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Mother and youth access (MAYA) maternal chlorhexidine, counselling and paediatric fluoride varnish randomized clinical trial to prevent early childhood caries

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Background. Mexican-American children have a higher caries prevalence than the US average. The Mothers and Youth Access (MAYA) study was a randomized clinical trial initiated to address this problem.

Aim. Comparison of the efficacy of two prevention interventions in reducing early childhood caries (ECC).

Design. All 361 randomized mother–child dyads received oral health counselling. Beginning at 4 months postpartum, intervention mothers received chlorhexidine (CHX) mouthrinse for 3 months beginning 4 months postpartum and children received fluoride varnish (FV) every 6 months from age 12–36 months. Control group children received FV if precavitated lesions developed. Salivary mutans streptococci (MS) and lactobacilli were assessed.

Results. No significant difference in children's 36-month caries incidence between groups; 34% in each group developed caries [(d_{2+}fs) > 0]. About half of control group developed precavitated lesions and received therapeutic FV. Maternal MS levels declined during CHX use, but increased when discontinued.

Conclusions. Maternal postpartum CHX regimen, oral health counselling and preventive child FV applications were not more efficacious than maternal counselling with child therapeutic FV for precavitated lesions for ECC prevention. FV for young children with brief maternal CHX use and oral health counselling may need to be combined with additional or longer-term therapies to significantly reduce ECC in high-risk populations.

Introduction

Early Childhood Caries (ECC) remains the most prevalent chronic US childhood disease,[1,2] increasing from 24% in 1988–1994 to 28% in 1999–2004.[3] ECC poses a serious threat to child welfare, particularly among economically disadvantaged, underserved children[4,5] with sequelae of pain, difficulty eating, speech impediment and being underweight.[3] Mexican-American children have ECC at 3.5–4.6 times greater prevalence than the US population.[6] ECC's adverse health effects, economic costs, and racial/ethnic and income disparities call for improved prevention strategies. Dental caries can begin in 10–12-month-old children.[7–9] Research showed caries in 2.5- to 3.8-year-olds may progress rapidly in two years.[10–12] Although ECC is a chronic, multifactorial, transmissible, infectious disease, it is largely preventable. When the Mothers and Youth Access (MAYA) study began, ECC prevention guidelines were not evidence-based. Ismail reviewed 130 papers from 1966 to 1997 concluding, 'No well-conducted clinical trial on the prevention of ECC was identified.'[13] Caries' biological mechanisms, fairly well understood in adults, may differ in young children with newly erupted teeth, vertical and horizontal bacteria transmission, fragile enamel, and immature immune systems.[14] Chlorhexidine (CHX)

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mouth rinses are broad-spectrum antimicrobials that can reduce plaque, caries and gingivitis.[15] It was hypothesized that reducing maternal salivary MS levels during a child's tooth emergence could delay or prevent MS colonizing their primary dentition, thereby decreasing caries incidence.[16] Fluoride varnish (FV) is a high-concentration topical fluoride (22,500 ppm) applied to teeth with a small brush that prevents demineralization and promotes remineralization of early enamel lesions.

At the San Ysidro Health Center (SYHC) in San Diego County, California, 69% of patients live below the federal poverty level; about 95% are Hispanic. In 2000, SYHC 2–4-year-olds had 68% caries prevalence (dmfs of 7.9)[17] compared with 28% national prevalence (dfs of 2.4) in 2–5-year-olds in 1999–2002.[18] The MAYA study was developed specifically for this community, which has high ECC prevalence, low socioeconomic status, limited dental care access, and no optimally fluoridated municipal water. Three interventions were selected: (1) CHX mouth rinse to reduce mothers' cariogenic bacteria and subsequent bacterial transmission to children, (2) FV to strengthen and remineralize child's tooth enamel, and (3) parental oral health counselling to encourage positive oral health behaviours.

In this dental examiner masked, randomized clinical trial for a population of primarily underserved Mexican-American pregnant women and their newborns, the objective was to compare the efficacy of two strategies in reducing caries incidence and increment; (1) the Control Group receiving (a) parental oral health counselling and (b) therapeutic ('rescue') FV (TherFV) in children developing precavitated lesions; and (2) the Intervention Group receiving (a) parental oral health counselling, (b) a three-month regimen of CHX mouth rinse for new mothers, and (c) preventive FV applications for children every six months from 12 to 36 months of age. We hypothesized that the more intensive intervention strategy geared at both reducing caries risk factors and increasing protective factors would be more efficacious than the control group's more limited intervention.

Methods

Mother and youth access was a collaboration involving the University of California, San Francisco (UCSF) and Los Angeles (UCLA), San Diego State University (SDSU), and San Ysidro Community Health Center (SYHC), with approval by three University Institutional Review Boards.

Study location and participants

The study was conducted at SYHC, a federally qualified health centre near the US–Mexico border and one of the world's busiest land border crossings.[19] Women were eligible if they were aged 18–33; in the second trimester of a normal, single-foetus pregnancy; resided in the South San Diego Bay area; supplied evidence of geographical stability; were registered patients at SYHC; and provided signed informed consent in English or Spanish. Women who were recent immigrants, who intended to return to Mexico to deliver their babies, who had high-risk pregnancies, or who had previous medical conditions, pregnancy complications, or pregnancy-related issues that required hospitalization were all excluded from study participation.

Recruitment

Given the highly transitory population, considerable efforts focused on recruitment and retention in three areas: (1) barrier reduction, (2) incentives and (3) relationship building. The Hispanic bicultural and bilingual principal investigator (PI), a US citizen with extensive research experience, led the team to develop culturally sensitive research materials. To gain acceptance and support, community members, including longstanding healthcare providers, the clinic board, local organization representatives, regional policy makers and local residents recommended recruitment strategies and hiring outreach coordinators. Further recruitment and retention details and success are described elsewhere.[20] Recruitment occurred between 2003 and 2004 and randomization between

2004 and 2006. The 36-month outcome assessment occurred between 2006 and 2008.

Randomization and masking

Mothers and their infants were randomly assigned (using computer-generated permuted blocks with varying block sizes securely stored in the web-based data management system-concealing group until assignment) to one of two treatment groups at 4 months postpartum. The timing of randomization was based on the Data and Safety Monitoring Board (DSMB) suggestion that randomization be moved from enrolment during the second trimester to 4 months post-partum to follow the principle of having randomization be as close to the initial intervention as possible. Thus, the period between enrolment and randomization would be akin to a run-in period and would reduce the post-randomization loss to follow-up. The MAYA dental examiners were masked to randomization and did not see participant charts. Separate physical locations for each activity aided examiner masking. Unblinded research team members counselled mothers and distributed CHX rinse. If the CHX caused enamel staining, the mother's teeth were cleaned prior to being seen by the examiner.

Data collection and intervention protocols

Study procedures for each mother–child dyad included multiple components: parent questionnaire, dental examination, saliva collection for bacterial levels, oral health counselling, monitoring for adverse events, referral of mother and/or child for dental treatment if signs of dental caries were identified, and chart review for possible FV applications outside the study (Fig 1). In addition, dyads in the intervention group received CHX mouth rinse for the mother (Peridex™ chlorhexidine gluconate 0.12% oral rinse; 3M ESPE OMNII Oral Pharmaceuticals, St Paul, MN, USA) and preventive FV for the child (CavityShield™ 5% NaF varnish, 3M ESPE OMNII Oral Pharmaceuticals, St Paul, MN, USA).

Parental questionnaires. Trained bilingual, bicultural MAYA staff, with strong community ties, administered questionnaires assessing mother and child oral health-related factors, in English or Spanish, with the mothers at each visit. Data were entered directly into a custom, secure, Web-based data management system with built-in validation rules. Questionnaire information included socio-demographics, medical, biological and behavioural factors potentially related to caries. At each visit, mothers were asked whether their children received FV elsewhere. If the answer was yes (there were six children receiving non-MAYA FV), they were advised to only obtain FV at MAYA visits. SYHC teams providing no-cost FV to children at WIC or health fairs asked parents whether they were MAYA participants; if yes, they did not receive FV.

Dental examinations, chart review and examiner reliability. The study's PI (FRG) trained and calibrated two MAYA dental examiners. Following universal infection control, examiners, masked to treatment group assignments, assessed mother and child caries status. NIDCR caries diagnostic criteria were used[21] supplemented with diagnostic criteria for non-cavitated (d_1) lesions.[22] A dental operatory was used. Mothers received an American Dental Association Council on Dental Health Type 3 Inspection with a mouth mirror, explorer and illumination. Child intra-/extra-oral examinations were conducted in the knee-to-knee position.[23]

Replicate exams were performed on dyads throughout the study for quality assurance (QA); 24-50 children participated per QA assessment. Inter-examiner caries reliability kappa statistics for any two examiners ranged from 0.58 to 0.95 and from 0.71 to 0.87 for intra-examiner reliability. Participants had 'reversals' (true remineralization or examiner variability) on 410 surfaces recorded initially as carious or demineralized and then sound at a subsequent visit. Of the 26,540 tooth surfaces, 310 demineralized surfaces (1.1%) were subsequently declared as sound; 19 cavitated carious surfaces (0.1%) declared as demineralized surfaces; and 81 cavitated carious surfaces

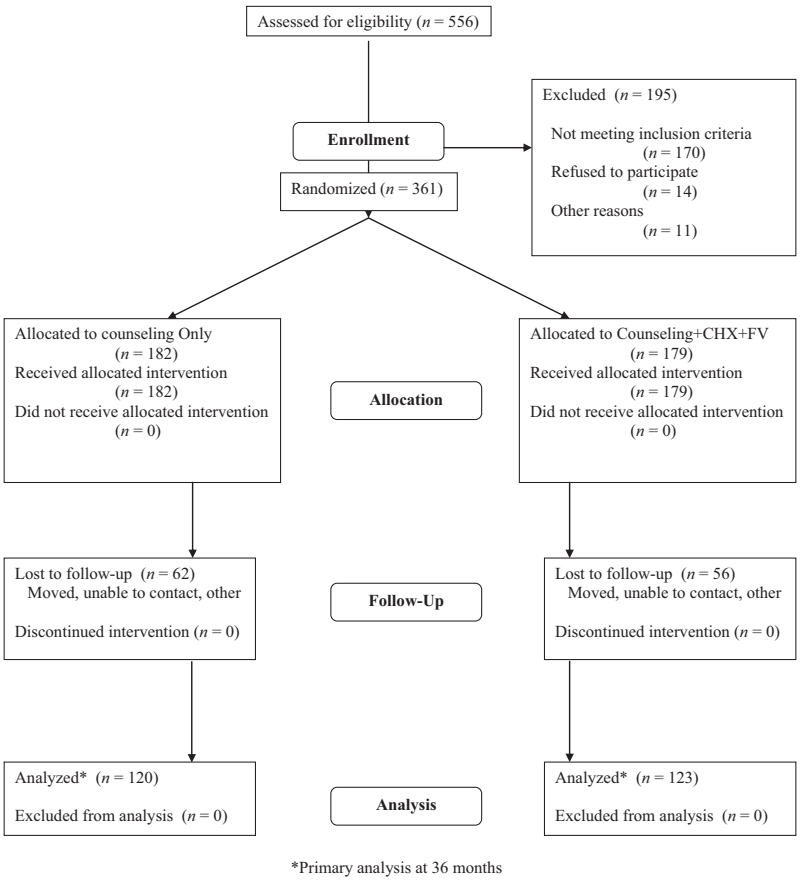

Fig. 1. MAYA flowchart.

(0.3%) declared sound; 2 (<0.1%) filled/restored tooth surfaces (in the same child) declared sound. Random audits revealed no systematic, consistent or frequent reversal pattern.

Saliva collection and microbial analysis. To assess changes in dyads' mutans streptococci levels (*S. mutans* and *S. sobrinus*) and lactobacilli levels, saliva samples of 1.5–2.0 mL were collected from all dyads. Mothers provided saliva samples at enrolment (about 3 months prior to giving birth), and with their children at 4, 9, 12, 24 and 36 months postpartum. Mothers expectorated saliva into a collection tube; children's saliva was obtained via a small plastic dropper or 'spitting' technique. For QA and reliability, split samples, one with the participant ID and one with a non-participant number, were both analysed masked to determine reliability (intraclass correlations from 0.60 to 0.98 for MS levels). Saliva remained refrigerated until transfer to UCSD Hospital Microbiology Laboratory, where within 24 h, serial dilutions and plating were performed to culture MS and LB.[24] Following 48–72-h anaerobic incubation, LB colonies were enumerated and MS plates were refrigerated and shipped every other week to UCSF for enumeration.

Interventions. Beginning at 4 months postpartum, intervention group mothers were instructed to rinse twice daily for 3 months with 0.5 oz. CHX on a 14-day rinse, 14-day rinse-free interval[25] based on the hypothesis that CHX would be most efficacious in reducing maternal bacteria levels and transmission during the initial eruption stages of the child's primary dentition. Mothers were counselled to maintain daily oral hygiene. Intervention group children received 0.25 mL FV containing 5.6 mg fluoride every 6 months, from 12 to 36 months.[26,27] Prior to treatment, as part

of the informed consent process, participants were advised of possible CHX and FV side effects. If a participant reported any CHX-related adverse event (AE), immediate stoppage was advised. All AEs were recorded and a MAYA dental provider evaluated the participant within a week. Once a CHX-related AE was confirmed, discontinuation was permanent. If the AE was unrelated to CHX, a two-week stoppage with resumption after symptoms dissipated was recommended.

At each visit, mothers received one-on-one oral health counselling in Spanish or English. Topics included were specific to the age-appropriate American Academy of Pediatric Dentistry recommendations for anticipatory guidance in paediatric dental care[4,28] and included oral hygiene, diet and nutrition, appropriate fluoride use, pacifier use, ECC, nursing bottle use, weaning at appropriate times, sugar's role in dental caries initiation, and injury prevention. Culturally suitable scripts were developed and tested before implementation. Four bilingual, age-specific educational brochures (prenatal, infants, toddlers and 2–5 years) reinforced oral health for the mothers and their children. During the clinical examination, the examiner instructed mothers on brushing children's teeth and tongue.

Control group children who developed precavitated 'white spot' lesions (d_1) immediately received therapeutic FV (TherFV) every 6 months as a 'rescue' treatment for the study duration. Precavitated lesions for intervention group children were recorded and subsequently monitored. Children with cavitated lesions (d_{2+}) received subsequent FV treatment every 6 months and were referred to the SYHC Paediatric Dental Clinic for treatment at discounted rates. The MAYA research assistant or case manager ensured SYHC dental appointments were made, but families were neither required to obtain treatment, nor barred from continuing in MAYA. Mothers' precavitated or cavitated lesions were noted in their charts and they were referred to SYHC for treatment, where discounted services were offered during pregnancy through 10 months postpartum. Participants, who met the financial criteria, continued to qualify for discounted dental services.

Sample size. Based on SYHC's 2000 data,[15] sample size was calculated for difference in dmft between intervention and control groups with a two-sided ($\alpha = 5\%$) *t*-test to provide 90% power, under various scenarios using a conservative age-specific standard deviation (5.37), mean dmft of the oldest age group for the control group (5.85) at age 3, and intervention group estimate of 3.5 at age 3, for 98 children per group completing the study. 1998 SYHC reports gave attrition estimates over time in this mobile, high risk population, so enrolment sample size was increased to 239 per group (478 total). The DSMB suggested randomizing at 4-months postpartum instead of during pregnancy corresponding to total enrolment of 512 and later recommended a 'rescue' therapeutic FV (TherFV) for ethical reasons on any control group participants with precavitated lesions, which would reduce effect size and required increasing total enrolment to 550, expected randomization total to 334 mother–child dyads, and those completing 36-month follow-up to 226.

Statistical analyses. Descriptive statistics summarized baseline factors. A multivariate test assessed baseline treatment group imbalance to avoid inflating alpha (Type I error). Intention-to-treat was used for primary analyses. Supplemental analyses utilized participants 'as treated'. Primary analysis compared 3-year children's caries increment (d_{2+}fs) between control and intervention groups with a negative binomial generalized linear model. Three-year caries incidence ($d_{2+}\text{fs} > 0$) was assessed between groups with a logit model. As treated, analyses used four groups to compare like with like, splitting each of the two randomized groups into whether they had a precavitated lesion and a subsequent FV application. 3-year increment used a negative binomial generalized linear model with a contrast averaging the control-intervention difference for those without precavitated lesions and that for those with precavitated lesions. 3-year incidence used a Mantel-Haenszel test stratifying on precavitated lesion status. Change in mother's $\log_{10}$ mutans streptococci (MS) level from 4-months postpartum to

9-months postpartum was compared between control and intervention groups with a 2-sample *t*-test to examine CHX use/compliance in the intervention group. The unmasked data analyst (WS) performed one DSMB interim analysis (Lan-DeMets alpha spending function and O'Brien-Flemming rule) about halfway to study completion with $\alpha = 0.003$; thus, $\alpha =$ ~~0.0047~~ applied to these final analyses.

Results:

Mother and youth access mothers' mean age at baseline was 29 years. Most were Spanish speaking, born in Mexico, who had at least a high school education, an annual household income less than $15,000, belonged to a dual parent household, and were recruited from within SYHC. Baseline variables were similar in both randomized groups (10 d.f. chi-square $P = 0.775$). (Table 1) Of the 556 initially pregnant enrollees, 361 (65%) were randomized at the 4-month postpartum visit. Exclusions prior to the 4-month visit were because of missed appointments or relocation (78%). Thereafter, the reasons were high-risk pregnancy (7%), voluntary exit (7%), abortion or child death (2%), and other (6%). Postpartum interval retention was 86% through 9 months, 97% through 12 months, 92% through 18 months, 94% through 24 months, 94% through 30 months, and 99% through 36 months. Overall post-randomization retention, 67.3% (243/361), was almost identical to the planned 67.7%.

Outcomes. The change (decline) in mothers' $\log_{10}$MS from 4 months when the intervention group mothers began CHX to 9 months when saliva was collected and bacteria levels enumerated, and the change during the same time period for mothers in the control group differed significantly by 0.39 ($P = 0.012$; 95% confidence interval of difference in change: 0.09–0.70). This decline provides evidence that the intervention group used CHX rinse during this time and CHX decreased salivary MS levels (Fig 2); however, once the CHX was discontinued, maternal MS levels began to steadily increase. By 36 months, 34.2% of the children in each group had cavitated caries incidence (d_{2+}fs > 0); most had untreated lesions (d_{2+}s > 0). (Table 2) A Fisher's exact test at 36 months of (d_{2+}fs) > 0 between the two groups ($P = 1.000$) and a negative binomial model of d_{2+}fs ($P = 0.731$) were both non-significant. A Mantel-Haenszel test at 36 months of (d_{2+}fs) > 0 between the two groups stratifying on $d_1 > 0$ before 36 months ($P = 0.817$) and a negative binomial model of d_{2+}fs with a contrast of the average difference between the two groups ($P = 0.808$) were also both not statistically significant (Table 3). In the control group, 67 (56%) of 120 children received therapeutic FV (TherFV)

Table 1. Baseline characteristics by randomized treatment group.

Characteristic	Counseling only* (n = 182)	Counseling + CHX + FV (n = 179)	Total (N = 361)
Mother's age (mean ± SD)**	28.9 ± 4.5	29.1 ± 4.6	29.0 ± 4.5
Race/ethnicity (%, *n*)			
Mexican/ Mexican-American	93.3 (167)	94.4 (167)	93.8 (334)
Other hispanic	3.4 (6)	1.1 (2)	2.2 (8)
Other/non-hispanic	3.4 (6)	4.5 (8)	3.9 (14)
Primary language at home (%, *n*)			
Spanish	60.6 (109)	62.9 (112)	61.7 (221)
English	6.7 (12)	3.9 (7)	5.3 (19)
Both	32.8 (59)	31.5 (56)	32.1 (115)
Other	0.0 (0)	1.7 (3)	0.8 (3)
Birth place (%, *n*)			
Mexico	57.7 (105)	63.6 (112)	60.6 (217)
Not Mexico	42.3 (77)	36.4 (64)	39.4 (141)
Education level (%, *n*)***			
<12th grade	39.3 (70)	41.4 (72)	40.3 (142)
≥12th grade/ vocational	60.7 (108)	58.6 (102)	59.7 (210)
Annual household income (%, *n*)			
<$15,000	51.8 (88)	50.9 (88)	51.3 (176)
$15,000–$49,999	45.9 (78)	47.4 (82)	46.7 (160)
>$50,000	2.4 (4)	1.7 (3)	2.0 (7)
Household composition (%, *n*)			
Two parents	64.1 (116)	68.0 (121)	66.0 (237)
Single parent	35.9 (65)	32.0 (57)	34.0 (122)

These data elements were not inclusion/exclusion criteria but the characteristics of the participants.
*Includes children who received therapeutic fluoride varnish.
**Participant's ages ranged from 18 to 37. The median age was near the mean. It is possible that younger women may have returned to Mexico for familial support with their young children.
***About 60% had at least a high school education (≥ HS) or vocational training, but fewer than 10% attended or graduated college. Few of the women had education beyond high school or vocational school.

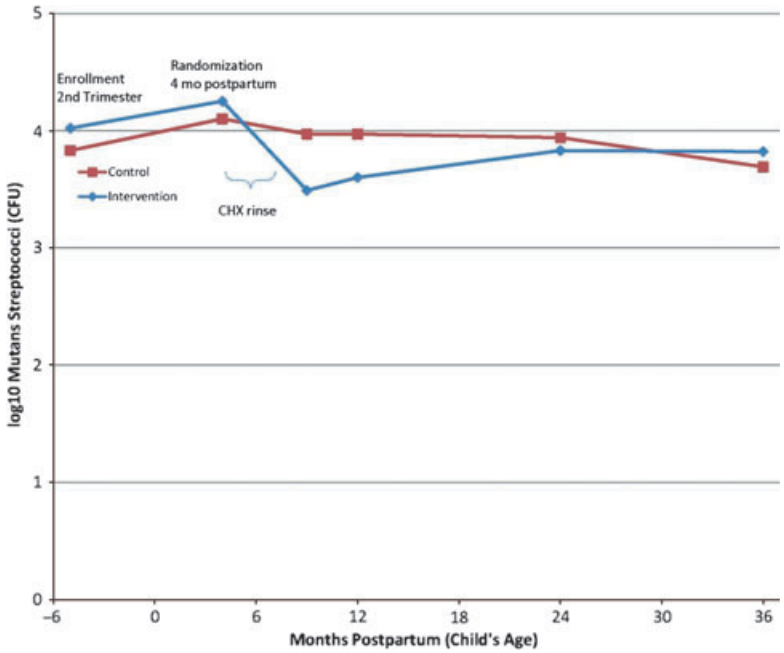

Fig. 2. Maternal MS levels.

Table 2. Intent to treat – children's caries incidence [percent (*n*)] and caries increment [mean (SD)] by 36 months.

Measure	Counseling Only* (*n* = 120)	Counseling + CHX + FV (*n* = 123)
(d_{2+}fs) > 0	34.2 (41)	34.2 (42)
(d_{2+}s) > 0	32.5 (39)	29.3 (36)
(d_{1+}fs) > 0	54.2 (65)	55.3 (68)
(d_{1+}s) > 0	53.3 (64)	54.5 (67)
d_{2+}fs	2.2 (5.2)	2.5 (5.5)
d_{2+}s	1.5 (3.2)	1.4 (3.4)
d_{1+}fs	4.4 (7.0)	4.5 (6.9)
d_{1+}s	3.7 (5.5)	3.4 (5.3)

d_{1+}s includes precavitated & cavitated lesions; d_{2+}s only includes cavitated lesions.
*Includes children who received therapeutic fluoride varnish if needed (TherFV)

subsequently, whereas 65 (53%) of 123 children in the intervention group had a demineralized lesion before 36 months. Caries incidence (d_{2+}fs > 0) compared over time between the groups with a GEE logit model was non-significant ($P = 0.959$) as was increment (d_{2+}fs) with a GEE negative binomial model ($P = 0.954$) (Table 4). Caries incidence (d_{2+}fs > 0) compared as an average difference between counselling only and C + CHX + FV groups as well as between C + TherFV and C + CHX + FV + TherFV groups over time with a GEE logit model was non-significant ($P = 0.782$); caries increment (d_{2+}fs) with a GEE negative binomial model was also non-significant ($P = 0.805$) (Table 5).

Mother and youth access had six serious adverse events (SAEs): three foetal mortalities, which were unrelated to study, prior to randomization; three intervention group mothers became pregnant post-randomization, discontinued CHX rinse, and had normal healthy deliveries. Moreover, there were 15 occurrences of mothers' dissatisfaction with dental staining likely associated with CHX; each mother was offered a free SYHC dental cleaning. A total of 917 FV applications were performed with no subject safety issues and no AEs, corresponding to a 1-sided upper 95% confidence interval of a FV application-related AE of 0.33%.[29]

Discussion

Interpretation

Mother and youth access began with a population at extremely high caries risk. About half the control group developed precavitated lesions and subsequently received therapeutic 'rescue' FV (TherFV) for ethical reasons in

Table 3. As-treated – children's caries incidence [percent (*n*)] and caries increment [mean (SD)] by 36 months.

Measure	Counsel only (*n* = 53)	Counsel + TherFV (*n* = 67)	C + CHX + FV (*n* = 58)	C + CHX + FV + ''TherFV''* (*n* = 65)
(d_{2+}fs) > 0	13.2 (7)	50.8 (34)	8.6 (5)	56.9 (37)
(d_{2+}s) > 0	13.2 (7)	47.8 (32)	6.9 (4)	49.2 (32)
(d_{1+}fs) > 0	26.4 (14)	76.1 (51)	29.3 (17)	78.5 (51)
(d_{1+}s) > 0	26.4 (14)	74.6 (50)	29.3 (17)	76.9 (50)
d_{2+}fs	0.3 (0.7)	3.7 (6.6)	0.3 (1.1)	4.5 (7.0)
d_{2+}s	0.3 (0.7)	2.5 (4.0)	0.2 (0.7)	2.5 (4.3)
d_{1+}fs	0.6 (1.3)	7.4 (8.2)	0.8 (1.7)	7.8 (8.0)
d_{1+}s	0.6 (1.3)	6.1 (6.3)	0.7 (1.3)	5.8 (6.3)

*C + CHX + FV + ''TherFV'' are children randomized to C + CHX + FV who would have gotten therapeutic FV at subsequent visit(s) if they had been randomized to the Counsel Only group. [this is needed to compare like with like; i.e. Counsel+TherFV *vs* C + CHX + FV + ''TherFV''].

Table 4. Intent to treat – children's caries incidence [percent (*n*)] by follow-up time.

Follow-up time (month)	(d_{2+}fs) > 0		(d_{1+}fs) > 0	
	Counsel only* (*n* = 120)	C + CHX + FV (*n* = 123)	Counsel only* (*n* = 120)	C + CHX + FV (*n* = 123)
12	0.0 (0)	0.0 (0)	6.8 (10)	8.3 (13)
18	5.8 (8)	6.5 (9)	20.3 (28)	20.1 (28)
24	11.7 (15)	16.5 (22)	29.7 (38)	30.8 (41)
30	23.5 (28)	21.4 (27)	46.2 (55)	46.8 (59)
36	34.2 (41)	34.2 (42)	54.2 (65)	55.3 (68)

Sample sizes listed are for 36 months; sample sizes for 12–36 months were respectively, Counsel only: 146, 138, 128, 119, 120; C + CHX + FV: 155, 139, 133, 126, 123.
*Includes children who received therapeutic fluoride varnish.

Table 5. As-treated – children's caries incidence [percent (*n*)] by follow-up time.

Follow-up time (months)	(d_{2+}fs) > 0				(d_{1+}fs) > 0			
	Counsel only (*n* = 53)	C + TherFV (*n* = 67)	C + CHX + FV (*n* = 58)	C + CHX + FV + ''TherFV'' (*n* = 65)	Counsel only (*n* = 53)	C + TherFV (*n* = 67)	C + CHX + FV (*n* = 58)	C + CHX + FV + ''TherFV'' (*n* = 65)
12	0.0 (0)	–	0.0 (0)	–	6.8 (10)	–	8.3 (13)	–
18	4.7 (6)	20.0 (2)	6.3 (8)	8.3 (1)	19.5 (25)	30.0 (3)	15.8 (20)	66.7 (8)
24	4.1 (4)	35.5 (11)	3.9 (4)	60.0 (18)	18.6 (18)	64.5 (20)	16.5 (17)	80.0 (24)
30	4.0 (3)	58.1 (25)	4.8 (4)	54.8 (23)	30.3 (23)	74.4 (32)	29.8 (25)	81.0 (34)
36	13.2 (7)	50.8 (34)	8.6 (5)	56.9 (37)	26.4 (14)	76.1 (51)	29.3 (17)	78.5 (51)

Sample sizes in the table are for 36 months; sample sizes for 12–36 months were, respectively, Counseling only: 146, 128, 97, 76, 53; C + TherFV: 0, 10, 31, 43, 67; C + CHX + FV: 155, 127, 103, 84, 58; C + CHX + FV + ''TherFV'': 0, 12, 30, 42, 65.
*C + CHX + FV + ''TherFV'' are children randomized to C + CHX + FV who would have gotten therapeutic FV at subsequent visit(s) if they had been randomized to the Counseling Only group.

these very young children. Thus, the 'intent-to-treat' analysis includes children in the control group with and without FV, diluting any possible differences between groups. The control group 'as treated' who did not develop precavitated lesions is at much lower caries risk. Although outside discounted dental services were available to MAYA mothers and children, mothers had an extremely high prevalence of caries and untreated cavitated lesions at study baseline.[30] Analysis showed a decrease in intervention group maternal bacteria levels during their 3-month CHX rinse regimen; however, once CHX was terminated, bacteria levels increased. Thus, bacterial transmission from mother to child was

not likely reduced for this 3-year study period. A longer CHX regimen may be warranted. Horizontal bacterial transmission to child participants from siblings and other children may have also occurred. CHX and/or FV interventions may have given parents a false sense of security differing from the control group. In addition, the lack or unavailability of effective child antibacterial regimens needs future research, as FV alone insufficiently addressed the extremely high baseline risk of these young children. From our previous FV study[8] and MAYA, there were no reports of any child's allergic reaction, sensitivity to FV, or any contraindications with asthmatic patients encountered during the study.

Mother and youth access participants had considerably reduced ECC compared with the same population historically. In 2000, SYHC caries prevalence in 2–4-year-olds was 68% (mean dmfs 7.9). In MAYA, at age 3, caries prevalence was 34% (mean d_{2+}fs 2.3 and the m component was very small in this age group). Although there were no differences between MAYA's control and intervention groups, participants had one-half the ECC prevalence and less than one-third the caries severity compared with their community's historical data. Well-timed and consistent maternal oral health counselling delivered in a culturally sensitive manner to both groups may have played an important role in reducing caries incidence for both groups. Importantly, our prior study demonstrating significant caries prevention from FV excluded children with caries at baseline when they were 6–44 months old[8] whereas this study included all children from 4 months of age; thus, this study included children at even higher risk than our previous study. Fluoride varnish for children and CHX and counselling for mothers did not provide significantly more caries prevention in this group than counselling alone. Because participants were recruited from a single, US-Mexico border city, with a similar sociodemographic profile, their cultural/social idiosyncrasies (e.g. dietary practices, social structures, and immigration status) may diminish the generalizability.

Overall evidence

Parental counselling, a 3-month maternal CHX regimen, and FV applied every 6 months to children's teeth was not shown to be sufficient to significantly reduce caries; and was not more efficacious than parental counselling alone combined with therapeutic fluoride varnish for precavitated lesions. The findings raise additional questions on how to effectively and substantially reduce caries in this extremely high caries risk population. A multi-faceted intervention programme may be required that considers the following: (1) an age-appropriate antibacterial regimen for very young children, (2) FV application frequency, (3) maternal CHX rinse duration, (4) length and type of parental oral health counselling, and (5) additional caries preventive strategies based on age and caries risk. Furthermore, the duration and continuum of these interventions may be significant for their cumulative effect on caries prevention.

What this paper or case report adds

- ~~Parental counselling, a 3-month maternal CHX regimen and FV applied semi-annually to the child's teeth were not shown sufficient in extremely high caries risk populations to significantly reduce caries and was not more efficacious than parental counselling alone combined with FV alone.~~
- ~~A multi-faceted caries intervention programme may not only be required, but the duration and continuum of treatment may be significant for their cumulative effect on caries prevention.~~

Why this paper or case report is important to paediatric dentists

- Colonization of bacteria occurred at a very early age, prior to the eruption of the first tooth, and continued to increase as the child aged.
- Bacterial testing can be considered a good predictor within a disease progression management model and is recommended as part of a comprehensive risk-assessment tool.
- Salivary MS & LB levels and mother's age were identified as significant ECC risk factors strongly predicting future caries incidence/increment.

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Conflict of interests

There are no conflicts of interest for any of the contributing author.

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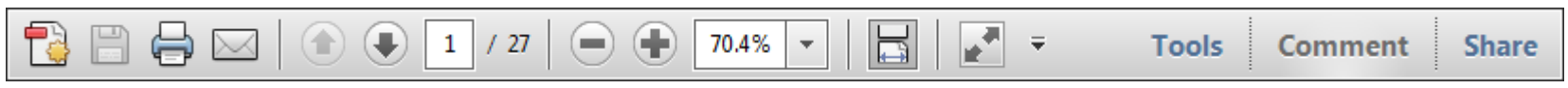
WILEY-BLACKWELL

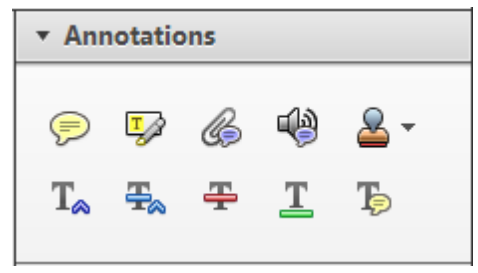
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

Required software to e-Annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 8.0 or above). (Note that this document uses screenshots from Adobe Reader X)
The latest version of Acrobat Reader can be downloaded for free at: http://get.adobe.com/reader/

Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:

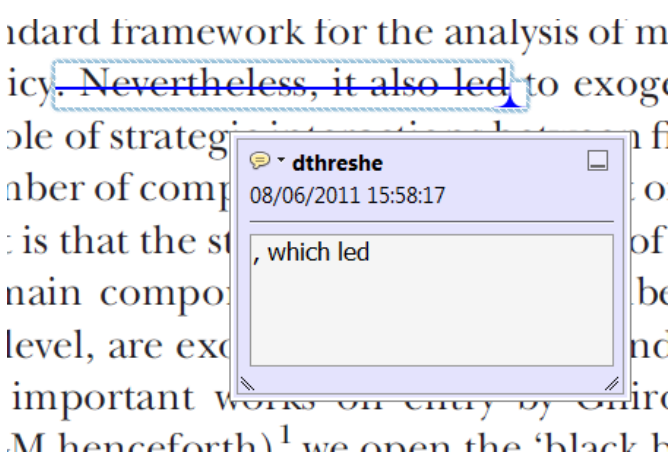


1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

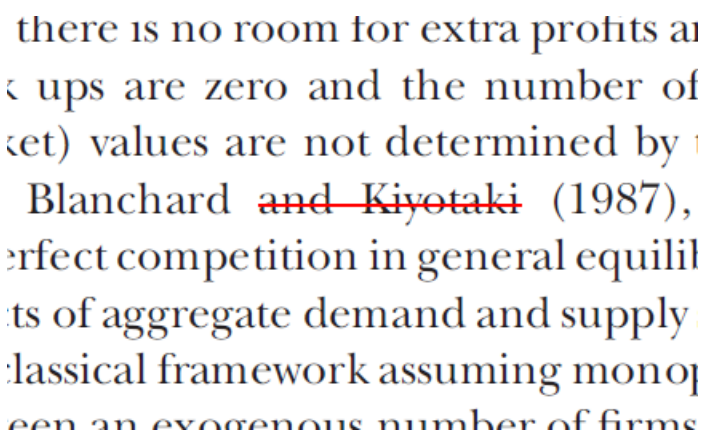


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

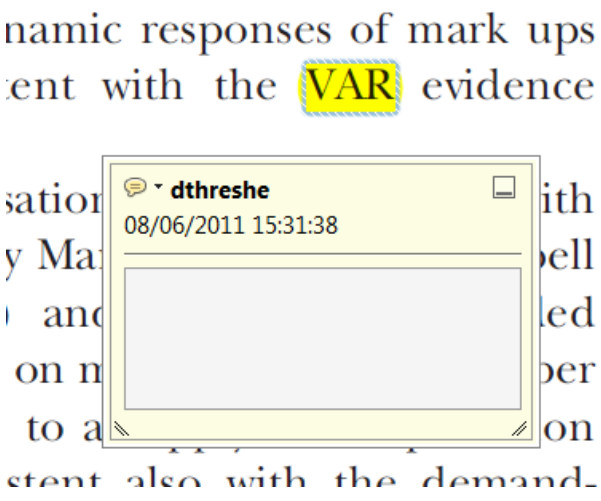


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

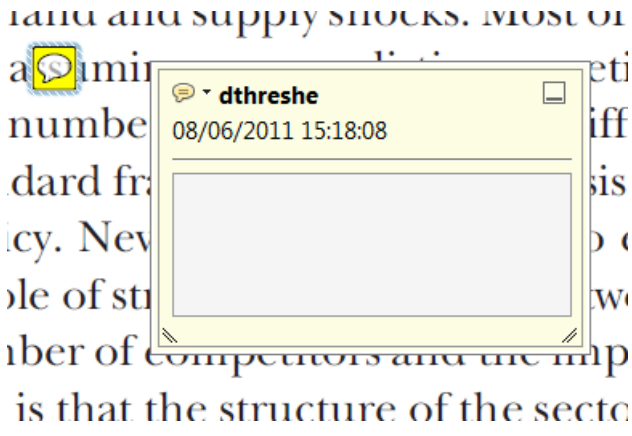


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



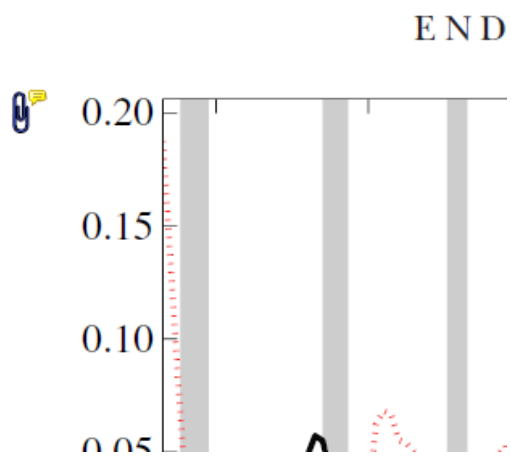
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the Add stamp icon in the Annotations section.
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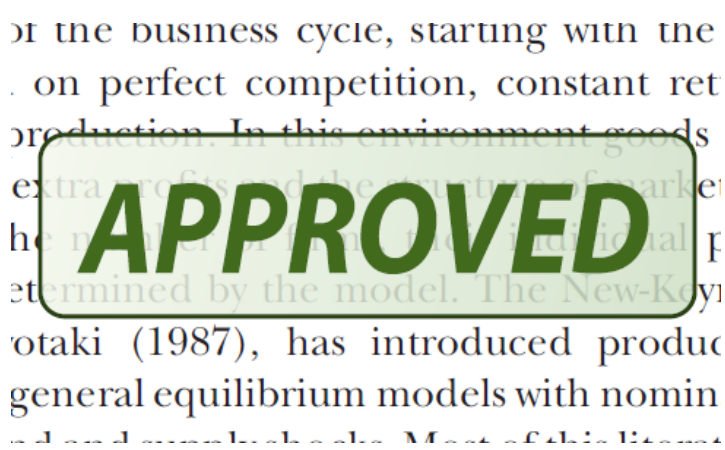
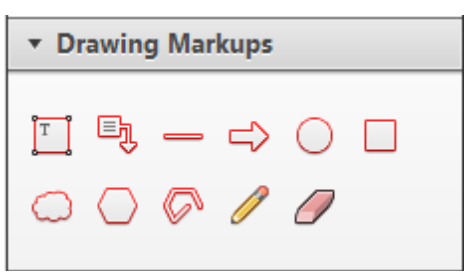


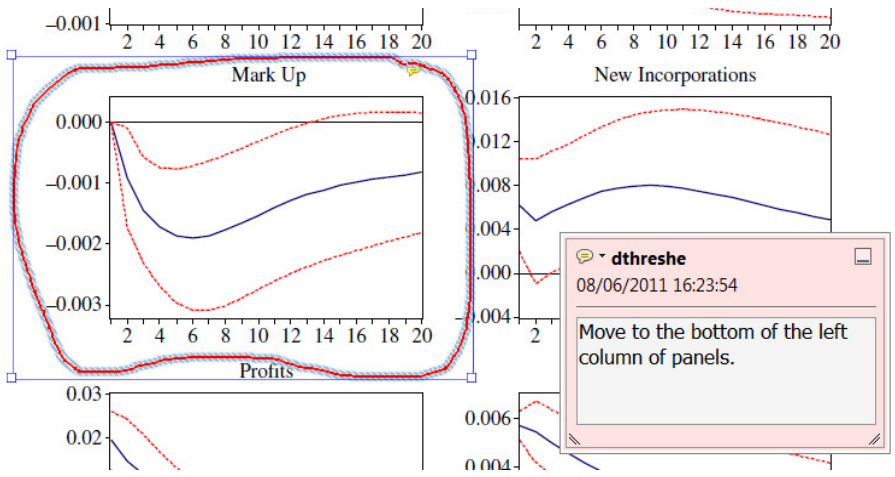


7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



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