Predicting Dental Caries Increment Using Salivary Biomarkers in a Remote Indigenous Australian Child Population

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Abstract

Background

The burden of childhood dental caries amongst Indigenous Australians is higher than that of other Australians. Because of differences in lifestyle and the evolutionary history of the oral microbiota associated risk indicators may differ. Our objective was to evaluate associations between caries increment, salivary biomarkers, and baseline caries status among a rural Indigenous community in Far North Queensland, Australia.

Methods

This study was part of a trial assessing effectiveness and cost-effectiveness of an intervention to prevent dental caries among children. A baseline epidemiological survey and application of topical caries preventive measures was conducted in 2015, followed-up in 2016 and 2017. Saliva flow rate, pH, buffering capacity and bacterial loads were measured. Caries was scored by the International Caries Detection and Assessment system. The outcome was caries increment. Explanatory variables were sex, being in experimental or comparison group, baseline caries status, saliva flowrate and buffering capacity, pH, and salivary loads of mutans streptococci (MS), Lactobacilli (LB), and yeast. Descriptive statistics assessed frequencies, means and percentages. Chi Square tests compared caries incidence in relation to explanatory variables and Generalised Linear Models with negative binomial regression and log-link explored associations between explanatory and outcome variables.

Results

Of children caries free at baseline, significantly fewer had incipient ($p=0.01$) and advanced ($p=0.04$) caries after two years. From Univariate analysis, children in the experimental group experienced fewer tooth surfaces with advanced caries ($p=0.02$) than children in the comparison group. Having caries at baseline ($p=0.02$) and low salivary flow rates ($p<0.001$) saw a significant increase in advanced caries after two years. Children with high salivary loads of MS ($p=0.03$) and LB ($p=0.004$) experienced more advanced carious surfaces. Multivariable analysis revealed 58% reduction ($p=0.001$) in advanced caries among children with high salivary flow rates. Caries increment was 61% ($p=0.03$) more for incipient and 121% ($p=0.007$) more for advanced caries among children who harboured higher loads of MS.

Conclusion

As with other ethnicities, children with low salivary flow rates and those with high loads of MS had higher incipient and advanced caries increments after two years. These risk assessments can facilitate targeted preventive interventions for such communities.

Trial registration

Australian New Zealand Clinical Trials Registry (ANZCTR), No: ACTRN12615000693527; date of registration: 3rd July 2015.

Background

In 2016, there were an estimated 798,400 Aboriginal and Torres Strait Islander people (hereinafter respectfully referred to as Indigenous Australians) living in Australia, representing 3.3% of the total Australian population [1]. The burden of communicable diseases and chronic health conditions amongst Indigenous Australians is substantially higher than that of other Australians [2], irrespective of age groups and health and well-being indices [3]. Reasons for these differences are multifactorial including socioeconomic disadvantage, poor access to health services, poor nutrition, heavy use of alcohol and smoking and genetic variations [4]. Similarly, Indigenous Australian children experience worse oral health than non-Indigenous children [5]. Regardless of many efforts, health inequalities, including oral health, between Indigenous and non-Indigenous Australians have remained for decades [6]. For example, the difference in prevalence of the dental caries indicators (26% for decayed teeth, 20% for missing teeth and 9% for filled teeth) [1] between Indigenous and non-Indigenous Australians was larger in Australia compared to Canada and New Zealand [7]. Recognising these historical disparities, the Council of Australian Governments (COAG), committed to 'close the gap' in disease levels and in life expectancy between Aboriginal and Torres Strait Islander and non-Indigenous Australians by 2030 [8]. The "Closing the Gap Framework" was initiated by the Australian Federal Government in 2008, aiming to reduce inequalities in life expectancy, children's mortality, education, employment, health, economic participation, healthy homes, and in governance and leadership of and by Indigenous Australians [9]. Encouragingly, in some parts of the country, there have been significant accomplishments in some key areas of health and education according to the most recent report from Queensland [10].

In the Northern Territory in 2020, it was reported that Indigenous children aged 5–10-years (44%) were more likely to have had at least one deciduous tooth with untreated decay than non-Indigenous children (26%), and among 6-to 14- year old's, Indigenous children were more likely to have had at least one permanent tooth with untreated decay than non-Indigenous children [11].

It is, therefore, clear that ways to identify and to predict Indigenous children at risk from severe dental caries are needed, so that targeted preventative measures can be undertaken. Saliva acts as the first line of defence, by dilution of toxins and via a wide range of non-specific [innate] and acquired immune mechanisms: it lubricates, assists mastication, swallowing, taste, and digestion of food [12]. As with blood and tissue biopsies, oral fluids are a source of biochemical data carrying prognostic and diagnostic information. It is an easily accessible and a non-invasive alternative to traditional diagnostic sources [13]. Saliva is the main biological factor protecting teeth against demineralization [13] by diluting and buffering acids and provides the calcium and phosphate ions for remineralisation. Caries risk assessment has, therefore, mainly focused on saliva as a diagnostic fluid, in addition to measures of past dental disease [14]. Commonly used biomarkers are concentrations of mutans Streptococci (MS) and lactobacilli (LB) in saliva, saliva flow rate and buffering capacity and, especially, past caries

experience as a clinical marker [15-17]. Even though many studies have shown that past caries experience is the strongest predictor of future caries [18], salivary biomarkers are promoted by many, especially the dental industry which sells easy-to-use chairside kits for the purpose [12]. Perhaps the greatest value of these is in monitoring compliance with good oral hygiene and dietary practices [12].

The balance of risk factors, and thus the utility of salivary biomarkers as risk indicators among remote Indigenous communities in Australia [19] may or may not be similar to other non-Indigenous and Indigenous communities around the world [20-22]. Colonisation and trans-generation inequalities have negatively impacted the oral and general health of Indigenous Australians. Therefore, it cannot be assumed that the oral microbiome and its potential for disease and maintenance of oral health is the same as that described in modern Western populations, as the oral microbiota of Indigenous Australians has been adapted over thousands of years of evolution [23]. Hence, the objective of this study was to evaluate associations between dental caries increment and salivary load of bacteria (MS, LB), yeasts, salivary pH, flow rate and buffering capacity among children living in a remote Indigenous community in Far North Queensland. This study is unique in that we were able to measure caries increment among an Indigenous child population after two years. The results will help determine the ability of salivary biomarkers and of past caries experience in predicting future caries in this community. More sophisticated analyses of the oral microbiota are published elsewhere [23].

Methods

This study was approved by the Griffith University Human Research Ethics Committee (GU Ref No: DOH/05/15/HREC) and the Far North Queensland Human Research Ethics Committee (FNQ HREC/15QCH/39–970). The Department of Education and Training (Queensland Government) also provided formal permission to approach potential research participants in schools. Site Specific Approval was authorised by the Torres and Cape Hospital and Health Service. Signed informed consent was obtained from parents or guardians of all children in the study. Consent for publication is covered by the Ethics Approvals and the Information and Consent Forms signed by Parents or Guardians of the children.

Study design and participants

This study was part of a trial assessing the effectiveness and cost-effectiveness of a single annual professional intervention for the prevention of childhood dental caries in a remote rural Indigenous community [24]. All school children attending the two primary and one secondary school campuses in the Northern Peninsula Area (NPA) of Far North Queensland (FNQ) were invited to participate. All consenting children underwent a detailed dental examination and an investigation of salivary biomarkers using commercially available chairside test kits to measure flow rate, pH, buffering capacity and later cultured for bacterial assessment [25]. Dental caries status was assessed by three trained and calibrated examiners using the International Caries Detection and Assessment system (ICDAS-II) [26]. The intervention study involved provision of any necessary restorative treatment, provided by the research team, followed by two annual applications of fissure sealant to appropriate teeth, swabbing the dentition

with povidone iodine and application of fluoride varnish to the intervention group. The usual care was provided for a comparison group, which was generated by children who attended for epidemiological assessments but who declined to receive the preventative treatments: usual care consisted of availability of symptomatic treatment from a public sector dentist available one or two days per week. The baseline survey was carried out in late 2015 and subsequent follow-up visits were conducted at the same time of year in 2016 and 2017. A total of 600 children were invited to participate and at baseline there were 408 participants of which 292 were compliant with saliva analysis. At the 2-year follow up, a total of 208 children participated in clinical examinations and were used for the current analysis.

Outcome variable

The outcome of the present analysis is caries increment. This was defined as the sum of tooth surfaces that had an ICDAS code of 0 (sound tooth surface: no evidence of caries after air drying for 5 seconds) at baseline and had any ICDAS codes from 1 to 6 at 2-year follow up (1= first visual change in enamel, opacity or discoloration, white or brown, visible at the entrance to the pit or fissure after prolonged air drying; 2= distinct visual change in enamel when wet or dry; 3 = localized enamel breakdown without clinical visual signs of dentinal involvement; 4 = underlying dark shadow from dentine; 5= distinct cavity with visible dentine; 6 = extensive cavity with visible dentine) in both deciduous and permanent dentitions [27]. Caries increment was calculated for each individual child in the sample. This paper assesses explanatory variables in relation to two specific outcome variables: 1) incipient caries - determined as the number of tooth surfaces that had no caries at baseline (ICDAS 0) but showed ICDAS codes of 1 or 2 at the 2-year follow up; and 2) overt caries – defined as the number of tooth surfaces that had no caries at baseline (ICDAS 0) but presented with any of the ICDAS scores 3 to 6 at the 2-year follow up. For bivariate analysis, caries incidence was used as the outcome, which is defined as the number of participants having one of more tooth surfaces with incipient or overt caries at follow-up [28]. ICDAS 1 and 2 are initial non-cavitated lesions and it is possible that the predictors for these incipient lesions could be different from those for advanced caries, viz: scores from ICDAS 3 to 6. Inter-examiner reliability was tested using Kappa statistics, where 5% of children were re-examined by the three participating examiners. The overall Kappa was 0.837, indicating a high level of agreement.

Explanatory variables

Information on all explanatory variables was collected at baseline. Saliva flow rate, buffering capacity and pH were recorded before the oral examination. A sample of stimulated saliva (by chewing on a piece of wax) was collected over 5 minutes and used to measure the variables with GC SalivaCheck test kits (http://www.gcaustralasia.com/Products/97/Prevention/Saliva-Check-BUFFER). Saliva was expectorated into a collection cup and the volume per 5 minutes calculated. Buffering capacity was measured by placing three drops of saliva on three GC test pads followed by matching the colour against the manufacturer's chart after 5 minutes. The litmus strip from the test kit was dipped into the collection cup and pH recorded after 30s. Caries Risk Test (CRT) agar plates were used to quantify cariogenic bacteria and yeast colonies (http://www.ivoclarvivadent.com/en/p/all/products/prevention-care/caries-risk/crt-

bacteria). Agar plates were coated with saliva and incubated for 48 hours at 37^0 C according to manufacturer guidelines. Colony forming units (CFU) were determined by referring to CRT guideline charts. For MS and LB this was recorded as < 10^5 CFU/ml saliva or ≥ 10^5 CFU/ml: saliva and Yeast counts were categorized into either none/light or moderate/heavy. In addition, sex, being in either the intervention or the comparison group and baseline caries experience (caries experience from ICDAS 1-6) were taken as explanatory variables.

Statistical analyses

SPSS 26.0 (IBM, New York) was used. Descriptive statistics were carried out to estimate the frequencies and means, as required. Sex, being in either the intervention or comparison group, presence or absence of caries at baseline, baseline salivary pH (≤6.6 and > 6.6), flow rate (≤5 ml/5 min and > 5 ml/5 min), buffering capacity (≤9 and > 9), salivary loads of MS and LB (> 10^5 and ≤ 10^5 CFU/ml saliva) and yeast (moderate or heavy v none or light) counts were dichotomised for descriptive analysis and presented as numbers and percentages. Chi Square tests were performed to compare caries incidence in relation to explanatory variables. We also used Generalised Linear Models with negative binomial regression and log link to explore the association between the explanatory variables and the outcomes. At first, a univariate analysis was conducted to assess the effect of each explanatory variable on the outcome variable, by entering one variable at a time. A multivariable analysis was then conducted to explore the association between explanatory variables where all variables were entered at once. Exponential estimates of the negative binomial regression with log link analyses are presented as Incidence Rate Ratios (IRR) with 95% confidence intervals (95% CI). For univariate and multivariable negative binomial regression analyses, salivary pH, buffering capacity and flow rate were used as scale measurements and for all tests, a p-value of < 0.05 was considered statistically significant.

Results

Out of a total 408 participants at baseline, only 208 were present and provided a saliva sample at the 2-year follow-up visit: all of these are included in the current analysis. There were more female participants (n=119): 91 children in the group which received the preventative intervention; 117 in the comparison group (Table 1).

Table 1 Characteristics of the participants at baseline

Variable	N (%)
Sex	
Male	85 (41.7)
Female	119 (58.3)
Comparison Vs Experimental group	
Experimental group	117 (56.2)
Comparison group	91 (43.8)
Baseline caries	
Without caries (ICDAS 0)	14 (6.7)
With caries (ICDAS 1-6)	194 (93.3)
Salivary pH at baseline	
<6 (low)	29 (13.9)
≥6 (high)	179 (86.1)
Salivary flow rate per 5 minutes at baseline	
<5ml (low)	148 (71.2)
≥5ml (high)	60 (28.8)
Total buffering capacity at baseline	
<9 (low)	83 (39.9)
≥9 (high)	125 (60.1)
Salivary MS levels at baseline	
≥ 10^5 CFU/ml saliva (high risk)	102 (69.4)
<10^5 CFU/ml saliva (low risk)	45 (30.6)
Salivary LB levels at baseline*	
≥ 10^5 CFU/ml saliva (high risk)	52 (35.4)
< 10^5 CFU/ml saliva (low risk)	95 (64.6)
Salivary yeast counts	
Moderate or heavy	24 (16.7)
None or light	120 (83.3)

Most had carious tooth surfaces (ICDAS 1 to 6) at baseline (93%). Nearly 90% of children had salivary pH levels over 6 and ~60% had a high salivary buffering capacity at baseline (Table 1). The majority of the children showed high levels of MS, LB and yeast in their saliva. Table 2 presents the results from the Chi-square analyses with the caries incidence in relation to sex, group allocation, baseline caries status, salivary pH, flow rate, buffering capacity, and salivary bacteria and yeast levels.

Table 2 Caries increment at 2 years in relation to sex, baseline salivary characteristics and caries status

Variable	Incipient caries (ICDAS 1 to 2)			Advanced caries (ICDAS 3 to 6)		
	No caries at 2 year follow up (%)	With caries at 2 year follow up (%)	p	No caries at 2 year follow up (%)	With caries at 2 year follow up (%)	p
Sex			0.89			0.76
Male	12(14.1)	73 (85.9)		39 (45.9)	46 (54.1)	
Female	16 (13.4)	103 (86.6)		52 (23.7)	167 (76.3)	
Comparison Vs Experimental group			**0.01**			**0.02**
Experimental group	22 (18.8)	95 (81.2)		61(52.1)	56 (47.9)	
Comparison group	6 (6.6)	85 (93.4)		33(36.3)	58 (63.7)	
Baseline caries status			**0.01**			**0.04**
Without caries(ICDAS 0)	5 (35.7)	9 (64.3)		10 (71.4)	4 (28.6)	
With caries (ICDAS 1-6)	23 (11.9)	171 (88.1)		84 (43.3)	110 (56.7)	
Salivary pH at baseline			0.52			0.45
≤6	5 (9.6)	24 (82.8)		15 (51.7)	14(48.3)	
>6	23 (12.8)	156 (87.2)		79 (44.1)	100 (55.9)	
Salivary flowrate per minute at baseline			0.97			0.21
≤5ml	20 (13.5)	128 (86.5)		71 (48.0)	77 (52.0)	
>5ml	8 (13.3)	52 (86.7)		23 (38.3)	37 (61.7)	
Total buffering capacity at baseline			0.37			0.89
≤9	9 (10.8)	74 (89.2)		38 (45.8)	45 (54.2)	
>9	19 (15.2)	106 (84.8)		56 (44.8)	69 (55.2)	
Salivary MS levels at baseline			**0.04**			**0.01**

> 10^5 CFU/ml saliva	10 (9.8)	92 (90.2)		42 (41.2)	60 (58.8)	
≤10^5 CFU/ml saliva	10 (22.2)	35 (77.8)		29 (64.4)	16 (35.6)	
Salivary LB levels at baseline			0.97			0.70
>10^5 CFU/ml saliva	7 (13.5)	45 (86.5)		24 (46.2)	28 (53.8)	
≤ 10^5 CFU/ml saliva	13 (13.7)	82 (86.3)		47 (49.5)	48 (50.5)	
Salivary Yeast counts			0.23			0.55
Moderate or heavy	5 (20.8)	19 (79.2)		13 (54.2)	11 (45.8)	
None or light	15 (12.5)	105 (87.5)		57 (47.5)	63 (52.5)	

X^2 Chi Square statistics, figures in bold are significant (p<0.05)

There were significant differences between experimental and comparison groups with regards to caries increment at the 2-year follow-up. Fewer children in the experimental group demonstrated advanced caries (ICDAS 3-6) at the follow-up than in the comparison group (p=0.02). Amongst those who were caries free at baseline (ICDAS 0), significantly fewer children presented with incipient (p=0.01) and advanced (p=0.04) caries after two years than those children who had caries at baseline. A greater proportion of children with high salivary loads of MS at baseline had new incipient (90%) and advanced (59%) caries at two years than their counterparts who had low salivary loads of MS (Table 2).

Table 3 Univariate analysis with caries increment at 2 years as the outcome variable and salivary characteristics as explanatory variables

Variable	Incipient caries (ICDAS 1 to 2)			Advanced caries (ICDAS 3 to 6)		
	B (SE)	IRR (95%CI)	P	B (SE)	IRR (95% CI)	P
Sex						
Male	0.11 (0.16)	1.12 (0.82-1.51)	0.47	0.09 (0.17)	1.09 (0.77-1.55)	0.61
Female	0[a]			0[a]		.
Comparison Vs Experimental; group						
Experimental group	-0.23 (0.15)	0.80 (0.59-1.07)	0.13	**-0.38 (0.17)**	**0.67 (0.48-0.95)**	**0.02**
Comparison group	0[a]			0[a]		
Baseline caries status	0.01 (0.01)	1.01 (0.99-1.02)	0.31	**0.02 (0.01)**	**1.02 (1.01-1.04)**	**0.02**
Salivary pH at baseline	-0.12 (0.20)	0.88 (0.60-1.30)	0.53	-0.02 (0.22)	0.97 (0.63-1.50)	0.91
Salivary flowrate per minute at baseline	0.08 (0.18)	1.08 (0.76-1.53)	0.66	**-0.86 (0.22)**	**0.43 (0.27-0.65)**	**0.000**
Total buffering capacity at baseline	-0.05 (0.05)	0.95 (0.86-1.05)	0.35	0.06 (0.05)	1.06 (0.95-1.18)	0.27
Salivary MS levels at baseline						
≥ 10^5 CFU/ml saliva	**0.42 (0.19)**	**1.53 (1.03-2.24)**	**0.03**	**0.50 (0.23)**	**1.66 (1.04-2.65)**	**0.03**
<10^5 CFU/ml saliva	0[a]		.	0[a]		.
Salivary LB levels at baseline						
≥ 10^5 CFU/ml saliva	0.35 (0.18)	1.42 (0.99-2.05)	0.06	**0.61 (0.21)**	**1.84 (1.20-2.80)**	**0.004**
< 10^5 CFU/ml saliva	0[a]		.	0[a]		.
Salivary Yeast counts						

Moderate or heavy	0.03(0.24)	0.97 (0.60-1.56)	0.90	-0.42(0.31)	0.65 (0.35-1.18)	0.16
None or light	0[a]		.	0[a]		.

0[a] Reference category, p values in bold are significant

Univariate analysis (Table 3) revealed that children in the experimental group experienced fewer surfaces with advanced caries by a factor of 33% (IRR:0.67; CI: 0.48-0.95) compared to children in the comparison group. Having caries experience (ICDAS 1-6) at baseline, increased advanced caries by 2% (IRR:1.02; CI: 1.01-1.04). With each unit increase in salivary flow rate, advanced caries increment decreased by 57% (IRR:0.43; CI: (0.27-0.65). Compared to their counterparts who had lower salivary loads of MS, children with higher loads of MS experienced more advanced carious surfaces by 66% (IRR:1.66; CI: 1.04-2.65). Similarly, children with higher loads of salivary LB levels had more surfaces with advanced caries than those with low salivary LB loads (IRR:1.84; CI: 1.20-2.80).

Table 4 Multivariable analysis with caries increment at 2 years as the outcome variable and salivary characteristics as explanatory variables

Variable	Incipient caries (ICDAS 1 to 2)			Advanced caries (ICDAS 3 to 6)		
	B (SE)	IRR (95%CI)	P	B (SE)	IRR (95%CI)	P
Sex						
Male	0.12 (0.20)	1.13 (0.75-1.68)	0.56	0.23 (0.25)	1.26 (0.77-2.08)	0.36
Female	0[a]	.		0[a]	.	
Comparison Vs Experimental group						
Experimental group	-0.29 (0.20)	0.75 (0.51-1.12)	0.16	0.35 (0.25)	1.43 (0.89-2.31)	0.15
Comparison group	0[a]	.		0[a]		
Baseline caries status	-0.007 (0.01)	0.99(0.97 -1.01)	0.54	-0.007 (0.01)	0.99 (0.97 -1.02)	0.62
Salivary pH at baseline	-0.07 (0.23)	0.93 (0.59 -1.46)	0.76	-0.044 (0.25)	0.96 (0.58 -1.57)	0.86
Salivary flowrate per minute at baseline	-0.04 (0.20)	0.96 (0.65 -1.42)	0.85	-0.870 (0.27)	**0.42 (0.25 -0.71)**	**0.001**
Total buffering capacity at baseline	-0.04 (0.05)	0.96 (0.87 -1.07)	0.45	0.005 (0.07)	1.01 (0.88 -1.14)	0.94
Salivary MS levels at baseline		.			.	
≥ 10^5 CFU/ml saliva	**0.48 (0.22)**	**1.61 (1.04 -2.50)**	**0.03**	**0.79 (0.29)**	**2.21 (1.24 -3.92)**	**0.007**
<10^5 CFU/ml saliva	0[a]	.		0[a]	.	
Salivary LB levels at baseline						
≥ 10^5 CFU/ml saliva	0.34(0.21)	1.40 (0.93 -2.11)	0.11	0.453 (0.25)	1.57 (0.96 -2.57)	0.07
< 10^5 CFU/ml saliva	0[a]	.		0[a]	.	
.,/Salivary Yeast counts						
Moderate or heavy	0.02 (0.25)	1.02 (0.62 -1.68)	0.94	-0.351 (0.33)	0.70 (0.37 -1.34)	0.28
None or light	0[a]	.		0[a]	.	

0[a] Reference category, p values in bold are significant

In the fully adjusted multivariable model (Table 4), there was a 58% reduction (IRR:0.42; CI:0.25 -0.71) in advanced caries among children with increased salivary flow rates. Caries increment in those with higher salivary MS counts was 61% (IRR:1.61; CI:1.04 -2.50) more for incipient caries and 121% (IRR:2.21; CI:1.24 -3.92) more for advanced caries than those with lower salivary loads of MS.

Discussion

In our study population high salivary loads of MS were significantly associated with the increment of both incipient and advanced caries, after adjusting for the effect of other confounding variables. There is a dearth of literature which has explored the risk of caries increment in relation to oral microbial loads in Indigenous populations. Most studies have assessed socio-economic, dietary, behavioural and family characteristics [29-32]. Nevertheless, we found that having ≥10^5 CFU/ml salivary loads of MS were significantly associated with an increment of tooth surfaces with incipient and advanced caries after two years. High loads of salivary LB were also associated with advanced caries increment when compared to those with low salivary loads of LB. This is consistent with our baseline study where caries experience was approximately twice in those with higher counts of salivary MS than those with lower counts [33], demonstrating that simple chair side assessment of microbial loads would assist in determining the risk for future caries. Furthermore, preventive interventions to reduce the bacterial loads were found to be equally effective in this community [34] and advanced investigations suggested that this could be due to a reduction of oral microbial diversity as a result of improved oral health of the children [23].

Despite the fact that only our univariate analysis indicated a significant association between having caries at baseline and an increment in advanced caries, previous decay has been one of the most consistently demonstrated, significant predictors in caries risk assessment [35, 36]. This has been observed among Indigenous children in the US, Canada and Australia [22, 37], as well as amongst non-Indigenous children in these countries [35, 38]. Similar results were observed in recent studies in a population of low socio-economic African American children [39], in Brazil [40] and in Mexico [41].

Variations of saliva flow rate have been evaluated as a risk indicator for dental caries, especially among Caucasian populations [42], but there is a scarcity of such literature from Indigenous communities. Here, we have demonstrated a significant negative association between salivary flow rate and the increment of both incipient and advanced caries. Mean levels of salivary flow were reported to be lower in a population of Mexican adolescents with a caries score of DMFT≥5 [43] and similar findings were reported in an Indian [44] and among Ugandan adolescents [42].

When highest and lowest salivary loads were considered, the agreement between a chairside commercial test and real-time quantitative PCR for MS was nearly equal [45]. If participants followed the user instructions, the sensitivity and specificity of these chair-side caries risk assessment kits are high [46], and the results can be used to assess caries risk, allowing targeted preventive interventions [47]. These simple risk assessment tools can be used in communities with limited resources. However, there are challenges in Indigenous communities due to limited access to dental services, and barriers to health

services in general, which include individual, organizational and policy level determinants [48]. Even though chair-side tests are easy to use and quick [49], in rural underserved Indigenous communities, prerequisite equipment and resources may be unavailable [48, 50]. In addition, Indigenous Australians face many challenges and barriers in the utilization of oral health services, among them differences in perception of benefit within traditional health beliefs [48]. In this regard, ways in which Indigenous communities' access public oral health services are important for execution: e.g. whether caries risk assessment should be done within mainstream or within Indigenous-specific clinics [51] or, as in our studies, in schools.

Conclusion

Children who had reduced salivary flow rates and those with higher loads of salivary MS had a higher incipient and advanced caries increment after two years. As per unadjusted model, higher loads of salivary LB, low salivary flow rates, having a history of caries experience and being in the comparison group increased the risk of developing more surfaces with new advanced caries. We recommend that caries risk assessment and targeted preventive interventions be carried out in this and similar populations based on identified risk indicators. However, for effective and lasting impact, the many challenges faced by these communities related to wider public health access and service provision in the context of relevant and specific social and cultural factors need to be addressed with multidisciplinary interventions.

Abbreviations

Northern Peninsula Area (NPA)

Far North Queensland (FNQ)

International Caries Detection and Assessment system (ICDAS-II)

Caries Risk Test (CRT)

Mutans streptococci (MS)

Lactobacillus (LB)

Colony forming units (CFU)

Declarations

Ethics approval and consent to participate

All procedures were performed in accordance with the Declaration of Helsinki and relevant guidelines from the health and educational authorities in Queensland, including those promulgated by relevant Indigenous authorities. Formal Ethics Approvals were granted by Griffith University (GU Ref:

DOH/05/15/HREC); Far North Queensland (FNQ HREC/15QCH/39-970); Department of Education and Training (Queensland Government) to approach participants at schools; and the Torres and Cape Hospital and Health Service for Site Specific Approval.

Signed informed consent was obtained from parents or guardians of all children in the study.

Consent for publication.

This is covered by the Ethics Approvals and the Information and Consent Forms signed by Parents or Guardians of the children.

Availability of data and materials.

All authors have full access to the all data (including statistical reports and tables) and gave final approval and agree to be accountable for all aspects of the work. Access to raw data may be requested from the corresponding author n.johnson@griffith.edu.au or from the Griffith University Human Research Ethics Committee (Ref: DOH/05/15/HREC) at research-ethics@griffith.edu.au

Competing interests

All authors declare no competing interests.

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Author Contributions

S Fernando contributed to design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; S. Tadakamadla contributed to data analysis and interpretation, and critically revised the manuscript; J Kroon, R Lalloo and NW Johnson contributed to design, data acquisition, data interpretation and critically revised the manuscript. All authors have full access to all data (including statistical reports and tables) and gave final approval and agree to be accountable for all aspects of the work.

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References

1. Australian Bureau of statistics: **Estimates of Aboriginal and Torres strait Islander Australians**. In: *Indigenous Status (individual categories).* Edited by Australian Bureau of statistics. Canberra; 2016.
2. Australian Institute of Health and Welfare: **Indigenous Australians**. In: *An Overview.* Edited by Australian Institute of Health and Welfare. Canberra; 2020.
3. Haag D, Schuch H, Ha D, Do L, Jamieson L: **Oral Health Inequalities among Indigenous and Non-Indigenous Children**. *JDR Clinical & Translational Research* 2020:2380084420939040.
4. Trout M, Henson G, Senthuran S: **Characteristics and outcomes of critically ill Aboriginal and/or Torres Strait Islander patients in North Queensland**. *Anaesthesia and Intensive Care* 2015, **43**(2):216-223.
5. de Silva AM, Martin-Kerry J, Geale A, Cole D: **Flying blind: trying to find solutions to Indigenous oral health**. *Australian Health Review* 2016, **40**(5):570-583.
6. Roberts-Thomson KF, Spencer AJ, Jamieson LM: **Oral health of aboriginal and Torres Strait islander Australians**. *Medical Journal of Australia* 2008, **188**(10):592.
7. Jamieson L, Elani H, Mejia G, Ju X, Kawachi I, Harper S, Thomson W, Kaufman J: **Inequalities in indigenous oral health: findings from Australia, New Zealand, and Canada**. *Journal of dental research* 2016, **95**(12):1375-1380.
8. **Closing The Gap** [https://healthinfonet.ecu.edu.au/learn/health-system/closing-the-gap/history-of-closing-the-gap/]
9. Australian Government: **Closing the gap report**. In: *The annual report to Parliament on progress in Closing the Gap.* Edited by Australian Government. Canberra; 2020.
10. Queensland Aboriginal and Islander Health Council: **Annual report 2019-2020**. In: *Annual Report 2019-2020 QAIHC.* Edited by Council, Queensland Aboriginal and Islander Health Council. Brisbane: QAIHC; 2020.
11. Australian Institute of Health and Welfare: **Oral health and dental care in Australia**. In: *Northern Territory Remote Aboriginal Investment: Oral Health Program July 2012 to December 2018.* Edited by Australian Institute of Health and Welfare. Canberra: Australian Institute of Health and Welfare; 2019.
12. Gao X, Jiang S, Koh D, Hsu CYS: **Salivary biomarkers for dental caries**. *Periodontology 2000* 2016, **70**(1):128-141.
13. Buzalaf MAR, Ortiz AdC, Carvalho TS, Fideles SOM, Araújo TT, Moraes SM, Buzalaf NR, Reis FN: **Saliva as a diagnostic tool for dental caries, periodontal disease and cancer: is there a need for more biomarkers?** *Expert Review of Molecular Diagnostics* 2020, **20**(5):543-555.
14. Sanche-Pere L, Golubov J, Irigoyen-Camacho ME, Mocteuma PA, Acosta-Gio E: **Clinical, salivary, and bacterial markers for caries risk assessment in schoolchildren: a 4-year follow-up**. *International journal of paediatric dentistry* 2009, **19**(3):186-192.
15. Leone CW, Oppenheim FG: **Physical and chemical aspects of saliva as indicators of risk for dental caries in humans**. *Journal of dental education* 2001, **65**(10):1054-1062.

16. Edelstein BL, Ureles SD, Smaldone A: **Very high salivary Streptococcus mutans predicts caries progression in young children**. *Pediatric Dentistry* 2016, **38**(4):325-330.
17. Basha S, Mohamed RN: **Past Caries Experience and Salivary Levels of Streptococcus mutans in Caries Prediction; a 3-Year Follow-Up**. *EC Dental Science* 2018, **17**:804-809.
18. Zemaitiene M, Grigalauskiene R, Andruskeviciene V, Matulaitiene ZK, Zubiene J, Narbutaite J, Slabsinskiene E: **Dental caries risk indicators in early childhood and their association with caries polarization in adolescence: a cross-sectional study**. *BMC Oral Health* 2017, **17**(1):1-6.
19. Jamieson LM, Roberts-Thomson K, Sayers S: **Dental caries risk indicators among Australian Aboriginal young adults**. *Community dentistry and oral epidemiology* 2010, **38**(3):213-221.
20. Christian B, Blinkhorn A: **A review of dental caries in Australian Aboriginal children: the health inequalities perspective**. *Rural Remote Health.* 2012 Oct;12(4):2032. Epub 2012 Oct 16.
21. Corraini P, Baelum V, Pannuti CM, Pustiglioni AN, Romito GA, Pustiglioni FE: **Tooth loss prevalence and risk indicators in an isolated population of Brazil**. *Acta Odontologica Scandinavica* 2009, **67**(5):297-303.
22. Parker EJ, Jamieson LM, Broughton J, Albino J, Lawrence HP, Roberts-Thomson K: **The oral health of Indigenous children: a review of four nations**. *Journal of paediatrics and child health* 2010, **46**(9):483-486.
23. Skelly E, Johnson NW, Kapellas K, Kroon J, Lalloo R, Weyrich L: **Response of Salivary Microbiota to Caries Preventive Treatment in Aboriginal and Torres Strait Islander Children**. *Journal of Oral Microbiology* 2020, **12**(1):1830623.
24. Lalloo R, Kroon J, Tut O, Kularatna S, Jamieson LM, Wallace V, Boase R, Fernando S, Cadet-James Y, Scuffham PA and Johnson NW *: **Effectiveness, cost-effectiveness and cost-benefit of a single annual professional intervention for the prevention of childhood dental caries in a remote rural Indigenous community**. *BMC Oral Health* 2015, **15**(1):1-8.
25. Walsh LJ, Tsang A: **Chairside testing for cariogenic bacteria: current concepts and clinical strategies**. *Journal of Minimum Intervention in Dentistry* 2008, **1**(2):26.
26. Pieper K, Weber K, Margraf-Stiksrud J, Heinzel-Gutenbrunner M, Stein S, Jablonski-Momeni A: **Evaluation of a preventive program aiming at children with increased caries risk using ICDAS II criteria**. *Clinical oral investigations* 2013, **17**(9):2049-2055.
27. Wang K, Pang L, Fan C, Cui T, Yu L, Lin H: **Enamel and Dentin Caries Risk Factors of Adolescents in the Context of the International Caries Detection and Assessment System (ICDAS): A Longitudinal Study**. *Frontiers in Pediatrics* 2020, **8**:419.
28. Machiulskiene V, Campus G, Carvalho JC, Dige I, Ekstrand KR, Jablonski-Momeni A, Maltz M, Manton DJ, Martignon S, Martinez-Mier EA: **Terminology of dental caries and dental caries management: consensus report of a workshop organized by ORCA and Cariology Research Group of IADR**. *Caries research* 2020, **54**(1):7-14.
29. Pierce A, Singh S, Lee J, Grant C, de Jesus VC, Schroth RJ: **The burden of early childhood caries in Canadian children and associated risk factors**. *Frontiers in Public Health* 2019, **7**.

30. Pascoe L, Kim Seow W: **Enamel hypoplasia and dental caries in Australian aboriginal children: prevalence and correlation between the two diseases**. *Pediatric Dentistry* 1994, **16**:193-193.
31. Ju X, Do L, Ha D, Jamieson L: **Association of Modifiable Risk Factors With Dental Caries Among Indigenous and Nonindigenous Children in Australia**. *JAMA network open* 2019, **2**(5):e193466-e193466.
32. Ha DH, Do LG, Roberts-Thomson K, Jamieson L: **Risk indicators for untreated dental decay among Indigenous Australian children**. *Community dentistry and oral epidemiology* 2019, **47**(4):316-323.
33. Lalloo R, Tadakamadla S, Kroon J, Tut O, Kularatna S, Boase R, Kapellas K, Gilchrist D, Cobbledick E, Rogers J and Johnson NW*: **Salivary characteristics and dental caries experience in remote Indigenous children in Australia: a cross-sectional study**. *BMC Oral Health* 2019, **19**(1):21.
34. Lalloo R, Tadakamadla SK, Kroon J, Jamieson LM, Ware RS, Johnson NW.: **Impact of a caries preventive intervention in remote Indigenous Australian children**. PLoS One. 2021 Jan 28;16(1):e0244927. doi: 10.1371/journal.pone.0244927. eCollection 2021.PMID: 33507984.
35. Fernando S, Tadakamadla S, Bakr M, Scuffham P, Johnson N: **Indicators of risk for dental caries in children: a holistic approach**. *JDR Clinical & Translational Research* 2019, **4**(4):333-341.
36. Obregón-Rodríguez N, Fernández-Riveiro P, Piñeiro-Lamas M, Smyth-Chamosa E, Montes-Martínez A, Suárez-Cunqueiro MM: **Prevalence and caries-related risk factors in schoolchildren of 12-and 15-year-old: a cross-sectional study**. *BMC oral health* 2019, **19**(1):120.
37. Schroth RJ, Harrison RL, Moffatt ME: **Oral health of indigenous children and the influence of early childhood caries on childhood health and well-being**. *Pediatric Clinics* 2009, **56**(6):1481-1499.
38. Kopycka-Kedzierawski D, Billings R, Feng C: **Development of a prognostic model for caries onset and progression from early childhood caries incidence in urban preschool children**. *European Archives of Paediatric Dentistry* 2019, **20**(4):303-309.
39. Ghazal TS, Levy SM, Childers NK, Carter KD, Caplan DJ, Warren JJ, Kolker JL: **Survival analysis of caries incidence in African-American school-aged children**. *Journal of public health dentistry* 2019, **79**(1):10-17.
40. Reyes LT, Knorst JK, Ortiz FR, Mendes FM, Ardenghi TM: **Pathways influencing dental caries increment among children: a cohort study**. *International Journal of Paediatric Dentistry* 2020.Sep 23. doi: 10.1111/ipd.12730.
41. López-Gómez SA, Villalobos-Rodelo JJ, Ávila-Burgos L, Casanova-Rosado JF, Vallejos-Sánchez AA, Lucas-Rincón SE, Patiño-Marín N, Medina-Solís CE: **Relationship between premature loss of primary teeth with oral hygiene, consumption of soft drinks, dental care and previous caries experience**. *Scientific reports* 2016, **6**(1):1-7.
42. Ndagire B, Mayanja-Kizza H, Ssenyonga R, Nakanjako D: **Comparative Study of Saliva Flow Rate, pH and Buffer Capacity in Caries-Free and Caries-Active Ugandan Adolescent Students**. *International Journal Dental and Medical Sciences Research (IJDMSR)* 2020, **4**(1):29-35.
43. Pineda AG-A, Pérez AG, García-Godoy F: **Salivary parameters and oral health status amongst adolescents in Mexico**. *BMC Oral Health* 2020, **20**(1):1-7.

44. Pyati SA, Naveen Kumar R, Kumar V, Praveen Kumar N, Parveen Reddy K: **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* 2018, **42**(6):445-449.
45. Li Y, Saraithong P, Chen Z, Leung E, Pattanaporn K, Dasanayake A: **Comparison of real-time quantitative PCR with a chairside test for Streptococcus mutans assessment**. *Chin J Dent Res* 2017, **20**(4):199-210.
46. Strickland M, Duda P, Merdad HE, Pelaez-Shelton RE, Rosivack RG, Markowitz K: **The clinical performance of chairside caries risk assessment kits**. *Quintessence International* 2017, **48**(2).
47. Yoon RK, Smaldone AM, Edelstein BL: **Early childhood caries screening tools: a comparison of four approaches**. *The Journal of the American Dental Association* 2012, **143**(7):756-763.
48. Bastani P, Sarikhani Y, Ghanbarzadegan A, Ostovar F, Jamieson L: **Challenges in Oral Health Provision and Utilization in the Australian Indigenous Population: A Scoping Review**. Research Square; 2020. DOI: 10.21203/rs.3.rs-42699/v1.
49. Pappa E, Vastardis H, Rahiotis C: **Chair-side saliva diagnostic tests: An evaluation tool for xerostomia and caries risk assessment in children with type 1 diabetes**. *Journal of Dentistry* 2020, **93**:103224.
50. Gwynn J, Skinner J, Dimitropoulos Y, Masoe A, Rambaldini B, Christie V, Sohn W, Gwynne K: **Community based programs to improve the oral health of Australian Indigenous adolescents: a systematic review and recommendations to guide future strategies**. *BMC health services research* 2020, **20**:1-14.
51. Martin-Kerry JM, Whelan M, Rogers J, Raichur A, Cole D, de Silva AM: **Addressing disparities in oral disease in Aboriginal people in Victoria: where to focus preventive programs**. *Australian Journal of Primary Health* 2019, **25**(4):317-324