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Physical and chemical properties of saliva and its role in Early Childhood caries – A systematic review and meta-analysis

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A B S T R A C T

Background: Early Childhood Caries is a chronic disease of childhood and salivary parameters are considered as one of the prime etiological factors of Early Childhood Caries.
Aim: To develop a systematic review based on the relation between physical and chemical properties of saliva and Early childhood caries by comparing children with and without Early childhood caries.
Methods: PubMed, Cochrane, Lilacs, Embase, Scopus, and additional manual search was done up to April 2021 to identify the original cross-sectional observational studies published in English. The risk of bias and quality of the included papers were assessed based on New castle Ottawa guidelines.
Results: From a total of 1709 identified studies, only 22 articles were included in this systematic review and 10 studies were qualified for meta-analysis. Eight studies were classified as ''moderate risk of bias'' and fourteen studies were classified as ''high risk of bias''.
Conclusion: There was a significant difference in physical and chemical properties of saliva in children with and without Early childhood caries. Since wide disparity were evident in available studies, further studies are needed to arrive to a definitive conclusion.

1. Introduction

Saliva is a complex body fluid and maintains the health of the oral cavity with its organic and inorganic constituents. The various functions of saliva include lubrication, serving as an ion reservoir, cleansing action, digestion of carbohydrates, antimicrobial action, buffering capacity, pellicle formation and maintaining water balance.[1] Saliva contains various hormones, antibodies, growth factors, enzymes and microbes similar to serum. Importantly, saliva is used as a diagnostic tool due to its ease of availability, low cost, minimal risk of cross contamination and is more economical in terms of storage as compared to serum.[2] Salivary characteristics and its components have been considered as a predisposing factor for the development of Early Childhood Caries (ECC) in children.[3,4] ECC is a complex disease of childhood in children below 6 years of age and affects the newly erupted immature teeth, leading to the development of hypoplastic defects.[5]

The physical properties of saliva, namely its pH and buffering capacity neutralizes the acid produced by the cariogenic bacteria, whereas, the flow rate and viscosity provide a flushing effect and helps in eliminating bacteria and food debris from the tooth surfaces. Saliva acts as a source of calcium and phosphate and plays an imperative role in remineralization of incipient carious lesions. These inorganic ions of saliva improve post-eruptive maturation of enamel by influencing the precipitation or dissolution of Hydroxy Apatite Crystals (HAP).[6–8] Salivary proteins and peptides have several anti-bacterial properties and help to prevent development of dental caries. Salivary amylase, an important salivary enzyme, is available in abundance in the oral cavity and helps in modulating the bacterial activity, and promotes clearance of bacteria, thereby, reducing the incidence of caries.[9] Salivary immunoglobulin, namely secretory IgA acts as a first line of defence and protects the oral mucosa from bacterial adhesion by neutralizing the bacterial toxins and enzymes.[10,11] Saliva and Gingival Crevicular Fluid (GCF) contains several antimicrobial peptides, namely, α, β defensins, histatins and human cathelicidin LL-37, which possess both bactericidal and/or bacteriostatic activities against oral pathogens.[12–14] Histatins 1, 3 and 5 are natural antimicrobial peptides present in the saliva and it destabilizes the bacterial cell membrane. One of the important functions of histatin is the inhibition of matrix metallo proteinases (MMP) and

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promoting wound healing.[15] LL-37 is present both in saliva and GCF and studies have reported that LL-37 has antimicrobial activity against Streptococci and Lactobacillus.[14] Carbonic Anhydrase (CA) VI, a salivary isoenzyme maintains the physiologic pH of saliva and its pH alteration capacity is based on its concentration in saliva and not based on its activity.[16–18] Studies identified that the concentration of CA VI was higher in caries active children, the possible explanation could be that this isoenzyme adheres to the enamel pellicle and biofilm, regulates the PH and neutralizes the acids produced by oral pathogens.[19,20] Salivary lysozymes are an antimicrobial enzyme with a molecular weight of 14.7 kDa. It degrades the bacterial cell wall by activation of bacterial autolysins and bacterial aggregation.[21–24] A study done by Lertsirivorakul et al. reported that the salivary lysozyme levels were found to be higher in children with Severe ECC (S ECC), explaining its possible association with ECC.[25]

To date, several cross-sectional and longitudinal studies have been published, providing insight into the salivary components and its role in ECC. However, it is difficult to interpret whether the ECC is associated only with increase/decrease of individual parameter. Because of different parameters assessed in each study under different circumstances, the results and conclusions obtained in many studies are sometimes conflicting and may be difficult to compare and interpret. Due to the presence of wide range of salivary factors and its role in ECC, this systematic review was undertaken to answer the following important questions:

What is the role of the physical and chemical properties of saliva in association with ECC?

Are there variations in the physical and chemical properties of saliva in children with and without ECC?

Furthermore, meta-analysis of selected parameters was done to find out a possible relationship between salivary factors and ECC. We hypothesize that there is a difference in the physical and chemical properties of saliva in children with and without ECC.

2. Materials and methods

The present systematic review was carried out according to the protocols of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta Analysis) GUIDELINES.[26]

2.1. Search strategies

The literature search was performed using broad MeSH terms and keywords and covered data up to April 2021. The data were obtained using the MEDLINE (PubMed) search engine, Cochrane, Lilacs, Embase, Scopus as well as an additional manual search. The MeSH terms and the key words used were (children) AND (physicochemical properties) OR (inorganic ions) OR (saliva) OR (biochemical parameters) OR (salivary proteins) OR (salivary peptides) OR (salivary enzymes) AND (with and without early childhood caries) OR (caries free and caries active children). The studies which were identified through the electronic search using the cited keywords but did not evaluate the relation between physical and chemical properties of saliva and ECC were excluded from this systematic review as they were considered to have a different research focus. To identify grey literature, www.opengrey.eu and Google Scholar were also searched for any unpublished material. We hand searched several key journals with the help of an experienced librarian to identify articles that could have been missed from the electronic data base search. The reference lists of the included articles were also checked to identify any potential article which could have been missed in the electronic search. The reference lists of the retrieved articles were also checked for additional studies.

2.2. Selection criteria

This systematic review and meta-analysis included cross-sectional observational studies which evaluated the relation between salivary physical and chemical properties and ECC by comparing children with and without ECC. The selected literature covers the data up to April 2021. Studies included all healthy children regardless of race, gender, socioeconomic status from birth to six years of age. Children without intake of any medication were selected, as medication could affect the salivary composition. Articles on genetic studies, reviews, case reports, abstracts, letters and conference proceedings were excluded. Articles published in English were only considered to include in the systematic review during electronic and hand search. The search identified a total of 1709 studies to be included in the present systematic review. Following the removal of 1083 duplicates, 626 records were screened based on the title, abstract, and keywords. Of these, 576 records were eliminated due to different outcome variable. The remaining 50 papers were assessed completely. The reason for exclusion of 28 articles at this stage were due to reasons such as different age groups, treatment comparison, genetic studies, different sample selection and comparison with rampant caries. After a full text review, 22 studies were included in the present systematic review. Fig. 1 depicts the PRISMA flow diagram of study identification process.

2.3. Inclusion criteria

Criteria for inclusion was based on PECO strategy, as described below.

P (participants): Healthy children less than 6 years of age.

E (Exposure) - Children with ECC.

C (comparison): Children without ECC.

O (outcome): Saliva, physicochemical properties, inorganic ions, salivary proteins, salivary peptides, salivary enzymes.

2.4. Data extraction and quality assessment of included studies

Data extraction was done by two independent reviewers. DR and PR assessed all the articles independently with respect to the inclusion and exclusion criteria. Any conflicts were resolved by a third reviewer RG, and required input was provided. The data extraction was done using electronic Excel spread sheet (Excel 10, Microsoft Corp., Redwood City, Calif., USA). Studies that required more information were obtained through communication with the author through Research Gate. After removing duplicates, the titles and abstracts of the records were screened based on the eligibility criteria to decide upon the inclusion for further full-text reading. Quality assessment was performed based on New Castle Ottawa guidelines for cross-sectional studies.[27] The New Castle Ottawa checklist was used by both the reviewers for coding the data and these reviewers were calibrated for inter-examiner agreement (Kappa- 0.9). Studies were categorized as having low, moderate, and high methodological quality, according to the number of stars allotted to each study. Studies were considered to have low risk of bias if the studies were allotted with over 7 stars, moderate risk if between 5 and 7 stars, and high risk if under 5 stars. Review Manager 2012 statistical software (Revman version 5.3, London, UK) was used to enter the data for meta-analysis and the mean difference was plotted in Forest plot. The mean difference of individual salivary parameters with 95% confidence interval (CI) were calculated and pooled in meta-analysis. Chi-square and I^2 test were done to assess the clinical heterogenecity of the studies. An I^2 value between 50% and 100% was considered to have statistical heterogenecity. Random effect generalized linear models with 95% CI were used to estimate effect size. Review Manager 2012 statistical software (Revman version 5.3, London, UK) was used to evaluate publication bias. The publication bias was examined visually using funnel plot and Egger's test was used to assess the degree of asymmetry.

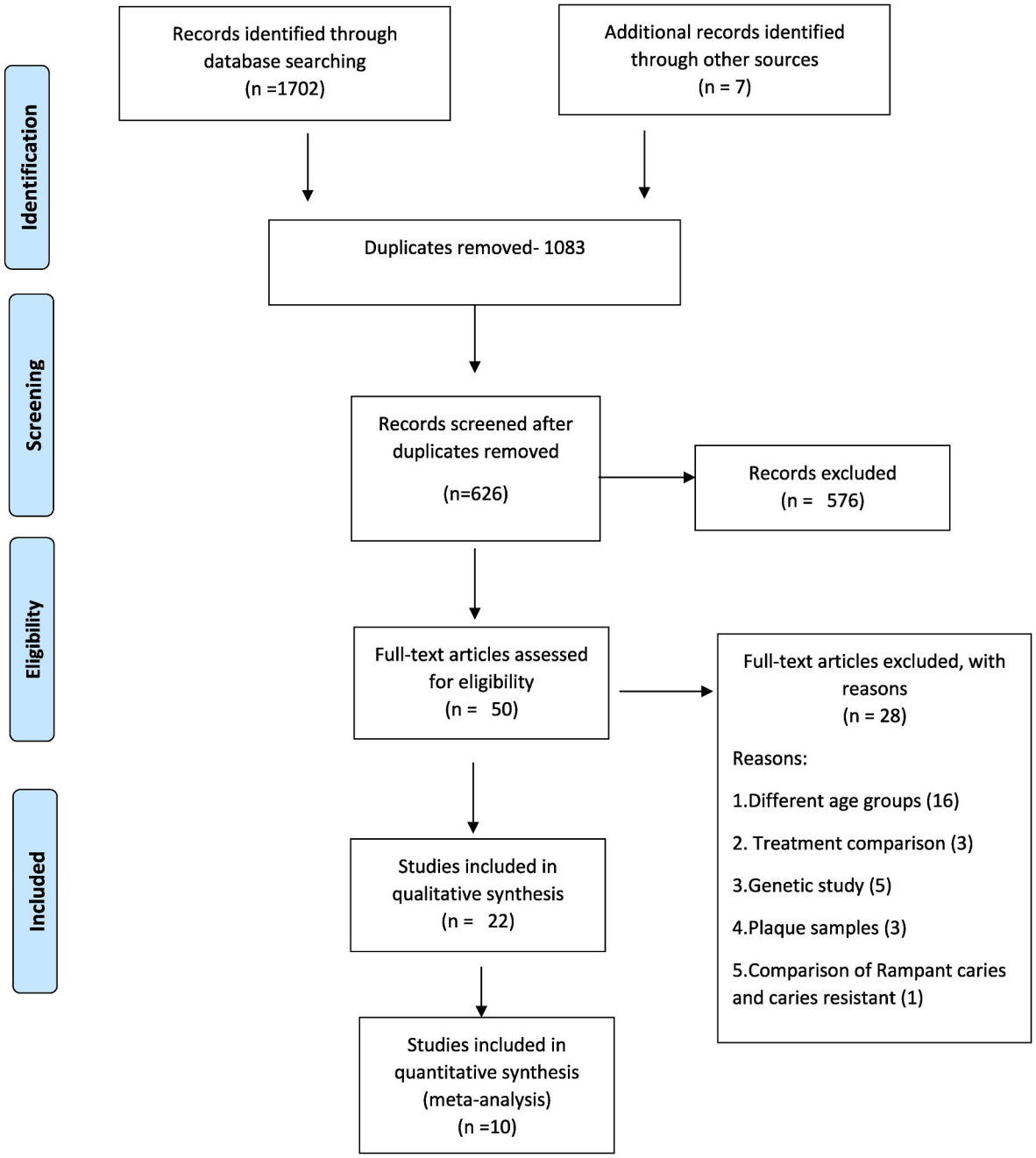

Fig. 1. PRISMA Search Flow chart.

3. Results

3.1. Study selection

A total of 22 studies were identified for inclusion in the review. The electronic search provided a total of 1702 articles, and manual search provided 7 articles; hence there were a total of 1709 articles. Of these 1709 articles, 1083 articles were excluded after identification of duplicates. Out of 626 articles, 576 articles were excluded after reading the title, abstracts, and full texts as they did not meet the inclusion criteria. The full texts of the included 22 articles were reviewed in detail. Fig. 1 depicts the flow chart of complete data collection and search process.

3.2. Characteristics of included studies

Information regarding the study characteristics is given in Table 1. This systematic review and meta-analysis included the studies that discusses the physical and chemical properties of saliva. In addition, other parameters evaluated in the studies include total antioxidant capacity, salivary fluoride, microbial profile and analysis of bacterial protein. Sample size notably varies between all the studies. Children less than 6 years were included in the study and only one study has evaluated children in 2 age groups, but the data of children between 3 and 5 years were included in the review.[28] Eight studies have evaluated salivary pH, 6 studies have assessed flow rate, 8 studies have estimated buffering capacity, 2 studies have evaluated salivary viscosity, 5 studies have measured the salivary calcium, 6 studies have assessed salivary phosphorus, 2 studies estimated alkaline phosphatase and 11 studies looked

Table 1
Characteristics of included studies.

Author/Year Study	group (n)	Age (Months/ years)	Parameters evaluated	Other parameters	Data of outcome variable (p value)	Results
de Farias DG, 2003	40 Children 20-ECC 20-caries-free children	12–47 months	Salivary antibodies, Salivary amylase and salivary proteins	–	IgA - $p < 0.05$* IgG - $p < 0.05$* IgM – $p > 0.05$ Total proteins - $p > 0.05$ Amylase (U/L) - $p > 0.05$	Salivary IgA and IgG were higher in children with ECC. No significant difference in IgM, proteins and salivary amylase between the groups
Shahrabi M, 2008	75 Children 25-caries free 25- Moderate caries 25- Severe caries	3–5 Years	Salivary Calcium, Phosphate Alkaline Phosphatase	–	Salivary calcium – p value – 0.9 Salivary phosphate – p value – 0.2 Alkaline phosphatase – p value – 0.07	Salivary phosphate and alkaline phosphatase were found to be higher in caries free children though it was not statistically significant
Bagherian A, 2008	90 Children caries free- 45 ECC-45	36–70 months	SIgA, IgG	–	SIgA – p value – 0.01* SIgG – p value – 0.04*	Salivary SIgA and IgG were significantly higher in children with ECC
Shifa et al., 2008	20 Children caries free- 10 ECC-10	3–6 years	sIgA	–	sIgA – p value – 0.76	No significant difference in sIgA level between the groups
Sharaf AA, 2010	90 children ECC-60 caries -free-30	36–71 months	Salivary flow rate Buffering capacity	Bacterial counts (*Mutans Streptococci and Lactobacilli*)	Salivary flow rate – p value – 0.06 Salivary Buffering capacity – p value – 0.75	No statistically significant difference in the salivary buffering capacity and salivary flow rate between the groups.
Martínez-Pabón MC, 2010	201 Children ECC- 143 Caries-free- 58	2–5 years	Salivary flow rate pH Buffering capacity	Bacterial counts	Salivary flow rate – p value – 0.12 Salivary pH - p value – 0.61 Buffering capacity- p value – 0.70	No significant difference in salivary pH, buffering capacity and flow rate between ECC and non ECC group
Bhalla S, 2010	100 Children caries free- 50 ECC-50	4–6 years	Salivary flow rate, pH mean protein concentration, and the electrophoretic profile of salivary proteins	–	Salivary flow rate - $p > 0.05$ Salivary pH - $p > 0.05$ Mean protein - $p > 0.05$ Proline rich protein – p value – 0.02 * Amylase – p value – 1.00 Glyco protein – p value – 0.02*	A significant inverse correlation between the mean protein concentration and the whole salivary flow rate. Proline rich proteins were found to be higher in caries-free children and glycoprotein was found to be higher in children with ECC.
Bagherian A, 2012	90 Children caries free- 45 ECC-45	36–70 months	Salivary pH, Buffering capacity, Calcium, Phosphate sIgA	–	Salivary pH- p value −0.002* Salivary Buffering capacity – p value - 0.002* sIgA- p value – 0.015* Salivary calcium – p value - 0.84 Salivary Phosphate – p value – 0.34	Salivary pH and Buffering capacity were high in caries free children. No significant difference in salivary calcium and phosphate level between the groups sIgA concentration was significantly higher among the ECC group
Kaur et al., 2012	60 Children caries free- 30 ECC-30	4–6 years	Flow rate pH Buffering capacity Viscosity, Calcium Phosphate Alkaline phosphatase	–	Flow rate - $p < 0.001$* pH- $p < 0.001$* Buffering capacity- $p < 0.001$* Viscosity - $p < 0.001$* Calcium - $p > 0.05$ Phosphate- $p < 0.001$* Alkaline phosphatase - $p < 0.05$	PH, Buffering capacity, alkaline phosphatase were found to be high in caries -free children. Salivary viscosity, calcium and phosphorus were found to be higher in children with ECC
Jolly et al., 2014	30 children ECC-15 caries-free-15	3–6 years	Salivary calcium and phosphorus	–	Salivary calcium in stimulated saliva p value – 0.05 Salivary calcium in unstimulated saliva – p value −0.02* Salivary phosphorus in stimulated saliva – 0.01* Salivary phosphorus	Salivary calcium and inorganic phosphorus were found to be higher in caries free children.

(*continued on next page*)

Table 1 (*continued*)

Author/Year Study	group (n)	Age (Months/ years)	Parameters evaluated	Other parameters	Data of outcome variable (p value)	Results
					in unstimulated saliva- p value - 0.8	
Jayaraj D,2015	100 Children caries free- 50 ECC-50	Under 6 years of age	alivary flow rate, Salivary pH and Buffering capacity	–	Salivary flow rate – p value – 0.77 Salivary pH- p value - 0.24 Buffering capacity – p value – 0.30	No significant difference was evident in salivary pH and buffering capacity between the groups.
Muchandi S, 2015	50 Children caries free- 25 SECC-25	3–5 years	Salivary pH	TAC	Salivary pH- p value - <0.0001*	Salivary pH was higher in caries- free group
Jurczak A, 2015	82 Children 41- ECC 41-caries-free children	ECC- 5 ± 2.5 Caries-free-5 ± 1.5	histatin-5 β-defensin-2	Bacterial Profile	Histatin –5 – p value – 0.0002* β-defensin-2- p value - 0.04*	Significant increase in the concentration of histatin-5 and β-defensin-2 ECC group The increase in the level of histatin-5 and β-defensin-2 is positively correlated with the progression of the disease.
Lertsirivorakul et al., 2015	64 Children caries free- 32 S ECC-32	4–6 years	Salivary Flow rate Total protein Salivary lysozyme	–	Salivary flow rate – p value - 0.93 Total protein – p value - 0.98 Lysozyme- p value -< 0.001*	No significant difference in salivary flow rate and mean protein concentration was evident between the groups. Salivary lysozyme values were found to be increased in children with S-ECC
Colombo et al., 2016	57 Children caries free- 19 ECC-17 S-ECC- 21	36–60 months	Total salivary IgA levels	Microbial culture Detection of salivary IgA antibody reactive with S. *mutans* GbpB	Salivary IgA – p value – 0.125	No significant difference in the salivary IgA level between caries-free, ECC and S ECC group Children with severe early childhood caries and high levels of *mutans streptococci* have reduced salivary IgA response to *S. mutans* GbpB
Colombo et al., 2016	83 Children caries free- 29 ECC-25 S-ECC- 29	36–60 months	Salivary concentrations of cathelicidin LL-37, human β-defensin 2 (Hbd-2), human β-defensin 3 (Hbd-3) and human-histatin 5 (HTN-5)	–	LL-37 – p value - 0.007* hBD-2 – p value - 0.01* hBD-3 – p value - 0.10 HTN-5 – p value - 0.68	Weak correlation of antimicrobial peptides among CF, ECC and S-ECC groups.
Makawi Y, 2017	120 Children Divided into high caries and low caries group	3–5 years 13–15 years	pH Buffering capacity	Carbonic anhydrase	pH – p value - < 0.001* Buffering capacity -p value - < 0.001*	Significant difference in pH and Buffering capacity between both the groups
Villavicencio et al., 2018	124 children ECC-69 CARIES-FREE-55	3–4 years	Buffering capacity	CFU, Plaque index	Buffering capacity – p value – 0.3	Though it was not statistically significant, buffering capacity was found to be higher in caries- free group
Bachtiar EW,2018	32 Children Caries free-16 ECC-16	3–5 Years	Salivary viscosity Salivary protein profile	–	Not mentioned	Salivary viscosity was higher in children with ECC
Jayakaran TG, 2020	86 Children caries free-43 ECC-43	3–6 years	salivary peptide HNP1	–	Salivary peptide HNP1 p value - < 0.001*	Statistically significant difference in salivary peptide HNP1 in children with and without ECC. A decrease in salivary peptide HNP1 was observed in children with ECC.
Abbas MJ,2020	77 children Caries free- 39 ECC- 38	37–72 months	Salivary flow rate, pH, Buffering capacity, phosphate	Salivary Fluoride	Salivary pH- p value – 0.9 Buffering capacity – p value –0.71 Salivary flow rate – p value – 0.32 Salivary phosphate – p value – 0.29	No significant difference in salivary flow rate, pH, buffering capacity and phosphate between the groups
Aruna S, 2020	18 Children caries free-9 ECC = 9	3–6 Years	Salivary Calcium, Phosphorus	–	Not mentioned	Salivary Calcium and phosphorus were higher in caries-free group than in children with ECC

into salivary proteins and peptides.

3.3. Risk of bias assessment

Information regarding quality assessment of included study is explained in Table 2. New Castle Ottawa guidelines for cross-sectional studies has been used to assess the quality of included study. Ascertainment of exposure was not considered for quality assessment, as all the included studies were cross-sectional, so exposure could not be identified. Eight studies were found to have moderate risk of bias and 14 studies were found to have high risk of bias and low level of evidence.

3.4. Description of meta analysis results

Fig. 2a shows relationship between ECC and salivary pH levels from 2 studies containing 57 children with ECC and 84 caries-free children.

Table 2
Quality assessment and Risk of Bias evaluation.

Criteria	de Farias, 2003	Shahrabi M, 2008	Bagherian A, 2008	Shifa S et al., 2008	Sharif AA, 2010	Martínez-Pabón MC, 2010	Bhalla S, 2010	Bagherian A, 2012	Kaur A,2012	Jolly LR et al., 2014
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 group. * (non-random sampling)										
c.Selected group of users/convenience sample	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
d.No description of sample strategy										
2.Sample size:										
a.Justified and satisfactory * (including sample size calculation).										
b.Not justified.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3.Non-respondents:										
Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory.										
a.The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory.										
b.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**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
b.Non-validated measurement tool, but the tool is available or described*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.No description of the measurement tool	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5.Comparability: (Maximum 2 stars)										
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 *	*	*	*	*	*	*	*	*	*	*
b.The study control for any additional factor*			*			*				
6.Outcome: (Maximum 3 stars)										
Assessment of outcome:										
a.Independent blind assessment**										
b.Unblinded assessment **	**	**	**	**	**	**	**	**	**	**
c.Self-report*										
d.No description										
7.Statistical test:										
a.Statistical test used to analyse the data clearly described, appropriate and measures of association presented including confidence intervals and probability level (p value).*	*	*	*	*	*	*	*	*	*	*
b.Statistical test not appropriate, not described or incomplete.										

Criteria	Jayaraj D, 2015	Muchandi S, 2015	Jurczak A, 2015	Lertsirivorakul J et al., 2015	Colombo NH et al., 2016	Colombo NH et al., 2016	Makawi Y,2017	Villavicencio J, 2018	Jayakaran TG, 2020	Bachtiar EW, 2018	Abbas MJ, 2020	Aruna S, 2020
Selection (Maximum 5 stars)												
1. Representativeness of the Sample												
a.Truly representative of the average in the target population. * (all subjects or random sampling)												NA
b.Somewhat representative of the average in the target group. * (non-random sampling)												NA
c.Selected group of users/convenience sample	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NA
d.No description of sample strategy												NA
2.Sample size:												
a.Justified and satisfactory * (including sample size calculation).	*			*					*		*	NA

(*continued on next page*)

Table 2 (*continued*)

Criteria	Jayaraj D, 2015	Muchandi S, 2015	Jurczak A, 2015	Lertsirivorakul J et al., 2015	Colombo NH et al., 2016	Colombo NH et al., 2016	Makawi Y,2017	Villavicencio J, 2018	Jayakaran TG, 2020	Bachtiar EW, 2018	Abbas MJ, 2020	Aruna S, 2020
b.Not justified.		✓	✓		✓	✓	✓	✓		✓		NA
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**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
b.Non-validated measurement tool, but the tool is available or described.*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
c.No description of the measurement tool	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5.Comparability: (Maximum 2 stars) 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 *	*	*	*	*	*	*	*	*	*	*	*	*
b.The study control for any additional factor*				*	*	*			*			
6.Outcome: (Maximum 3 stars) Assessment of outcome:												
a.Independent blind assessment**												
b.Unblinded assessment **	**	**	**	**	**	**	**	**	**	**	**	**
c.Self report*												
d.No description												
7.Statistical test:												
a.Statistical test used to analyse the data clearly described, appropriate and measures of association presented including confidence intervals and probability level (p value).*	*	*	*	*	*	*	*	*	*	*	*	*
b.Statistical test not appropriate, not described or incomplete.												

NA – Not applicable.

✓- Tick mark is given where stars cannot be given.

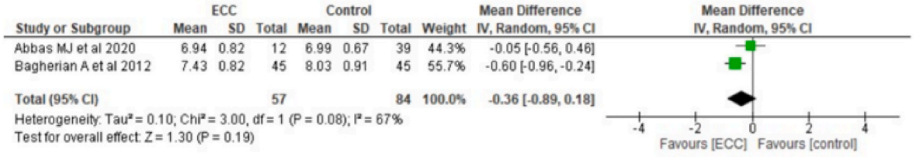

Fig. 2a. Relationship between ECC and PH.

Pooled data indicated that children with ECC had a low salivary pH level than controls (MD = 0.36, 95% CI: 0.89, 0.18). Fig. 2b depicts the relationship between SECC and salivary pH levels from 2 studies containing 51 children with SECC and 64 caries-free children. Pooled data showed that children with SECC had a low salivary pH (MD = 0.58, 95% CI:1.52,0.36).

Fig. 3 shows relationship between ECC and salivary flow rate from 3 studies containing 93 children with ECC and 90 caries-free children. Pooled data indicated that children with ECC had a low salivary flow than caries-free children (MD = 0.41, 95% CI:0.73, 0.09).

Fig. 4 shows relationship between ECC and salivary calcium from 4 studies containing 99 children with ECC and 99 caries free children. Forest plot shows a heterogenicity with I^2 value of 82%. (MD = 0.53, 95% CI: 1.68, 0.63). Forest plot depicts that calcium levels are low in children with ECC as compared to caries-free children. Outlier was detected in one study due to wide variation in calcium levels between ECC and caries-free group.

Fig. 5 shows relationship between ECC and salivary phosphate, and depicts that salivary phosphate level was found to be high in children ECC as compared to caries free children. Outlier was detected in one study due to wide variation in phosphate levels between ECC and caries-free group.

Fig. 6a represents the relationship between ECC and salivary IgA from 2 studies. The forest plot shows that salivary IgA was found to be high in children with ECC. Fig. 6b depicts the relationship between IgG and ECC and the IgG values were found to be high in children with ECC as compared to caries free children.

3.5. Publication bias

Fig. 7a, 8-11 shows a Symmetrical funnel plot distribution for all the studies evaluated pH, salivary flow rate, buffering capacity, calcium and phosphate. Asymmetrical distribution was evident in the funnel plot for the studies that had evaluated the pH of S ECC children and IgA levels. (Figs. 7b and 12). Publication bias in the aforementioned studies could be due to a smaller sample size or only the positive findings of the studies have been reported. .

4. Discussion

The studies selected for this systematic review and meta-analysis were those that best satisfied the minimum criteria to be able to evaluate the role of physical and chemical properties of saliva and ECC. Although several studies related to ECC can be found on the scientific databases, only 22 studies were considered suitable for systematic review. Among the included studies, few studies found no significant differences, while others found a significant difference in the evaluated physical and chemical properties and ECC. This systematic review did not include other types of studies such as longitudinal studies, or comparison of physical and chemical properties before and after treatment, in order to maintain the homogeneity with the study design. There was no publication bias evident from the screened studies.

4.1. Salivary pH and buffering capacity

The pH and buffering action of saliva can alter the low plaque pH, thus preventing demineralization of enamel. Hence pH and buffering capacity of saliva plays a vital role in preventing the initiation of dental caries. Salivary pH and buffering capacity in children with and without ECC have been studied extensively with diversified results. Since salivary pH and buffering capacity can be considered as a potential tool for caries risk assessment in children, studies are still being carried out due to its potential importance.

Studies have reported that pH and buffering capacity of saliva were high in caries free children as compared to caries active children[28–33] The Ph required for enamel dissolution to initiate dental caries should fall below critical pH, hence higher salivary pH in caries free children might be making the caries initiation difficult. Similarly, high buffering capacity of saliva neutralizes the acid produced by micro-organism and hence less chance of caries initiation.

On the contrary, studies also have reported that there was no significant difference in pH and buffering capacity of saliva in children with and without ECC.[34–39] The authors justify that, pH and buffering capacity solely cannot be considered as a risk factor for ECC. Importance should be given to diet, microbial flora, salivary protein, as these factors could dominate pH and buffering capacity.

4.2. Salivary flowrate

Salivary flushing is essential for maintenance of oral health and clearance of microorganisms and food components. The salivary flow rate is low in young children, hence, evaluating the salivary flow rate in young children helps us to identify its role in ECC.

Some studies have concluded that low salivary flow rate is a vital indicative of an increased risk of caries in children[29,31,32] However, other studies demonstrates that salivary flow rate is not a contributing factor in caries rate in children.[34,37,38] Tenovuo et al. had stated that, there exists a threshold limit of salivary flow rate and it is specific for each individual. So, considering the normal flow rate would be ideal for population level than while screening for individual patients. Similarly, studies state that, it is important to establish a reference value for salivary flow rate in children, because the salivary flow rate of children coincides with the reference value of hyposalivation in adults.[41] So, it is necessary to develop a reference value of salivary flow rate in children.

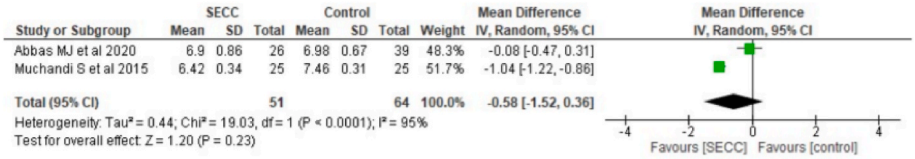

Fig. 2b. Relationship between severe ECC and PH.

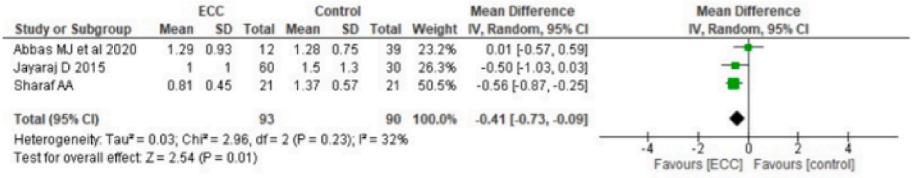

Fig. 3. Relationship between Salivary flow and ECC.

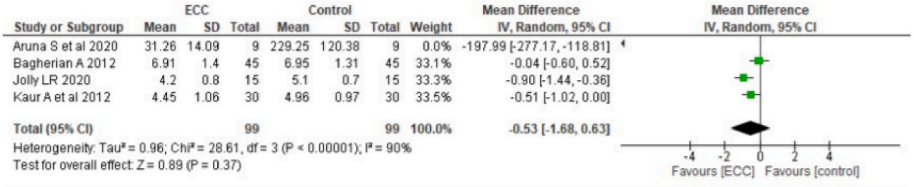

Fig. 4. Relationship between salivary calcium and ECC

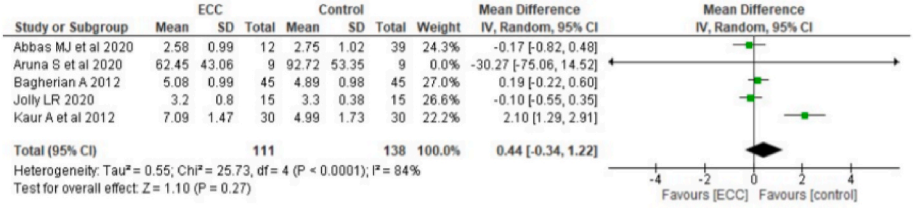

Fig. 5. Relationship between salivary phosphate and ECC.

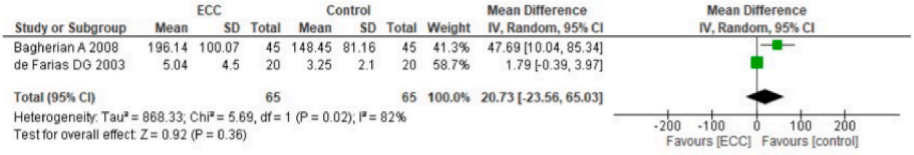

Fig. 6a. Relationship between salivary IgA and ECC

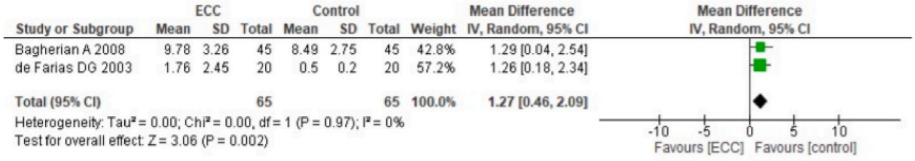

Fig. 6b. Relationship between IgG and ECC

In addition, seasonal temperature can affect the flow rate of saliva[42–44] There is no evidence that the above-mentioned studies have taken these points into consideration while conducting the studies. Hence, salivary flow rate is an important parameter for caries activity, and in future, it is important to take the above points into consideration while evaluating this parameter in children.

4.3. Salivary viscosity

Few studies have evaluated the salivary viscosity of children with and without ECC, and stated that salivary viscosity was higher in children with ECC and caries -free children have low viscous and watery saliva.[33,40,45] This highly viscous saliva is less effective in oral clearance and could be a contributing factor for ECC.

4.4. Inorganic ions of saliva

4.4.1. Salivary calcium, phosphate and alkaline phosphatase

Saliva acts as a source of calcium and phosphate and plays an imperative role in remineralization of incipient carious lesion. These inorganic ions of saliva improve post-eruptive maturation of enamel by influencing the precipitation or dissolution of Hydroxy Apatite Crystals (HAP).[46,47] Saliva maintains calcium and phosphate in supersaturated state and helps in neutralizing acids. Alkaline phosphatase is a non-specific enzyme and it maintains the level of calcium and phosphate to sustain the demineralization and remineralization process.

With regard to the role of salivary calcium and phosphorus levels in ECC, conflicting reports have been published. A study by Aruna et al. reported an increased salivary calcium and phosphorus levels in caries-free children, as opposed to children with ECC.[48] However, the results of

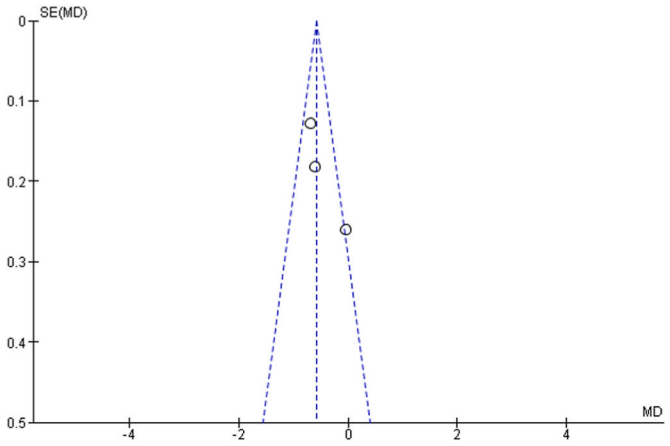

Fig. 7a. Funnel plot for pH of ECC and Caries free children.

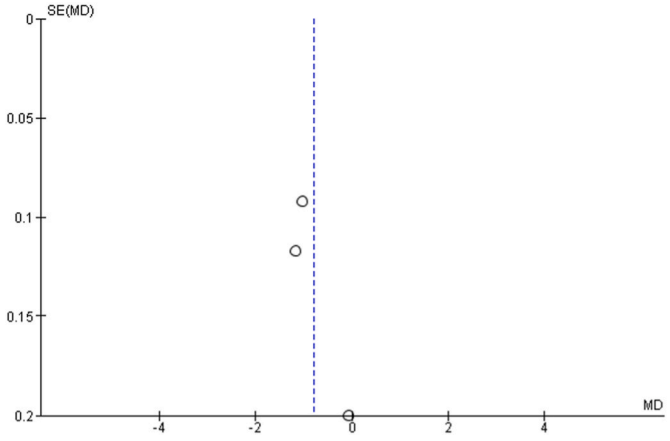

Fig. 7b. Funnel plot for pH of SECC and Caries free children.

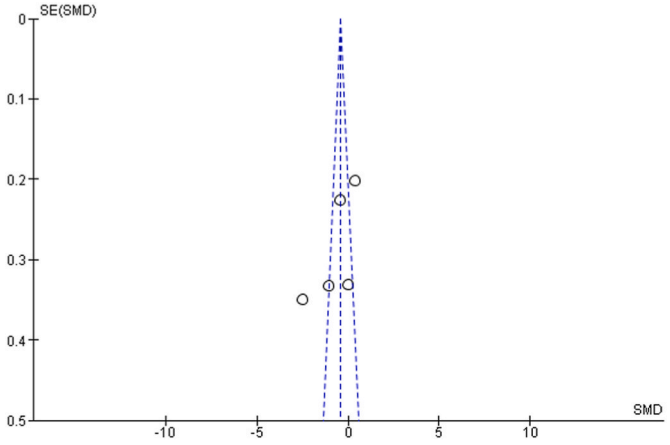

Fig. 8. Funnel plot for Salivary flow rate of ECC and Caries free children.

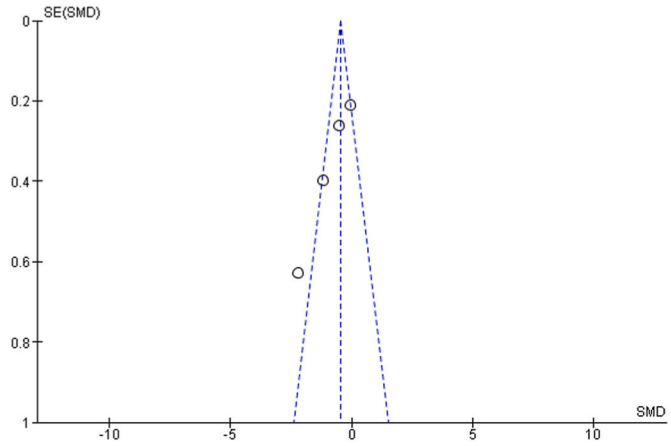

Fig. 9. Funnel plot for Salivary calcium of ECC and Caries free children.

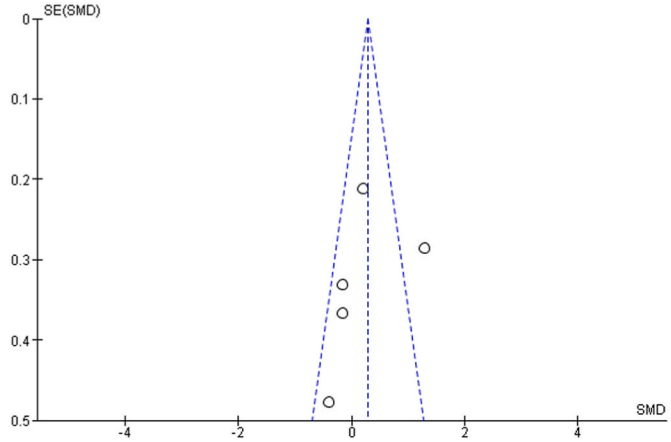

Fig. 10. Funnel plot for Salivary phosphate of ECC and Caries free children.

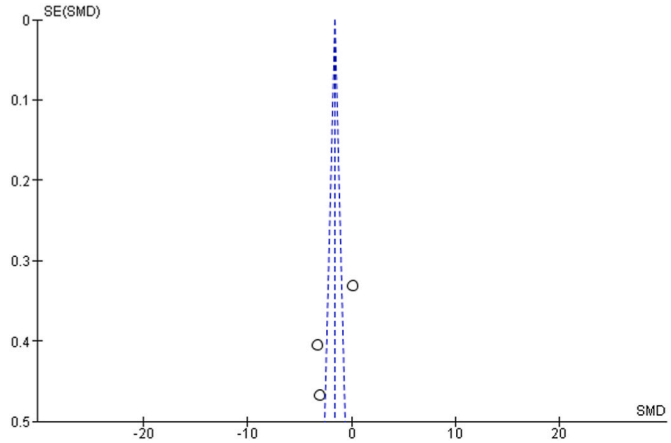

Fig. 11. Funnel plot for Buffering capacity of ECC and Caries free children.

this study should be evaluated with caution as it is a pilot study. Contrary to this, Turtola et al. and Elizarova and Petrovich reported an increase in salivary calcium in children with increased caries activity[49,50] Kaur et al. and Mahajana et al. reported an increase in salivary phosphate level in caries active children than the caries free children.[33,51] Jolly et al. evaluated salivary calcium and phosphorus and found an increase in salivary calcium levels in caries free children and no difference in salivary phosphorus between ECC and caries free children.[52] Similarly, Gandhy and Damle reported an increase in inorganic phosphate level in children with rampant caries.[53] The increase in salivary calcium levels in caries active children could be due to release of calcium from demineralized tooth, thereby increasing salivary calcium levels. On the other hand, few studies insisted that there was no difference in salivary calcium and phosphate level in caries-free and caries-active children.[28,36,54,55] One of the possible explanations for no difference in calcium in both the groups could be due to the fact that, saliva is a blood

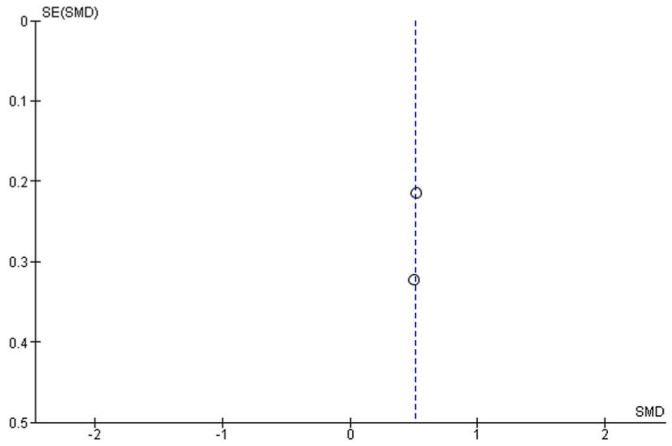

Fig. 12. Funnel plot for Salivary IgA of ECC and Caries free children.

filtrate and the unaltered level of calcium in children with ECC might be due to the regulatory role of parathyroid hormone (PTH), maintaining its level homogeneously in both ECC and caries free children.[56,57]

Kaur et al. and Shahrabi et al. estimated the level of salivary alkaline phosphatase between caries-free and caries-active children and reported a higher alkaline phosphatase activity in caries-free children.[33,54] The above-mentioned studies also reported a higher level of calcium and phosphorus in caries-free children and the reason could be due to higher alkaline phosphatase activity, or vice versa.

4.5. Salivary proteins and peptides

Out of the 22 included studies, 11 studies have assessed salivary proteins, enzymes and immunoglobulins. These salivary proteins and peptides possess an important function of resistance of oral mucosa to infection. Salivary immunoglobulins provide a host immune response and adaptive immunity against oral pathogens. In addition, it also enhances the activity of other salivary enzymes.[58]

Few studies have assessed the salivary immunoglobulins, namely IgA, IgG, IgM and sIgA and compared in children with and without ECC. De Farias et al., Bagherain et al. (2008 & 2012) has stated that the salivary IgA and IgG levels were significantly increased in children with ECC.[29,59,60] The long duration of carious process would have lodged numerous micro organisms and that could have stimulated immune response with secondary increase in immunoglobulin levels. Contrary to this, Shifa et al. and Colombo NH et al. found no correlation in salivary IgA levels between children with and without ECC.[61,62]

Bachtiar EW and Bhalla et al. et al. assessed the electrophoretic profile of salivary protein using SDS page and stated that Proline Rich Protein was found to be higher in caries free children, explaining its protective role.[37,45] In addition, Bachtiar et al. found a decreased frequency of occurrence of cysteine and albumin in children with ECC.[45]

Lertsirivorakul et al. evaluated activity of lysozyme in children with and without ECC and the author noticed an increased lysozyme activity in children with SECC.[25] Moslemi et al. analysed the salivary lysozyme levels in children before and after dental treatment, and found an increased lysozyme activity in caries free children. The author justified that it provides an antimicrobial effect, and it has an important role in dental caries prevention.[63] As only 2 studies had been conducted on salivary lysozymes in Iran and Thai children, and the available results are contradictory, further research is mandatory to explore the role of salivary lysozymes in the near future.

Colombo et al. estimated the levels of Antimicrobial Peptides (AMP) in a group of children with ECC, SECC and caries -free. The author found no significant difference in antimicrobial peptides between the group. The author further added, though there was no significant difference in individual peptide, salivary hBD-2 or HTN-5 are positively correlated with level of Mutans Streptococci.[64] Jayakaran et al. estimated the level of AMP namely salivary peptide HNP1 and stated that, the salivary peptide HNP1 was found to be low in children with ECC as compared to caries free children.[65] In contrast, Jurczak et al. found an increased level of AMP in children with ECC as compared to children with mild demineralization.[66] However, direct comparison cannot be done on the above 3 studies as one study compared the salivary lysozyme and caries progression, one study has assessed salivary peptide level and ECC, and one study compared the salivary lysozyme and level of *Mutans Streptococci and Lactobacilli*. Since wide disparity is evident in the available studies on AMP, further research is needed to explore its role in ECC.

5. Limitations

Though the results of the aforementioned studies regarding salivary physical and chemical properties and its relationship with ECC looks promising, there is a wide disparity in the study results. Several other etiological factors, namely diet, virulence of microorganisms and genetic patterns of salivary proteins and micro-organisms could have accounted for this difference. Similarly, all the included studies were cross-sectional, so in future, it is important to conduct a prospective cohort with adequate follow-up of the same children at different age groups to arrive at a definitive conclusion. As ECC is considered as a multifactorial etiology, a single variable cannot be considered as a predictor of ECC, since many confounding factors accounts for the variation. For E.g.: Systemic fluoride intake during the tooth eruption is considered as a confounding factor and many studies haven't accounted systemic fluoride while selecting the study sample and this parameter also varies between population in different studies. Hence a properly designed longitudinal study with adequate follow up of the same children along with consideration of the above-mentioned factors would give us a broader view on the role of physical and chemical properties of saliva in children.

6. Conclusion

With the light of available evidence, following conclusions can be drawn:

1. Physical and chemical properties of saliva play an important role in prevention of dental caries in children
2. Physical and chemical properties vary between children with and without ECClista
3. Further studies are needed with long term follow-ups of similar group children belonging to different age groups to find out the difference in physical and chemical properties in primary and permanent dentition
4. These parameters can be utilized in chair side salivary tests to evaluate the caries risk status of children

Declaration of competing interest

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