bradford method.			
Muchandi *et al.,* 2015	India	Cross-sectional study	50 children (3 to 5 years old)	S-ECC/ caries-free	**TAC:** TAC in saliva was significantly higher (p < 0.05) in S-ECC group compared to the caries-free group. The TAC was done using an antioxidant assay with the help of a spectrophotometer at wavelength 532 nm proposed by Koracevic et al., 2001. **pH:** Mean salivary pH in group caries-free was higher as compared to the average salivary pH level in group S-ECC. This difference in the average salivar pH in the two groups was found to be statistically significant (p-value < 0.0001). pH determination of saliva samples using pH indicator paper strips (Qualigens, Glaxo India Ltd, Mumbai, India) was due in the process.	Not reported	None	None
Pandey *et al.,* 2015	India	Cross-sectional study	60 children (7 to 10 years old)	Caries active (DMFS/dfs ≥5)/ caries-free	**TAC:** TAC in saliva was significantly higher (p < 0.05) in S-ECC group compared to the caries-free group, independently of the sex (male/female). Salivary antioxidant activity was measured with a spectrophotometer. **Salivary flow (unstimulated):** The	Not reported	None	None

| | | | | | difference was not statistically significant between the groups. Saliva samples were collected into a preweighed tube during a 5-min period. After collection, the tube was weighed again, and the flow rate calculated.

pH: The difference was not statistically significant between the groups. pH was measured by a manual pH meter

Buffer capacity: The difference was not statistically significant between the groups. Buffer capacity was determined by quantitative test using a hand-held pH meter method.

Total Protein Concentration: in saliva was significantly higher (p < 0.05) in S-ECC group compared to the caries-free group, independently of the sex (male/female). The total protein a levels of the samples were measured by autoanalyzer. Measurement of protein content was based on biuret method.

Calcium: Calcium in saliva was significantly higher (p < 0.05) in S-ECC group compared to the caries-free | | | |

					group, independently of the sex (male/female). The calcium levels of the samples were measured by autoanalyzer. Salivary calcium concentration was measured by Arsenazo-III method.			
Prabhakar *et al.,* 2009	India	Cross-sectional study	60 children (7 to 10 years old)	Caries active children having at least five decayed tooth surfaces/ caries-free	**TAC:** TAC in saliva was significantly higher ($p < 0.05$) in caries active group compared to the caries-free group, independently of the sex (male/female). Total salivary antioxidant levels were estimated by using a spectrophotometer. **Salivary flow** (unstimulated): Flow rate were slightly decreased in caries active children compared to caries-free children. The flow rate of saliva was estimated by asking children to spit into the preweighed plastic cylinders for 5 minutes. These plastic cylinders (containing saliva) were then weighed and the flow rate was calculated in gm/ml which is almost equivalent to ml/min. **pH:** pH was slightly decreased in caries active children compared to caries-free children. pH of saliva was measured by using manual pH meter.	Not reported	Not reported	Not reported

					Buffer capacity: Buffering capacity were slightly decreased in caries active children compared to caries-free children. Buffering capacity of saliva (by Ericsson method 1959). **Total Protein Concentration**: The total protein increased significantly in caries active children. Estimation of total protein was done by autoanalyzer which works on the principle of atomic absorption spectrophotometer. **Calcium:** Total calcium decreased significantly in caries active children. Estimation of calcium was done by autoanalyzer which works on the principle of atomic absorption spectrophotometer.			
Pyati *et al.,* 2018	India	Cross-sectional study	100 children (6 to 12 years old)	Caries active (DMFS/dfs ≥5)/ age and sex matched caries--free	**TAC:** TAC levels were increased in caries active children compared to caries-free (p < 0.05). Salivary TAC was estimated by spectrophotometric method. **MDA:** MDA levels were increased in caries active children compared to caries-free controls (p < 0.05). Salivary MDA was estimated by Thiobarbituric	Not reported	Not reported	Not reported

					acid (TBA) method.			
					*As the DMFS/ dfs score increases, levels of salivary MDA and TAC also increase, but this correlation was statistically not significant ($p > 0.05$).			
					Salivary flow (unstimulated): The levels of salivary flow rate, were decreased in caries active children when compared to caries-free controls and these changes were statistically significant ($p < 0.05$). The plastic cylinders containing saliva were then weighed & the flow rate was calculated.			
					pH: The levels of pH, were decreased in caries active children when compared to caries-free controls and these changes were statistically significant ($p < 0.05$). Salivary pH was estimated by using the digital pH meter (ELICO Ltd., Hyderabad, India.).			
					Buffer capacity: The mean value of salivary buffering capacity was decreased in cases when compared to controls, but the difference was statistically not significant ($p = 0.08$). Salivary buffering capacity			

					was estimated by Ericsson method (1959). **Total Protein Concentration**: The values of salivary total protein increased in caries active children when compared to caries-free controls and these changes were statistically significant ($p < 0.05$). Salivary total protein was estimated by Biuret method.			
Shaki *et al.,* 2020	Iran	Cross-sectional study	80 children (3 to 5 years old)	Caries active/ caries-free (dmft=0)	**TAC:** TAC levels were increased in caries active children compared to caries-free ($p < 0.05$). The total antioxidant capacity of saliva was determined by measuring the ability of plasma to reduce Fe^{3+} to Fe^{2+} using the Ferric reducing antioxidant power (FRAP) test. **pH:** Mean salivary pH in caries active group was lower in comparison with caries-free group. pH of saliva was measured by using commercial pH meter (paper strip manufactured by MERCK, Germany). **Total Protein Concentration**: Salivary total protein concentration was significantly higher ($p < 0.01$) in the caries active group when compared to caries-free group. Protein	Detection of dental caries was done by using dental mirror and explorer while radiographic examination was not performed because of lack of instruments and impossibility of using them in the place. Dental caries was identified only with clinical diagnosis.	None	This study supported by a grant from the Research Council of Mazandaran University of Medical Sciences, Sari, Iran (grant number: 2916).

					content was determined in saliva samples with Bradford method.			
					NO: Salivary nitric oxide levels were significantly lower (p < 0. 001) in the group of children caries active compared to caries-free group. Nitric oxide was evaluated by using the commercial kits based on the Griess reagent.			
Subramanyam *et al.,* 2018	India	Cross-sectional study	150 children (mean ages 5.46)	ECC/non-ECC	**MDA:** The MDA levels were not statistically difference (p > 0.05). MDA levels were estimated by Buege and Aust method by using thiobarbituric acid.	The study did not assess the correlation between oral hygiene levels and MDA levels in children with ECC, which is the limitation of the study.	None	None
Syed *et al.,* 2016	India	Cross-sectional study	100 children (6 to 12 years old)	Caries-free (dmft=0) / caries-active (dmft ≥3)	**NO:** The salivary nitric oxide level of the caries-free group was found to be significantly higher than the caries-active group (p = 0.000). The nitric oxide concentration was measured as total nitrates and nitrites by the Griess reaction method (Green et al. 1982).	Not reported	None	None
Tulunoglui *et al.,* 2006	Turkey	Cross-sectional study	40 children (7 to 10 years old)	Caries active as at least five decayed tooth surface requiring restoration/ caries-free (dmfs=0)	**TAC:** TAC values were not statistically difference (p > 0.05). Total salivary antioxidant activity was measured with an autoanalyser (Technicon RAXT, USA). **Salivay flow** (unstimulated): Salivary flow rates were almost	Not reported	Not reported	Not reported

					the same between the groups. The saliva was collected into a preweighed tube on ice during a 5-min period. After collection, the tube was weighed again and the flow rate calculated. **pH:** pH values were higher in the caries active group but the differences were not statistically significant ($p > 0.05$). The pH was measured by a manual pH meter (Hanna Instruments, Kehl am Rhein, Germany). **Buffer capacity:** Buffer capacity values were higher in the caries active group but the differences were not statistically significant ($p > 0.05$). The buffer capacity was determined by the method of Ericsson modified for smaller volumes. **Total Protein Concentration**: Total protein values were higher in the caries active group but the differences were not statistically significant ($p > 0.05$). The total protein levels of the samples were measured by auto analyser (Syncron CX7, Beckman Coulter, USA). The principle of the total protein			

				assay was based on the biuret method and included alkaline copper reagent. **Calcium:** The salivary concentration of calcium was significantly higher in the caries-free group (p< 0.05). The calcium levels of the samples were measured by auto analyser (Syncron CX7, Beckman Coulter, USA). The salivary calcium concentration was measured by the Arsenazo-lll method (Sigma-Aldrich, St Louis, MO, USA).			

Supplement 4: Risk of bias of individual studies.

Study Identification	Selection (5 stars)	Comparability (2 stars)	Outcome (3 stars)	Total (10 stars)	Risk of bias
Araujo *et al.,* 2020	4	2	3	9	Low risk of bias
Banda *et al.,* 2016	4	2	3	9	Low risk of bias
Silva *et al.,* 2016	4	2	3	9	Low risk of bias
Farghaly *et al.,* 2013	3	1	3	7	Low risk of bias
Geethika *et al.,* 2019	4	1	3	8	Low risk of bias
Hegde *et al.,* 2008	3	2	3	8	Low risk of bias
Hegde *et al.,* 2009	3	2	3	8	Low risk of bias
Jurczak *et al.,* 2017	4	2	3	9	Low risk of bias
Kumar *et al.,* 2011	3	2	3	8	Low risk of bias
Mahjoub *et al.,* 2014	4	2	3	9	Low risk of bias
Muchandi *et al.,* 2015	4	1	3	8	Low risk of bias
Pandey *et al.,* 2009	3	2	3	8	Low risk of bias
Prabhakar *et al.,* 2009	4	2	3	9	Low risk of bias
Pyati *et al.,* 2018	5	2	3	10	Low risk of bias
Shaki *et al.,* 2020	4	2	3	9	Low risk of bias
Subramanyam *et al.,* 2018	3	1	3	7	Low risk of bias
Syed *et al.,* 2016	4	2	3	9	Low risk of bias
Tulunoglui *et al.,* 2006	3	2	3	8	Low risk of bias

The risk of bias was performed by Newcastle Ottawa Scale (NOS) instrument for observational studies (cohort and case-control) and by the modified version of NOS for cross-sectional studies. Regarding the risk of bias, individual studies were assessed as low risk ($\geq$ 7 stars) or high risk ($<$ 7 stars).

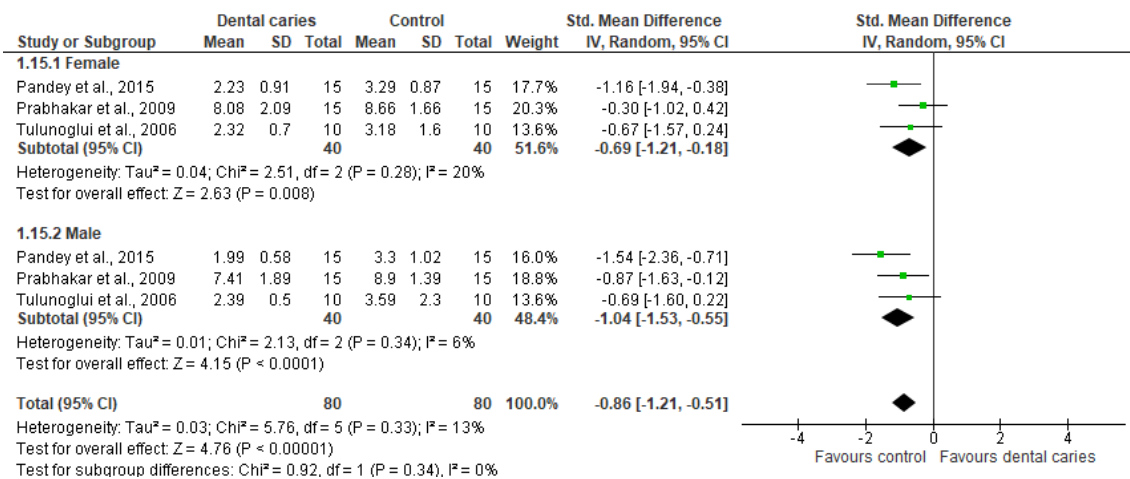**Supplement 5:** Forest plot of salivary parameters.
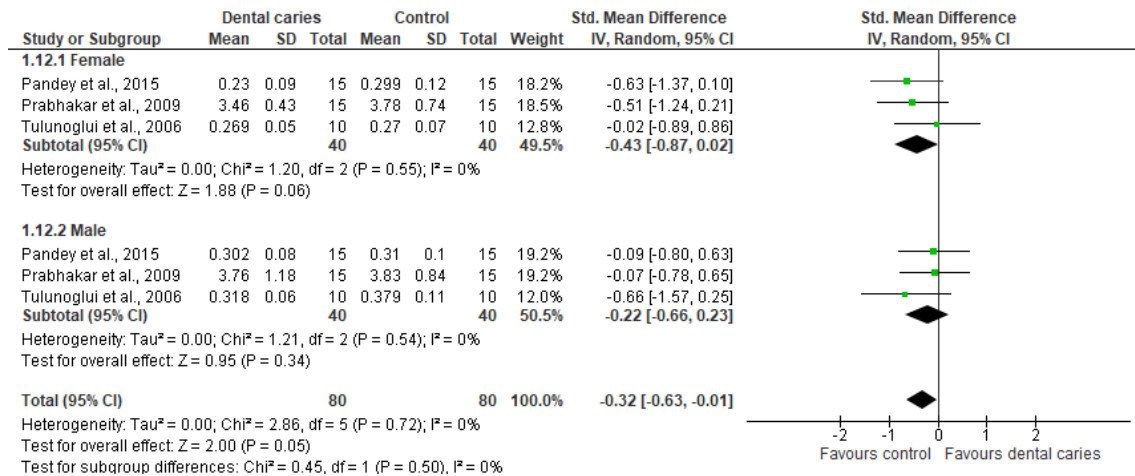
Suppl. 5. A: Forest plot of meta-analysis investigating salivary flow rate unstimulated (ml/min) in dental caries versus control by gender subgroups. CI= Confidence Interval, and IV= Inverse Variance method. The forest plot shows reduced salivary flow rate in the dental caries group. The subgroups were not statistically different.
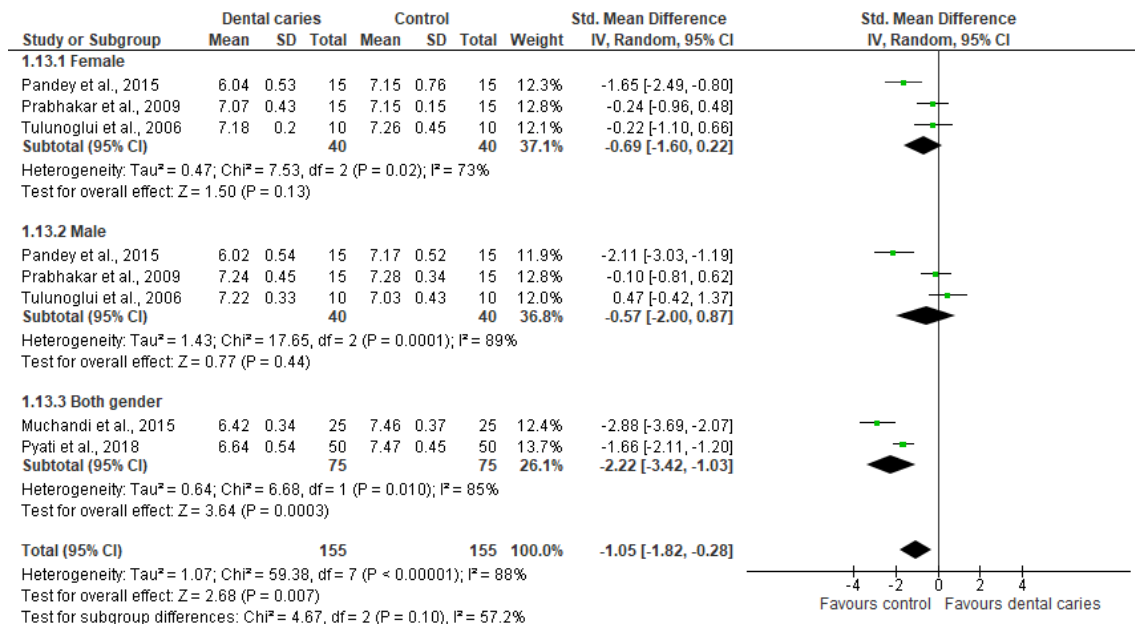
Suppl. 5. B: Forest plot of meta-analysis investigating pH in dental caries versus control by gender subgroups. CI= Confidence Interval, and IV= Inverse Variance method. The forest plot shows reduced pH levels in the dental caries group. The subgroups were not statistically different.

Study or Subgroup	Dental caries Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
1.14.1 Female								
Pandey et al., 2015	4.36	0.48	15	4.98	0.5	15	16.9%	-1.23 [-2.02, -0.44]
Prabhakar et al., 2009	5.32	0.4	15	5.44	0.43	15	18.0%	-0.28 [-1.00, 0.44]
Tulunoglui et al., 2006	4.62	0.5	10	5.09	0.5	10	14.9%	-0.90 [-1.83, 0.03]
Subtotal (95% CI)			40			40	49.8%	-0.78 [-1.36, -0.19]
Heterogeneity: Tau² = 0.10; Chi² = 3.15, df = 2 (P = 0.21); I² = 37%								
Test for overall effect: Z = 2.60 (P = 0.009)								
1.14.2 Male								
Pandey et al., 2015	4.48	0.58	15	5.25	0.51	15	16.7%	-1.37 [-2.18, -0.57]
Prabhakar et al., 2009	5.3	0.76	15	5.39	0.44	15	18.1%	-0.14 [-0.86, 0.58]
Tulunoglui et al., 2006	5.33	0.7	10	5.01	0.7	10	15.5%	0.44 [-0.45, 1.33]
Subtotal (95% CI)			40			40	50.2%	-0.37 [-1.37, 0.64]
Heterogeneity: Tau² = 0.63; Chi² = 9.48, df = 2 (P = 0.009); I² = 79%								
Test for overall effect: Z = 0.71 (P = 0.48)								
Total (95% CI)			80			80	100.0%	-0.58 [-1.13, -0.03]
Heterogeneity: Tau² = 0.30; Chi² = 13.89, df = 5 (P = 0.02); I² = 64%								
Test for overall effect: Z = 2.08 (P = 0.04)								
Test for subgroup differences: Chi² = 0.48, df = 1 (P = 0.49), I² = 0%								

Favours control Favours dental caries

Suppl. 5. C: Forest plot of meta-analysis investigating buffer capacity (mg/dl) in dental caries versus control by gender subgroups. CI= Confidence Interval, and IV= Inverse Variance method. The forest plot shows reduced buffer capacity in the dental caries group. The subgroups were not statistically different.

Study or Subgroup	Dental caries Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
1.15.1 Female								
Pandey et al., 2015	2.23	0.91	15	3.29	0.87	15	17.7%	-1.16 [-1.94, -0.38]
Prabhakar et al., 2009	8.08	2.09	15	8.66	1.66	15	20.3%	-0.30 [-1.02, 0.42]
Tulunoglui et al., 2006	2.32	0.7	10	3.18	1.6	10	13.6%	-0.67 [-1.57, 0.24]
Subtotal (95% CI)			40			40	51.6%	-0.69 [-1.21, -0.18]
Heterogeneity: Tau² = 0.04; Chi² = 2.51, df = 2 (P = 0.28); I² = 20%								
Test for overall effect: Z = 2.63 (P = 0.008)								
1.15.2 Male								
Pandey et al., 2015	1.99	0.58	15	3.3	1.02	15	16.0%	-1.54 [-2.36, -0.71]
Prabhakar et al., 2009	7.41	1.89	15	8.9	1.39	15	18.8%	-0.87 [-1.63, -0.12]
Tulunoglui et al., 2006	2.39	0.5	10	3.59	2.3	10	13.6%	-0.69 [-1.60, 0.22]
Subtotal (95% CI)			40			40	48.4%	-1.04 [-1.53, -0.55]
Heterogeneity: Tau² = 0.01; Chi² = 2.13, df = 2 (P = 0.34); I² = 6%								
Test for overall effect: Z = 4.15 (P < 0.0001)								
Total (95% CI)			80			80	100.0%	-0.86 [-1.21, -0.51]
Heterogeneity: Tau² = 0.03; Chi² = 5.76, df = 5 (P = 0.33); I² = 13%								
Test for overall effect: Z = 4.76 (P < 0.00001)								
Test for subgroup differences: Chi² = 0.92, df = 1 (P = 0.34), I² = 0%								

Favours control Favours dental caries

Suppl. 5. D: Forest plot of meta-analysis investigating calcium (mg/dl) in dental caries versus control by gender subgroups. CI= Confidence Interval, and IV= Inverse Variance method. The forest plot shows reduced calcium levels in the dental caries group. The subgroups were not statistically different.

Supplement 6: Summary of findings and certainty of evidence.

Summary of findings and certainty of evidence: Primary Outcome

Salivary biomarkers associated with oxidative stress in children with dental caries

Patient or population: Children up to 12 years-old

Intervention: Dental caries

Comparison: Caries-free

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with caries-free	**Risk with dental caries**				
Total antioxidant capacity (age) (TAC)	-	SMD **2.66 SD higher** (1.33 higher to 3.98 higher)	-	550 (8 observational studies)	⊕⊕⊕◯ MODERATE	Children with dental caries probably results in a large increase in total antioxidant capacity.
Total antioxidant capacity (gender) (TAC)	-	SMD **0.98 SD higher** (0.56 higher to 1.39 higher)	-	170 (3 observational studies)	⊕⊕⊕◯ MODERATE	Children with dental caries probably results in a large increase in total antioxidant capacity.
Malondialdehyde (MDA)	-	SMD **0.9 SD lower** (2.69 lower to 0.88 higher)	-	310 (3 observational studies)	⊕⊕◯◯ LOW	Children with dental caries may result in little to no difference in malondialdehyde biomarker.
Malondialdehyde (protein) (MDA)	-	SMD **16.51 SD lower** (29.02 lower to 4 lower)	-	120 (2 observational studies)	⊕⊕◯◯ LOW	Children with dental caries may result in a reduction in malondialdehyde (protein).
Superoxide Dismutase (SOD)	-	SMD **5.83 SD higher** (4.6 lower to 16.25 higher)	-	120 (2 observational studies)	⊕⊕◯◯ LOW	Children with dental caries may result in little to no difference in superoxide dismutase biomarker.
Superoxide Dismutase (protein) (SOD)	-	SMD **5.09 SD higher** (0.01 higher to 10.18 higher)	-	80 (2 observational studies)	⊕⊕◯◯ LOW	Children with dental caries may increase superoxide dismutase (protein).
Uric Acid (UA)	-	SMD **5 SD higher** (2.29 lower to 12.3 higher)	-	120 (2 observational studies)	⊕⊕◯◯ LOW	Children with dental caries may result in little to no difference in uric acid biomarker.

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **SMD:** Standardised mean difference

Summary of findings and certainty of evidence: Secondary Outcome

Salivary parameters in children with dental caries

Patient or population: Children up to 12 years-old

Intervention: Dental caries

Comparison: Caries-free

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with caries-free	**Risk with dental caries**				
Total protein concentration (age)	-	SMD **0.98 SD higher** (0.27 higher to 1.69 higher)	-	346 (7 observational studies)	⊕⊕⊕◯ MODERATE	Children with dental caries probably results in a large increase in total protein concentration.
Total protein concentration (gender)	-	SMD **0.77 SD higher** (0.45 higher to 1.1 higher)	-	160 (3 observational studies)	⊕⊕⊕◯ MODERATE	Children with dental caries probably results in a large increase in total protein concentration.
Salivary flow rate (unstimulated)	-	SMD **0.32 SD lower** (0.63 lower to 0.01 lower)	-	160 (3 observational studies)	⊕⊕◯◯ LOW	Children with dental caries may result in a slight reduction in salivary flow rate.
pH	-	SMD **1.05 SD lower** (1.82 lower to 0.28 lower)	-	310 (5 observational studies)	⊕⊕⊕◯ MODERATE	Children with dental caries probably results in a large reduction in pH.
Buffer capacity	-	SMD **0.58 SD lower** (1.13 lower to 0.03 lower)	-	160 (3 observational studies)	⊕⊕◯◯ LOW	Children with dental caries results in a slight reduction in buffer capacity.
Calcium	-	SMD **0.86 SD lower** (1.21 lower to 0.51 lower)	-	160 (3 observational studies)	⊕⊕⊕◯ MODERATE	Children with dental caries probably results in a large reduction in calcium.

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **SMD:** Standardised mean difference

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

ANEXOS

ANEXO A - Guide for Authors of Archives of Oral Biology

Editors-in-Chief:
Professor S W Cadden, Dundee, Scotland
Dr Fionnuala T. Lundy, Northern Ireland, UK

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Immediately after the abstract, provide a maximum of 6 keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

As Archives of Oral Biology is a journal with a multidisciplinary readership, abbreviations, except those universally understood such as mm, g, min. u.v., w/v and those listed below, should be avoided if possible. Examples of abbreviations which may be used without definition are: ADP, AMP, ATP, DEAE-cellulose, DNA, RNA, EDTA, EMG, tris.

Other abbreviations used to improve legibility should be listed as a footnote on the title page as well as being defined in both the abstract and the main text on first usage. Chemical symbols may be used for elements, groups and simple compounds, but excessive use should be avoided. Abbreviations other than the above should not be used in titles and even these should be avoided if possible. ***Acknowledgements***

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.) but who did not meet all the criteria for authorship (see Authorship section above). ***Formatting of funding sources***

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Bacterial nomenclature

Organisms should be referred to by their scientific names according to the binomial system. When first mentioned the name should be spelt in full and in italics. Afterwards the genus should be abbreviated to its initial letter, e.g. 'S. aureus' not 'Staph. aureus'. If abbreviation is likely to cause confusion or render the intended meaning unclear, the names of microbes should be spelt in full. Only those names which were included in the Approved List of Bacterial Names, Int J Syst Bacteriol 1980; 30: 225-420 and those which have been validly published in the Int J Syst Bacteriol since 1 January 1980 have standing in nomenclature. If there is good reason to use a name that does not have standing in nomenclature, the names should be enclosed in quotation marks and an appropriate statement concerning the nomenclatural status of the name should be made in the text (for an example see Int J Syst Bacteriol 1980; 30: 547-556). When the genus alone is used as a noun or adjective, use lower case Roman not italic, e.g.'organisms were staphylococci' and 'streptococcal infection'. If the genus is specifically referred to use italics e.g. 'organisms of the genus Staphylococcus'. For genus in plural, use lower case roman e.g. 'salmonellae'; plurals may be anglicized e.g.'salmonellas'. For trivial names, use lower case Roman e.g. 'meningococcus'

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• Submit graphics that are disproportionately large for the content.

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List: references should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

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Reference to a journal publication with an article number:

Van der Geer, J., Hanraads, J. A. J., & Lupton, R. A. (2018). The art of writing a scientific article. *Heliyon*, *19*, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.

Reference to a book:

Strunk, W., Jr., & White, E. B. (2000). *The elements of style.* (4th ed.). New York: Longman, (Chapter 4).

Reference to a chapter in an edited book:

Mettam, G. R., & Adams, L. B. (2009). How to prepare an electronic version of your article. In B. S. Jones, & R. Z. Smith (Eds.), *Introduction to the electronic age* (pp. 281–304). New York: E-Publishing Inc.

Reference to a website:

Cancer Research UK. Cancer statistics reports for the UK. (2003). http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ Accessed 13 March 2003.

Reference to a dataset:

[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T. (2015). *Mortality data for Japanese oak wilt disease and surrounding forest compositions.* Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

Reference to a conference paper or poster presentation:

Engle, E.K., Cash, T.F., & Jarry, J.L. (2009, November). The Body Image Behaviours Inventory-3: Development and validation of the Body Image Compulsive Actions and Body Image Avoidance Scales. Poster session presentation at the meeting of the Association for Behavioural and Cognitive Therapies, New York, NY.

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Statistical analysis

Authors should ensure that the presentation and statistical testing of data are appropriate and should seek the advice of a statistician if necessary. A number of common errors should be avoided, e.g.: -

• Use of parametric tests when non-parametric tests are required
• Inconsistencies between summary statistics and statistical tests such as giving means and standard deviations for data which were analysed with non-parametric tests.
• Multiple comparisons undertaken with multiple t tests or non-parametric equivalents rather than with analysis of variance (ANOVA) or non-parametric equivalents.
• Post hoc tests being used following an ANOVA which has yielded a non-significant result.
• Incomplete names for tests (e.g. stating "Student's t test" without qualifying it by stating "single sample", "paired" or "independent sample")
• n values being given in a way which obscures how many independent samples there were (e.g. stating simply n=50 when 10 samples/measurements were obtained from each of 5 animals/human subjects).
• Stating that $P=0.000$ (a figure which is generated by some computer packages). The correct statement (in this case) is $P<0.0005$.

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ANEXO B - PRISMA 2020 checklist

Section and Topic	Item #	Checklist item
TITLE		
Title	1	Identify the report as a systematic review.
ABSTRACT		
Abstract	2	See the PRISMA 2020 for Abstracts checklist.
INTRODUCTION		
Rationale	3	Describe the rationale for the review in the context of existing knowledge.
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.
METHODS		
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.

Section and Topic	Item #	Checklist item
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.
RESULTS		
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.
Study characteristics	17	Cite each included study and present its characteristics.
Risk of bias in studies	18	Present assessments of risk of bias for each included study.
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.
	20c	Present results of all investigations of possible causes of heterogeneity among study results.
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.
DISCUSSION		
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.
	23b	Discuss any limitations of the evidence included in the review.
	23c	Discuss any limitations of the review processes used.
	23d	Discuss implications of the results for practice, policy, and future research.
OTHER INFORMATION		
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.
	24c	Describe and explain any amendments to information provided at registration or in the protocol.
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.
Competing interests	26	Declare any competing interests of review authors.
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.

ANEXO C - Protocolo de registro

1. * Review title.
Give the title of the review in English
Salivary biomarkers of oxidative damage and antioxidant systems response in children with dental caries: systematic review and meta-analysis

2. Original language title.
For reviews in languages other than English, give the title in the original language. This will be displayed with the English language title.
English

3. * Anticipated or actual start date.
Give the date the systematic review started or is expected to start.
01/03/2021

4. * Anticipated completion date.
Give the date by which the review is expected to be completed.
01/06/2021

5. * Stage of review at time of this submission.
Tick the boxes to show which review tasks have been started and which have been completed. Update this field each time any amendments are made to a published record.
Reviews that have started data extraction (at the time of initial submission) are not eligible for inclusion in PROSPERO. If there is later evidence that incorrect status and/or completion date has been supplied, the published PROSPERO record will be marked as retracted. This field uses answers to initial screening questions. It cannot be edited until after registration.
The review has not yet started: No

6. * Named contact.
The named contact is the guarantor for the accuracy of the information in the register record. This may be any member of the review team.
Cristina Antoniali
Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence:
Profa. Dra. Cristina Antoniali

7. * Named contact email.
Give the electronic email address of the named contact.
cristina.antoniali@unesp.br

8. Named contact address
Give the full institutional/organisational postal address for the named contact.
Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry of Araçatuba, Araçatuba, São Paulo, Brazil. Rua José Bonifácio 1193, Vila Mendonca, Araçatuba, SP, cep:16015-050.

9. Named contact phone number.
Give the telephone number for the named contact, including international dialling code.
55-18-981505995

10. * Organisational affiliation of the review.
Full title of the organisational affiliations for this review and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.
Department of Basic Sciences and Department of Preventive and Restorative Dentistry, São Paulo StateUniversity (UNESP), School of Dentistry of Araçatuba, Araçatuba, São Paulo, Brazil

11. * Review team members and their organisational affiliations.
Give the personal details and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong. **NOTE: email and country now MUST be entered for each person, unless you are amending a published record.**
Miss Jordana Resende Martins. Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry Araçatuba, SP, Brazil
Mrs Beatriz Díaz-Fabregat. Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry Araçatuba, SP, Brazil
Mr Wilmer Ramírez-Carmona. Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry Araçatuba, SP, Brazil

Professor Douglas Roberto Monteiro. Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry Araçatuba, SP, Brazil. Graduate Program in Dentistry GPD - Master's Degree, University of Western São Paulo (UNOESTE), Presidente Prudente, SP, Brazil.

Assistant/Associate Professor Juliano Pelim Pessan. Department of Preventive and Restorative Dentistry, São Paulo State University (UNESP), School of Dentistry Araçatuba, SP, Brazil

Assistant/Associate Professor Cristina Antoniali. Department of Basic Sciences, São Paulo State University (UNESP), School of Dentistry Araçatuba, SP, Brazil

12. * Funding sources/sponsors.
Details of the individuals, organizations, groups, companies or other legal entities who have funded or sponsored the review.

The research was carried out with funds from the researcher herself

Grant number(s)
State the funder, grant or award number and the date of award

13. * Conflicts of interest.
List actual or perceived conflicts of interest (financial or academic).

None

14. Collaborators.
Give the name and affiliation of any individuals or organisations who are working on the review but who are not listed as review team members. **NOTE: email and country must be completed for each person, unless you are amending a published record.**

15. * Review question.
State the review question(s) clearly and precisely. It may be appropriate to break very broad questions down into a series of related more specific questions. Questions may be framed or refined using PI(E)COS or similar where relevant.

Salivary biomarkers associated with oxidative damage and antioxidant systems response would be increased in saliva of children with dental caries?

16. * Searches.
State the sources that will be searched (e.g. Medline). Give the search dates, and any restrictions (e.g. language or publication date). Do NOT enter the full search strategy (it may be provided as a link or attachment below.)

The systematic literature search was carried out by two independent researchers (JRM and BDF) according to the eligibility criteria in PubMed, Scopus, Web of Sciences, Embase, Cochrane, LILACS, Google Scholar, and Open Grey databases. In addition, the researchers (JRM and WRC) were performed a manual search through references. Any disagreement was resolved by consensus with a third researcher (CA). Search terms included "Child", "Child, Preschool", "Dental caries", "Biomarkers", "Saliva", and "Oxidative Stress"

17. URL to search strategy.
Do not make this file publicly available until the review is complete

18. * Condition or domain being studied.
Give a short description of the disease, condition or healthcare domain being studied in your systematic review.

Salivary biomarkers of oxidative damage (malondialdehyde), and enzymatic or/and non-enzymatic antioxidant systems response (superoxide dismutase, glutathione peroxidase, uric acid, total antioxidant capacity).

19. * Participants/population.
Specify the participants or populations being studied in the review. The preferred format includes details of both inclusion and exclusion criteria.

We considered the study population to be children from 0 to 12 years old.

20. * Intervention(s), exposure(s).
Give full and clear descriptions or definitions of the interventions or the exposures to be reviewed. The preferred format includes details of both inclusion and exclusion criteria.

Dental caries as localized destruction of the tooth surface initiated by decalcification of the enamel followed by enzymatic lysis of organic structures and leading to cavity formation, the cavity may penetrate the enamel and dentin and reach the pulp.

21. * Comparator(s)/control.
Where relevant, give details of the alternatives against which the intervention/exposure will be compared (e.g. another intervention or a non-exposed control group). The preferred format includes details of both inclusion and exclusion criteria.

Caries-free

22. * Types of study to be included.

Give details of the study designs (e.g. RCT) that are eligible for inclusion in the review. The preferred format includes both inclusion and exclusion criteria. If there are no restrictions on the types of study, this should be stated.
Observational studies

23. Context.
Give summary details of the setting or other relevant characteristics, which help define the inclusion or exclusion criteria.

24. * Main outcome(s).
Give the pre-specified main (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.
Salivary biomarkers (O_1: oxidative damage and O_2: antioxidant systems response)

* Measures of effect
Please specify the effect measure(s) for you main outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.
We measured mean difference

25. * Additional outcome(s).
List the pre-specified additional outcomes of the review, with a similar level of detail to that required for main outcomes. Where there are no additional outcomes please state 'None' or 'Not applicable' as appropriate to the review
None

* Measures of effect
Please specify the effect measure(s) for you additional outcome(s) e.g. relative risks, odds ratios, risk difference, and/or 'number needed to treat.
None

26. * Data extraction (selection and coding).
Describe how studies will be selected for inclusion. State what data will be extracted or obtained. State how this will be done and recorded.
For each manuscript we extract the following data: name of the first author, title of the journal, year of publication, study design, information on funding and conflict of interest, total number of participants, children with dental caries and caries-free, and levels of oxidative stress biomarkers.

27. * Risk of bias (quality) assessment.
State which characteristics of the studies will be assessed and/or any formal risk of bias/quality assessment tools that will be used.
The quality of the risk of bias was evaluated by Newcastle Ottawa Scale (NOS) for observational studies.

28. * Strategy for data synthesis.
Describe the methods you plan to use to synthesise data. This **must not be generic text** but should be **specific to your review** and describe how the proposed approach will be applied to your data. If meta-analysis is planned, describe the models to be used, methods to explore statistical heterogeneity, and software package to be used.
Meta-analysis was planned whenever studies would be considered combinable and relatively homogeneous. We measured Mean Difference using Inverse Variance as statistical method and the Fixed-Effects as analysis model, corresponding 95% confidence intervals were calculated (represented in forest plot graphical). Publication bias and small study effects were assessed by funnel plot graphical and Egger' regression test for any analyses that included at least 10 studies (p<0.10 was taken as statistical evidence of the presence of small study effects and potential publication bias).

29. * Analysis of subgroups or subsets.
State any planned investigation of 'subgroups'. Be clear and specific about which type of study or participant will be included in each group or covariate investigated. State the planned analytic approach.
There were subgroups according to outcome evaluated.

30. * Type and method of review.
Select the type of review, review method and health area from the lists below.
Type of review
Cost effectiveness
Meta-analysis
Yes
Systematic review
Yes

Oral health
Yes

Select each language individually to add it to the list below, use the bin icon to remove any added in error.
English

32. * Country.
Select the country in which the review is being carried out. For multi-national collaborations select all the countries involved.
Brazil

33. Other registration details.
Name any other organisation where the systematic review title or protocol is registered (e.g. Campbell, or The Joanna Briggs Institute) together with any unique identification number assigned by them. If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here. If none, leave blank.

34. Reference and/or URL for published protocol.
If the protocol for this review is published provide details (authors, title and journal details, preferably in Vancouver format) Add web link to the published protocol. Or, upload your published protocol here in pdf format. Note that the upload will be publicly accessible.
No I do not make this file publicly available until the review is complete
Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

35. Dissemination plans.
Do you intend to publish the review on completion?
Yes

Give brief details of plans for communicating review findings.?
36. Keywords.

38. * Current review status.
Update review status when the review is completed and when it is published. New registrations must be ongoing so this field is not editable for initial submission. Please provide anticipated publication date
Review_Ongoing

39. Any additional information.
Provide any other information relevant to the registration of this review.

40. Details of final report/publication(s) or preprints if available.
Leave empty until publication details are available OR you have a link to a preprint (NOTE: this field is not editable for initial submission). List authors, title and journal details preferably in Vancouver format. Give the link to the published review or preprint.

ANEXO D - Newcastle Ottawa Scale-modifield for cross sectional studies

Aadapted for CROSS SECTIONAL STUDIES

Selection: (Maximum 5 stars)
1) Representativeness of the sample:
a) Truly representative of the average in the target population. * (all subjects or random sampling)
b) Somewhat representative of the average in the target population. * (non-random sampling)
c) Selected group of users.
d) No description of the sampling strategy.
2) Sample size:
a) Justified and satisfactory. *
b) Not justified.
3) Non-respondents:
a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory. *
b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory.
c) No description of the response rate or the characteristics of the responders and the non-responders.
4) Ascertainment of the exposure (risk factor):
a) Validated measurement tool. **
b) Non-validated measurement tool, but the tool is available or described.*
c) No description of the measurement tool.

Comparability: (Maximum 2 stars)
1) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled.
a) The study controls for the most important factor (select one). *
b) The study control for any additional factor. *

Outcome: (Maximum 3 stars)
1) Assessment of the outcome:
a) Independent blind assessment. **
b) Record linkage. **
c) Self report. *
d) No description.
2) Statistical test:
a) The statistical test used to analyze the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and the probability level (p value). *
b) The statistical test is not appropriate, not described or incomplete.

ANEXO E - Referências da Introdução Geral

Albino J, Tiwari T. Preventing childhood caries: a review of recent behavioral research. J Dent Res. 2016;95(1):35-42.

Anil S, Anand PS. Early childhood caries: prevalence, risk factors, and prevention. Front Pediatr. 2017;5:157.

Arana C, Moreno-Fernández AM, Gómez-Moreno G, Morales-Portillo C, Serrano-Olmedo I, de la Cuesta Mayor MC, Martín Hernández T. Increased salivary oxidative stress parameters in patients with type 2 diabetes: relation with periodontal disease. Endocrinol Diabetes Nutr. 2017;64(5):258-64.

Araujo HC, Nakamune ACMS, Garcia WG, Pessan JP, Antoniali C. Carious lesion severity induces higher antioxidant system activity and consequently reduces oxidative damage in children's saliva. Oxid Med Cell Longev. 2020;2020:3695683.

Battino M, Ferreiro MS, Gallardo I, Newman HN, Bullon P. The antioxidant capacity of saliva. J Clin Periodontol. 2002;29(3):189–194.

Betteridge DJ. What is oxidative stress? Metabolism. 2000;49 (2 Suppl 1):3-8.

Brouwer F, Askar H, Paris S, Schwendicke F. Detecting secondary caries lesions: a systematic review and meta-analysis. J Dent Res. 2016;95(2):143-51.

Buczko P, Zalewska A, Szarmach I. Saliva and oxidative stress in oral cavity and in some systemic disorders. J Physiol Pharmacol. 2015;66(1):3-9.

Colak H, Dürgergil CT, Dalli M, Hamidi MM. Early childhood caries update: a review of causes, diagnoses, and treatments. J Nat Sci Biol Med. 2013;4(1):29-38.

Cunha-Cruz JD, Scott J, Rothen M, Mancl L, Lawhorn T, Brossel K, Berg J. Salivary characteristics and dental caries: evidence from general dental practices. J Am Dent Assoc. 2013;144(5):31–40.

Darczuk D, Krzysciak W, Vyhouskaya P, Kesek B, Galecka-Wanatowicz D, Lipska W, Kaczmarzyk T, Gluch-Lutwin M, Mordyl B, Chomyszyn-Gajewska M. Salivary oxidative status in patients with oral lichen planus. J Physiol Pharmacol. 2016;67(6):885-94.

Dodwad R, Betigeri AV, Preeti B. Estimation of total antioxidant capacity levels in

saliva of caries-free and caries-active children. Contemp Clin Dent. 2011;2(1):17-20.

Fenoll-Palomares C, Muñoz-Montagud JV, Sanchiz V, Herreros B, Hernández V, Mínguez M, Benages A. Unstimulated salivary flow rate, pH and buffer capacity of saliva in healthy volunteers. Rev Esp Enferm Dig. 2004;96(11):773-83.

Halliwell B. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic Res. 1999;31(4):261-72.

Hassaneen M, Maron JL. Salivary diagnostics in pediatrics: applicability, translatability, and limitations. Front Public Health. 2017;5:83.

Hoskin ER, Keenan AV. Can we trust visual methods alone for detecting caries in teeth? Evid Based Dent. 2016;17(2):41-2.

Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. *J Prosthet Dent.* 2001;85:162–9.

Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. J Oral Biol Craniofac Res. 2016;6(1):66-75.

Jurczak A, Kościelniak D, Skalniak A, Papież M, Vyhouskaya P, Krzyściak W. The role of the saliva antioxidant barrier to reactive oxygen species with regard to caries development. Redox Rep. 2017 Nov;22(6):524-533.

Kawashita Y, Kitamura Y, Saito T. Early childhood caries. Int J Dent. 2011;2011:725320.

Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. Adv Dent Res. 2000;14:40-7.

Machiulskiene V, Campus G, Carvalho JC, Dige I, Ekstrand KR, Jablonski-Momeni A, Maltz M, Manton DJ, Martignon S, Martinez-Mier EA, Pitts NB, Schulte AG, Splieth CH, Tenuta LMA, Ferreira Zandona A, Nyvad B. Terminology of dental caries and dental caries management: consensus report of a workshop organized by ORCA and Cariology Research Group of IADR. Caries Res. 2020;54(1):7-14.

Mahjoub S, Ghasempour M, Gharage A, Bijani A, Masrourroudsari J. Comparison of total antioxidant capacity in saliva of children with severe early childhood caries and caries-free children. Caries Res. 2014;48(4):271-5.

Malamud D. Saliva as a diagnostic fluid. Dent Clin North Am. 2011;55(1):159–78.

Manton DJ. Child dental caries: a global problem of inequality. EClinicalMedicine. 2018**;**17(1):3-4.

Roth G, Calmes R, editores. Glândulas salivares e saliva. In: Roth G, Calmes R, editores. Oral biology. St Louis: CV Mosby; 1981. p. 196-236.

Senthil Eagappan AR, Arun Prasad Rao V, Sujatha S, Senthil S, Sathiyajeeva J, Rajaraman G. Evaluation of salivary nitric oxide level in children with early childhood caries. Dent Res J. 2016;13(4):338-41.

Silva PVD, Troiano JA, Nakamune ACMS, Pessan JP, Antoniali C. Increased activity of the antioxidants systems modulate the oxidative stress in saliva of toddlers with early childhood caries. Arch Oral Biol. 2016;70:62-66..

Spielman N, Wong DT. Saliva: diagnostics and therapeutic perspectives. Oral Dis. 2011;17(4):345–54.

Subramanyam D, Gurunathan D, Gaayathri R, Vishnu Priya V. Comparative evaluation of salivary malondialdehyde levels as a marker of lipid peroxidation in early childhood caries. Eur J Dent. 2018;12(1):67-70.

Tartaglia GM, Gagliano N, Zarbin L, Tolomeo G, Sforza C. Antioxidant capacity of human saliva and periodontal screening assessment in healthy adults. Arch Oral Biol. 2017 Jun;78:34-38.

Tulunoglu O, Demirtas S, Tulunoglu I. Níveis totais de antioxidantes na saliva em crianças relacionados à cárie, idade e sexo. Int J Paediatr Dent. 2006;16:186-91.