COPYRIGHT AND USE OF THIS THESIS

This thesis must be used in accordance with the provisions of the Copyright Act 1968.

Reproduction of material protected by copyright may be an infringement of copyright and copyright owners may be entitled to take legal action against persons who infringe their copyright.

Section 51 (2) of the Copyright Act permits an authorized officer of a university library or archives to provide a copy (by communication or otherwise) of an unpublished thesis kept in the library or archives, to a person who satisfies the authorized officer that he or she requires the reproduction for the purposes of research or study.

The Copyright Act grants the creator of a work a number of moral rights, specifically the right of attribution, the right against false attribution and the right of integrity.

You may infringe the author’s moral rights if you:

- fail to acknowledge the author of this thesis if you quote sections from the work
- attribute this thesis to another author
- subject this thesis to derogatory treatment which may prejudice the author’s reputation

For further information contact the University’s Copyright Service.

sydney.edu.au/copyright
An investigation of the possible anti-cariogenic effect of raw sugarcane: an epidemiologic study of 12-year-old Punjabi Children, India

Amandeep Singh, BDS (Magadh, India)

A thesis submitted in partial fulfilment of the requirements for the degree of

MASTER OF DENTAL SCIENCE
Community Oral Health and Epidemiology

Faculty of Dentistry
The University of Sydney Australia

2006
Dedication

I dedicate my thesis to my father E.R. Haravtar Singh and to my mother Surinder Kaur for their constant support and courage to support me in studying further and helping me financially and emotionally in completing my degree.
Acknowledgements

I would like to express my sincere thanks to the following people for their contribution and help towards the research and preparation of the thesis:

- Associate Professor Wendell Evans, Head of Discipline, Community Oral Health and Epidemiology, University of Sydney for his guidance and assistance in completing the thesis.

- Azad Singh, School Principal, for his support in conducting the survey.

- Dr Karen Byth, Statistician, for her help in conducting the statistical analysis.

- Dr Brad Curtis, PhD student, for his help with the literature search.

- Ms Ramona Grimm, Administrative Officer, Community Oral Health and Epidemiology for her administrative support.

- Dr Sarkis Nalbandian, Dentist, for his encouragement and emotional support during the completion of the thesis.

- Dr Abhinandan Vashistha, dental colleague, for his constant support and encouragement during the writing of the thesis.

********************************************************************************
# Table of Contents

Dedication .................................................................................................................. ii
Acknowledgements .................................................................................................... iii
Table of Contents ...................................................................................................... iv
List of Tables ............................................................................................................. vi
List of Abbreviations ............................................................................................... vii
Foreword ..................................................................................................................... viii

**Part One: Review of Literature** ........................................................................... 1
  - In vitro studies .................................................................................................... 2
  - Cross-sectional uncontrolled studies ................................................................. 3
  - Cross-sectional controlled study ...................................................................... 5
  - Controlled cohort study .................................................................................... 6
  - Experimental cross over study ........................................................................ 6

Review of findings .................................................................................................... 8
References .................................................................................................................. 9

**Part Two: The cariogenicity of raw sugarcane – a study of Punjabi 12-year-olds, India** ................................................................................................................................. 11
  - Abstract ............................................................................................................ 12

Introduction ............................................................................................................... 13
Material and methods ............................................................................................... 18
Results ....................................................................................................................... 20
Discussion .................................................................................................................. 26
Conclusion ................................................................................................................. 29
References .................................................................................................................. 30
Appendices ................................................................................................................ 34

Appendix A. Correspondence with the District Education Officer seeking permission for conducting the survey ................................................................. 35
Appendix B. Letter of approval from the Human Research Ethics Committee, The University of Sydney, including, the Participant Information Sheet (English and Punjabi), the Parent/Guardian Consent Form (English and Punjabi), and the Need for Dental Care Form (English and Punjabi) ................................................................. 39
Appendix C. Clinical Examination Form. ................................................................. 48
Appendix D. Questionnaire (English and Punjabi).................................................. 50
Appendix E. Details of the multiple variable logistic regression analysis. .......... 56
Appendix F. List of schools surveyed in both the sugarcane and the non-sugarcane growing areas. ........................................................................... 59
Appendix G. Map of Amritsar (Punjab). ................................................................. 61
List of Tables

Table 1  Summary of studies investigating the possible anticariogenic effect of raw sugarcane chewing.

Table 2  Caries experience by residential area.

Table 3  Mealtime intake of water and sugar containing beverages.

Table 4  Exposure of the children to protective/risk factors for dental caries and their caries experience by residence location.

Table 5  Odds ratios from best fitting multiple variable logistic regression model (using the backward stepwise selection method) for dental caries experience (dichotomised as DMFT = 0 and 1+).
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMFT</td>
<td>Decayed, missing, and filled teeth</td>
</tr>
<tr>
<td>DMFS</td>
<td>Decayed, missing, and filled surfaces</td>
</tr>
<tr>
<td>F</td>
<td>Fluoride</td>
</tr>
<tr>
<td>mg/L</td>
<td>Milligram per litre</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
</tbody>
</table>
Foreword

This thesis is divided into two parts:

Part One: A review of the international literature on the relationship between sugarcane chewing and dental caries.

Part Two: Part two of this thesis is a paper that will be submitted for publication in Community Dentistry and Oral Epidemiology concerned with an epidemiologic study of 12-year-old Punjabi school children, India. The purpose of this study was to investigate the effect of raw sugarcane on caries experience. It was a parallel study of 12-year-olds residing in (1) the sugarcane belt and in (2) a non-sugarcane growing area of the Amritsar District in the Punjab, India.
Part One:

Review of Literature
Review of Literature

In vitro studies

The first of the studies on sugarcane and dental caries were conducted by Osborn et al. (1937). They incubated healthy human teeth at 37°C with a mixture of saliva and crude sugarcane juice (15% sucrose) or with saliva and refined pure sugar juice (15% sucrose). The mixtures were renewed daily and were agitated continuously by means of bubblers. After 2-8 weeks of this treatment it was found that of 32 teeth exposed to the saliva-refined sugar mixture, 15 became decalcified, whereas only 3 of 32 teeth became decalcified by the saliva-crude sugarcane juice preparation. They concluded that, at least under those in vitro conditions, the raw sugarcane juice must have protective effect against the development of caries.

Jenkins et al. (1959) conducted in vitro studies on crude and refined sugars to determine the influence of the refinement of carbohydrates on their cariogenicity. Pure sucrose and crude cane juice were compared. In the procedure for incubation experiment 50 mg calcium phosphate mixed with 7 ml saliva was added to each of 5 ml of (a) Sugarcane juice (20% sucrose) (b) pure sucrose solution (20% sucrose). After incubation for 24 hours, the concentration of phosphate in solutions (a) and (b) increased by 9 and 79 micrograms/ml, respectively. The protection of teeth was clearly seen by visual inspection as well as by chemical analysis.
Cross-sectional uncontrolled studies

In the study conducted by Samuel Dreizen et al. (1952) in west central Cuba. Dental decay was investigated in a group of subjects who were originally from that region and chewed sugarcane daily, and also consumed raw sugarcane juice in large quantities daily, especially in harvesting season. Daily diet since birth was high in natural and unrefined carbohydrate foods. Crude brown sugar was used exclusively for seasoning and cooking. Of the147 total number of subjects chosen, only 10 were edentulous and 137 had a complete or partial natural dentition. The DMFT score was 15.1. Only one person was caries free. Extensive amounts of tooth destruction and high caries incidence in the group did not support their null hypothesis “that raw sugarcane and unrefined carbohydrates contain a protective factor in amounts sufficient to prevent tooth decay”.

In another study, conducted by Harris and Cleaton-Jones (1978) on 98 Bantu sugarcane chewers in South Africa, the rural labourers lived and worked in sugarcane farms in the Pongola district. All ate a typical Bantu diet consisting of maize meal supplemented by legumes and meat. Fluoride level in drinking water was <0.1 ppm. On average 4-5 kg of sugarcane was chewed per day (=500g sucrose), however the mean DMFT scores for adolescents, young adults, middle, and old age groups were: 0.9, 3.1, 6.0, and 8.1, respectively. The mean DMFT across all age groups in this male population was 2.8.

Nörmark and Mosha (1989) conducted a study to investigate the relationship between habits and dental health among rural Tanzanian children. A total of
399 children of standard two were examined average age was 10.9 yrs. Fifty-three percent of children examined were caries free. Seventy-three percent of the caries observed were in occlusal surfaces of molars. No fillings were present and no teeth were missing due to caries. Fifty-nine percent of children ate sugarcane everyday. Children eating cakes, biscuits, and/or sweets at least three times a day seemed to have more caries than the children who did not, their DMFS being 1.5 and 1.1 respectively. The distribution of DMFS by sugarcane was random. They concluded that “consumption of sugarcane did not bear any relation to the caries experience”.

Rugarabamu et al. (1990) assessed caries experience in 12 and 15-year-old children living on a sugar estate in Kilombero, Tanzania. The average sugar consumption per capita per year was estimated to be six times higher (35kg v/s 6kg) for the estate population than for the Tanzanian population as a whole. At that time, refined sugar was regarded as a “very precious commodity” because not everyone had easy access to it. However, sugar plant workers were given a 10kg bag of sugar per month as an incentive. (It cannot be assumed however, that they consumed the total sugar given to them because it was reported that it may have been given to friends or relatives). The fluoride concentration level in the area varied between 0.23 - 0.27 ppm (dry season) and 0.57 - 0.65 ppm (wet season). The percentage of caries-free children was higher in 12-year-olds (68%) than in 15-year-olds (60%). Compared with the mean DMFT score of 0.48 in this study for 12-year-old children, similar DMFT scores of 0.51 and 0.67 for rural and urban 12-year-olds, respectively, have been reported in relation to other communities.
investigated in Tanzania by the same authors (Manji et al., 1988; Frencken et al., 1986). It might have been expected that the caries experience of the children in the sugarcane estate would be higher than elsewhere due to their much higher consumption of refined sugar. However, the results supported the author's (Rugarabamu) conclusion that the caries experience of children in the sugarcane estate was low and in keeping with data elsewhere in Tanzania. Nevertheless, it cannot be overlooked that the caries experience of the estate children was low because of the possible caries protective effect afforded by sugarcane consumption.

**Cross-sectional controlled study**

Frencken and Rugarabamu (1989) conducted a study to investigate the effect of sugar cane chewing on the development of dental caries in Tanzania. Two groups, 77 sugar cane cutters and 68 sisal workers, were compared. The daily mean numbers of sugarcane pinglee (sections of a sugarcane stick) consumed by the cane cutters and sisal workers were estimated to be 8.1 and 0.8 respectively. It was claimed that the diets of the two groups were otherwise comparable and that the both groups consumed around 4 gm of refined sugar per day. Socio-economic status and fluoride levels in drinking water in both the groups were similar. The caries experience of the sugarcane cutters and sisal workers were 3.5 and 2.0 DMFT (p=0.01) and 13.9 and 7.8 DMFS (p=0.02) respectively. Greater pinglee consumption was associated with higher mean DMFS (p=0.05) and DMFT (p=0.02) scores. The authors concluded that the data "suggest that sugar cane chewing in large quantities
over a long period of time has caries-promoting effecting a population with low caries prevalence".

**Controlled cohort study**

Harris *et al.* (1967) conducted a clinical trial of 5-17 year old boarding school children in Queensland, Australia. Two groups, 361 controls and 166 treatments were compared. The treatment group was exposed to a diet in which calcium sucrose phosphate was substituted for dietary sugar. Both the groups were followed for a period of 3 years. Medical investigation showed no differences in the physical status and general health between the children of both groups. Compared with children in the control groups, scores for DMFT, DMFS, and DMFS (proximal surfaces) for 9-13 year old children were less by 15.3%, 17.9%, and 29.5%, respectively in the group in which calcium sucrose phosphate was substituted for dietary sugar.

**Experimental cross over study**

Fejerskov (1992) conducted a study on five rural Kenyan children aged 7 to 14 years, to investigate the effect of sugar cane chewing on plaque pH. The selected children had poor oral hygiene and had two or more open carious cavities. The children were asked to rinse with 10ml of 10% sucrose solution on day 1 and to chew sugarcane for 3 min on day 2. Each day, the plaque pH measurements were taken before the experiment (the resting pH) and at 2, 5, 10, 15, 20, and 30 mins after rinsing or chewing. pH responses were measured at interproximal sites in the maxilla and the mandible. The main results were that following sugarcane chewing, plaque pH dropped and then
recovered to resting values in 5-10 mins or before, at all sites, whereas, pH values took longer than 30 minutes to recover following the sucrose rinses. It was concluded that the result was probably not due to a protective factor derived from the sugarcane but that the rapid pH rise was a natural result of an increased saliva flow rate which, in this case, was stimulated by the combination of the sweet cane juice and the vigorous chewing action required to masticate the tough cane.
Review of findings

The analysis of the literature summarized in Part One of this thesis is reported on in the introduction section in Part Two.
References


Part Two:

The cariogenicity of raw sugarcane – a study of Punjabi 12-year-olds, India
The cariogenicity of raw sugarcane – a study of Punjabi 12-year-olds, India

A SINGH, R.W.EVANS.

Abstract

Introduction: There is laboratory evidence that compared with unrefined carbohydrates, refined carbohydrates are more cariogenic. It was reported that sugarcane workers in South Africa, who consumed large quantities of raw sugarcane daily, had a low caries experience. However, other epidemiologic studies of this relationship both in Africa and elsewhere have given mixed results and to date the question concerning the cariogenicity of raw sugarcane remains equivocal.

Objective: The purpose of the present study was to investigate further the hypothesis that children who reside in a sugarcane growing region will have a lower caries experience than those residing in an otherwise comparable environment outside of the cane growing region.

Material and methods: A total of 404 Children aged 12 years were selected from government schools in both sugarcane and non-sugarcane regions of Amritsar district in the state of Punjab, India. The drinking water in both regions contained fluoride at 0.5 ppm. Data on eating habits of the children were obtained by questionnaire and caries experience was determined during a clinical examination. Informed consent for the survey was obtained from the parents of the children.

Results: The mean DMFT scores for the sugarcane and non-sugarcane groups were 0.41 and 0.70 respectively (p<0.002). Neither the potential confounding variables of lollie consumption, dietary added sugar, and mealtime consumption of sugar-containing beverages were significantly associated with caries experience.

Conclusion: The null-hypothesis is rejected; the results of this study support the hypothesis that the chewing of raw sugarcane is associated with reduced dental caries.

To be submitted for publication in Community Dentistry and Oral Epidemiology.
Introduction

On the basis of an examination of skulls dating from the bronze-age onwards, Hardwick demonstrated that dental caries was rare in England and that its prevalence fluctuated with rises and falls in cereal production (1). Lesions were confined mainly to cervical margins whereas coronal caries did not generally occur until sugar came into common usage in the 17th century. Several 20th century studies of remote hunting communities have detailed dramatic rises in caries experience coinciding with diet changes following a sudden exposure to flour and sugar containing foodstuffs (2, 3).

Prior to the development of an understanding of the role of fluoride in caries prevention, research centred on the diet-caries relationship which indicated that the upsurge in sugar consumption was mainly responsible for the caries pandemic that swept western cultures during the first half of the 20th century (4). The acid theory regarding the etiology of dental caries was first postulated by Miller (1890) who demonstrated that when extracted teeth were incubated in a mixture of saliva and carbohydrate, they were subject to demineralization (5). In 1937, Osborne and Noriskin (6) reported on the low caries experience of cane cutters in South Africa in a region where the fluoride concentration in the drinking water was less than 0.1 mg/L. These workers chewed up several sugarcane sticks daily and consumed large quantities of sugarcane juice. On average, workers consumed 4.5kg of sugarcane daily (500gm sucrose) (7). Osborn et al. (8) demonstrated via in vitro studies that the incubation of mixtures of saliva and pure sucrose readily results in the
production of organic acids and that the addition of teeth or calcium phosphate to these mixtures results in their dissolution. On the other hand, when such experiments are repeated in which pure sucrose is replaced with raw sugarcane juice, the dissolution does not occur despite the acidic environment. As a result, it was postulated that phosphates which are removed during the sugar refining process, are protective against dental caries (9). It was later shown that, in fact, the acid yield on fermentation of unrefined carbohydrate was about double that derived from the refined products (10).

Animal studies demonstrate that dietary phosphate additives are protective against experimentally induced caries (11), but epidemiologic studies of caries experience in sugarcane chewers (see Table 1 for summary), that have been cited in discussions of the hypothesis that consumption of raw sugarcane is protective against caries, give mixed results (12).

Possible support for a protective effect was reported more recently among 100 Tanzanian 12-year-olds living on a sugarcane estate (13). Caries experience there was reported to be similar to that of other rural and urban Tanzanian children examined by the same authors (14, 15), but it was also reported that workers at the sugarcane estate received refined sugar as a work incentive, and that refined sugar consumption by the estate population was six times the national average. Had it not been for the possible protective effect of the sugarcane, it might have been expected that the caries
Table 1. Summary of studies investigating the possible anticariogenic effect of raw sugarcane chewing.

<table>
<thead>
<tr>
<th>First author</th>
<th>Study type</th>
<th>Location</th>
<th>Method and results</th>
<th>Support</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osborn (7)</td>
<td>In vitro</td>
<td>South Africa</td>
<td>32 teeth were added to each of mixtures of saliva and (a) crude sugarcane juice (15% sucrose) and (b) refined pure sugar solution (15% sucrose). After 8 weeks incubation, 3 and 15 teeth in solutions (a) and (b) respectively were decalcified.</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Driesen (18)</td>
<td>Cross sectional</td>
<td>Cuba</td>
<td>Study of 147 sugarcane cutters who chewed and drank large quantities of sugarcane juice daily. Crude brown sugar was also used extensively in cooking and for food seasoning. Mean DMFT was 15.1, 10 were edentulous, and 1 was caries free.</td>
<td>No</td>
<td>No control for non-cane cutters and other sugar use</td>
</tr>
<tr>
<td>Jenkins (22)</td>
<td>In vitro</td>
<td>United Kingdom</td>
<td>50 mg calcium phosphate mixed with 7 ml saliva was added to each of 5 mins of (a) sugarcane juice (20% sucrose) and (b) pure sucrose solution (20% sucrose). After incubation for 24 hours, the concentration of phosphate in solutions (a) and (b) increased by 9 and 79 micrograms/ml, respectively.</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Harris R (15)</td>
<td>Controlled cohort</td>
<td>Australia</td>
<td>Boarding school children aged 9-13 years were exposed to (a) diet in which calcium sucrose phosphate was substituted for dietary sugar and (b) normal diet. Compared with children on diet (b) scores for DMFT, DMFS, and DMFS (proximal surfaces) were less by 15.3%, 17.9% and 29.5%, respectively.</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Harris S (6)</td>
<td>Cross sectional</td>
<td>South Africa</td>
<td>Study of 98 male and female sugarcane chewers aged 14-&gt;60 years. Their daily mean consumption of sugarcane was 4.5kg (500gm sucrose). Normal diet comprised maize, legumes, meat, and water (&lt;0.1 ppm F). DMFT scores for adolescents, young adults, middle and old age groups were: 0.9, 3.1, 8.0, and 8.1, respectively.</td>
<td>Yes</td>
<td>High intake of sucrose and low caries experience hints at protective effect.</td>
</tr>
<tr>
<td>Nörmack (19)</td>
<td>Cross sectional</td>
<td>Tanzania</td>
<td>Study of 399 children aged 7-15 years. The distribution of DMFS by sugarcane was as random</td>
<td>No</td>
<td>No control for age.</td>
</tr>
<tr>
<td>Frencken (20)</td>
<td>Cross sectional controlled</td>
<td>Tanzania</td>
<td>Study of (a) 77 sugarcane cutters and (b) 68 salal labourers. Diet, said to be comparable, included 4gm of refined sugar daily. The ratio of daily sugarcane eaten (a);(b) was 8:1:0.8. DMFS for (a) and (b) were 13.9 and 7.8, respectively.</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Rugaramb (12)</td>
<td>Cross sectional</td>
<td>Tanzania</td>
<td>Study of 100 sugarcane estate children aged 12 years, where estate consumption of refined sugar was 6 times higher than the national average. Mean DMFT of 0.48 was comparable to scores of 0.51 and 0.67 for other rural and urban 12-year-olds, respectively.</td>
<td>Possible</td>
<td>Sugarcane chewing may have counter-acted the effect of exposure to refined sugar.</td>
</tr>
<tr>
<td>Fejerskov (17)</td>
<td>Experimental cross over</td>
<td>Kenya</td>
<td>Study of children aged 7-14 years with 2 or more open cavities. On day 1, they rinsed with 10% sucrose solution, and on day 2, chewed sugar cane for 3 minutes. Plaque pH was measured at non-carious proximal sites before and up to 30 minutes following both rinsing and chewing. After rinsing with sucrose solution, pH values recovered after 30 minutes, but fell less and recovered in 5-10 minutes following chewing sugarcane.</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

*1. Support for hypothesis that raw sugarcane chewing is anticariogenic.*
experience of the estate children would be higher than among the children from elsewhere.

Strong support for a sugarcane-related protective effect was shown in Australia (16) where a controlled cohort study was conducted among 399 boarding school children to investigate the effect of supplementing dietary sugar with calcium sucrose phosphate, the organic phosphate which is presumed to be removed from sugarcane during the refining process. Children attending the control schools followed their usual diet while those belonging to the experimental group were exposed to a diet in which the added sugar was supplemented with calcium sucrose phosphate. After three years, the DMFT, DMFS, and DMFS (proximal surfaces) were 15.3, 17.9, and 29.5% less in the experimental children aged 9-13 years compared with the control children of the same age. While these results seem impressive, Craig (17) reported that during the course of the study, the groups became unbalanced and that the results could be biased. A more recent in vivo study of Kenyan children, aged 7-14 years, with cavitated teeth confirmed the anticariogenic potential of chewing sugarcane (18). On one day, they rinsed with 10% pure sucrose and on the next day they chewed sugarcane for 3 minutes. Measurements at interproximal sites on both days showed that pH falls following sucrose rinses were deeper than those following the chewing of sugarcane. Further, the depressed pH induced by the sucrose exposure took longer than 30 minutes to return to baseline levels compared with return times of 5-10 minutes following sugarcane chewing. It was concluded that the result was probably not due to a protective factor derived from the sugarcane but
that the rapid pH rise was a natural result of an increased saliva flow rate
which, in this case, was stimulated by the combination of the sweet sugarcane
juice and the vigorous chewing action required to masticate the tough cane.

On the other hand, in Cuba (19), where brown sugar is used extensively in
cooking and for food seasoning, extensive caries experience and tooth loss
was reported among sugarcane workers. This study did not, however, include
non-sugarcane eating controls, nor was the dietary added sugar controlled for.
The results of another cross sectional study of sugarcane consumption among
Tanzanian children aged 7 to 15 years failed to demonstrate a correlation with
caries experience, however it is not clear whether either age-related
consumption of sugarcane or age-related caries experience was controlled for
in this study (20). In a controlled cross sectional study of adults, also
conducted in Tanzania, a caries protective effect among 77 cane cutters was
not evident (21). In this study, the caries experience of 68 sisal labourers was
also investigated. Although the ratio of sugarcane consumption by the cane
cutters and the sisal labourers was 10:1, the diets of both groups were
otherwise said to be comparable and low in refined carbohydrates. The
respective mean DMFS scores of the sugarcane and sisal workers were 13.9
and 7.8.

The purpose of the present study was to investigate further the hypothesis
that children who reside in a sugarcane-growing region will have a lower
caries experience than those residing in an otherwise comparable
environment outside of the cane-growing region.
Material and methods

This survey of 12-year-old children attending government schools was conducted in the Amritsar District of the State of Punjab, India. They were selected from villages surrounding a sugar mill, close to the town of Mehta in the sugarcane belt along the Beas River. Sugarcane chewing occurs during two periods each year totalling about 6 months when the cane is harvested. Parents of children attending the largest seven village schools in this area were requested to give permission for their 12-year-olds to participate. Similarly, consent was obtained from parents of the reference children who attended the six largest schools in villages around Attari, a non-sugarcane growing region. Attari is a small town, 50 km southwest of Mehta, near the border with Pakistan. In both areas, the fluoride concentration in the drinking water was less than 0.5 mg/L. Families subsist on their land from one generation to the next, so that the children examined were life-long residents of their respective areas. Although the children in both regions were either Sikhs (the majority) or Hindus, they live in rural villages and share the same cultural beliefs and practices; in particular, their diets are the same. Only government schools were selected, and only children of lower socio-economic groups attend government schools in Punjab.

At the selected schools, the children were interviewed according to a structured questionnaire to obtain information on their diet and tooth brushing habits. This was followed by a clinical examination. These were conducted outside in natural daylight by a single examiner (AS) who had been calibrated.
before the survey. The children, none of whom had experienced previous
dental care, were seated on a chair and their teeth were examined with the
aid of a wooden spatula but without drying. Caries was diagnosed by visual
inspection alone whereby teeth were recorded as decayed on the evidence of
presence of cavitation (22). The data were later entered into an electronic
database for subsequent statistical analysis.

Intra-examiner calibration was carried out during return visits to two schools
in each region where randomly selected children were re-examined under the
same conditions as previously.

Following data checking, the mean DMFT scores of children in the two
locations were calculated and compared. The effect of potential confounding
variables on the relationship between residential location and dental caries
experience was conducted. Firstly, the univariate association of each of the
potential confounders (total added dietary sugar, lollie consumption, mealtime
consumption of sugar containing beverages, tooth brushing frequency, and
use of fluoridated toothpaste) with DMFT was assessed. Secondly, the
potential confounders that were shown to be significantly associated with
differences in DMFT were included in a multiple variable regression analysis.
As the data were highly skewed (the majority of the children were caries free)
a logistic regression model (caries experience was dichotomised as DMFT = 0
or 1+) was developed using the backward stepwise selection option. The
analyses were carried out using SAS statistical software, Release 8.2.
Results

A total of 404 12-year-olds participated in the study. The children living in the sugarcane belt chewed around three to four sticks of sugarcane daily during the cane harvesting seasons. None of the children had received dental care. The DMFT scores reflected decayed or missing teeth only. Caries experience in both the sugarcane and non-sugarcane groups, which was confined almost exclusively to the first permanent molars, is presented in Table 2. The mean DMFT of the non-sugarcane group was 0.70 (sd=1.17) compared with 0.41 (sd=0.96) in sugarcane group. That is, the mean DMFT was 71% higher in non-sugarcane group and further, the proportion of children who had one or more DMFT was 62% higher in the non-sugarcane group. The kappa value relating to diagnostic reliability, based on 51 duplicate examinations, was 0.73.

The distribution of mealtime risk factors for caries experience, namely sugar containing beverages, which are consumed at breakfast, tiffin (lunch), and dinnertime, is shown in Table 3. Except for yoghurt (12% versus 3.9%), the consumption of all other sugar containing beverages was greater in the sugarcane group than in the non-sugarcane group at breakfast (p<0.001). At tiffin (lunch), more milk with added-sugar was consumed by the non-sugarcane group (43% versus 19%) and at dinnertime, more sweetened tea was consumed by non-sugarcane group (56% versus 27.5%) (p<0.001).
Table 2. Caries experience by residential area.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>DMFT</th>
<th>(sd)</th>
<th>DMFT = 0</th>
<th>n</th>
<th>%</th>
<th>DMFT = 1+</th>
<th>n</th>
<th>%</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sugarcane</td>
<td>200</td>
<td>0.70</td>
<td>1.17</td>
<td>127</td>
<td>63.5</td>
<td>73</td>
<td>36.5</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugarcane</td>
<td>204</td>
<td>0.41</td>
<td>0.96</td>
<td>158</td>
<td>77.5</td>
<td>46</td>
<td>22.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square = 9.45, DF = 1
### Table 3. Mealtime intake of water and sugar-containing beverages

<table>
<thead>
<tr>
<th>Meal</th>
<th>Sugarcane group</th>
<th>Water n</th>
<th>Water %</th>
<th>Milk n</th>
<th>Milk %</th>
<th>Tea n</th>
<th>Tea %</th>
<th>Yoghurt n</th>
<th>Yoghurt %</th>
<th>Total</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>Non-sugarcane</td>
<td>84</td>
<td>42.0</td>
<td>5</td>
<td>2.5</td>
<td>87</td>
<td>43.5</td>
<td>24</td>
<td>12.0</td>
<td>200</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Sugarcane</td>
<td>78</td>
<td>38.2</td>
<td>22</td>
<td>10.8</td>
<td>96</td>
<td>47.1</td>
<td>8</td>
<td>3.9</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>Tiffin</td>
<td>Non-sugarcane</td>
<td>21</td>
<td>10.5</td>
<td>86</td>
<td>43.0</td>
<td>86</td>
<td>43.0</td>
<td>7</td>
<td>3.5</td>
<td>200</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Sugarcane</td>
<td>56</td>
<td>27.5</td>
<td>39</td>
<td>19.1</td>
<td>102</td>
<td>50.0</td>
<td>7</td>
<td>3.4</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td>Non-sugarcane</td>
<td>32</td>
<td>16.0</td>
<td>32</td>
<td>16.0</td>
<td>112</td>
<td>56.0</td>
<td>24</td>
<td>12.0</td>
<td>200</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Sugarcane</td>
<td>56</td>
<td>27.5</td>
<td>25</td>
<td>12.3</td>
<td>56</td>
<td>27.5</td>
<td>67</td>
<td>32.8</td>
<td>204</td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square (across milk, tea, and yoghurt), DF = 2
Table 4 shows the distribution of exposure to additional caries risk factors namely tooth brushing habits, lollie consumption, and total daily added-sugar to children in both groups. Both groups had almost identical tooth brushing habits regarding brushing frequency and use of fluoride toothpaste. One quarter of the children reported that they brushed daily and 60% reported that they used fluoridated toothpaste. More children in the non-sugarcane group (88% versus 70%) consumed 4 or more lollies per day than in the sugarcane group (p<0.001). An almost identical amount of total daily added-sugar was consumed by both groups; the median amount was 8 teaspoons per day. This amount included sugar that is eaten by itself, as a sweet, directly after a meal. The mean DMFT scores are lower in sugarcane group in relation to each of the risk/protective factors.

In the multivariable logistic regression analysis of risk factors for a caries experience (Table 5), all but one of the factors were dropped from the model, leaving residence area alone as the sole significant predictor of experiencing one or more DMFT (see Appendix E for full details of the model). Compared with children in the reference group, the children residing in the sugarcane growing region were 49% less likely to have decayed teeth (OR=0.51; 95%CI= 0.33, 0.78)
Table 4. Exposure of the children to protective/risk factors for dental caries and their caries experience by residence location.

<table>
<thead>
<tr>
<th>Risk/protective factor</th>
<th>Exposure</th>
<th>Sugarcane</th>
<th>Non-sugarcane</th>
<th>p value</th>
<th>Mean DMFT (SD)</th>
<th>Sugarcane</th>
<th>Non-sugarcane</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toothbrushing frequency</td>
<td>Daily</td>
<td>54</td>
<td>26.5</td>
<td>53</td>
<td>26.5</td>
<td>0.916</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weekly</td>
<td>150</td>
<td>73.5</td>
<td>147</td>
<td>73.5</td>
<td></td>
<td>0.48</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.04)</td>
<td>(0.93)</td>
<td>(0.93)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.14)</td>
<td>(1.19)</td>
<td>(1.19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toothpaste use</td>
<td>Fluoride</td>
<td>118</td>
<td>58.5</td>
<td>119</td>
<td>59.5</td>
<td>0.590</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-fluoride</td>
<td>88</td>
<td>43.1</td>
<td>81</td>
<td>40.5</td>
<td></td>
<td>0.45</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.04)</td>
<td>(0.91)</td>
<td>(0.91)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.84)</td>
<td>(1.35)</td>
<td>(1.35)</td>
</tr>
<tr>
<td>Lollies</td>
<td>2 per day</td>
<td>64</td>
<td>30.0</td>
<td>23</td>
<td>12.0</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 per day</td>
<td>140</td>
<td>70.0</td>
<td>177</td>
<td>88.0</td>
<td></td>
<td>0.39</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.95)</td>
<td>(0.97)</td>
<td>(1.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.65)</td>
<td>(1.15)</td>
<td>(1.15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total daily added sugar (number of</td>
<td>0 - 4</td>
<td>21</td>
<td>10.3</td>
<td>12</td>
<td>6.00</td>
<td>0.383</td>
<td></td>
<td></td>
</tr>
<tr>
<td>teaspoons)</td>
<td>5 - 6</td>
<td>42</td>
<td>20.6</td>
<td>45</td>
<td>22.5</td>
<td></td>
<td>0.28</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.64)</td>
<td>(0.83)</td>
<td>(0.83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.58)</td>
<td>(1.03)</td>
<td>(1.03)</td>
</tr>
<tr>
<td></td>
<td>7 - 8</td>
<td>79</td>
<td>38.7</td>
<td>74</td>
<td>37.0</td>
<td></td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.04)</td>
<td>(1.04)</td>
<td>(1.39)</td>
</tr>
<tr>
<td></td>
<td>9+</td>
<td>62</td>
<td>30.4</td>
<td>69</td>
<td>34.5</td>
<td></td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.03)</td>
<td>(1.07)</td>
<td>(1.07)</td>
</tr>
</tbody>
</table>
Table 5. Odds ratio from best fitting* multiple variable logistic regression model (using the backward stepwise selection method) of risk factors for dental caries.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Variable category</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residence</td>
<td>Sugarcane area</td>
<td>0.507</td>
<td>0.327, 0.784</td>
<td>0.0023</td>
</tr>
<tr>
<td></td>
<td>Non-sugarcane area (reference category)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The effects of sugar consumption at breakfast, tiffin (lunch) and dinner, and the effect of lollie consumption were dismissed during successive steps (p>0.05).
Discussion

Although overall caries experience of children in both regions was low, a clear cut association between exposure to raw sugarcane and caries experience was evident. The important question is: whether or not this association is causal. In this cross-sectional study, the caries experience outcome was measured directly on the day of the survey, while exposures to the variables of interest were inferred on the basis of the current habits of the children. The weakness of this research design is the assumption that measures of exposure, based on current habits, may be biased estimates of previous habits. Hence, the inference we have drawn on the basis of the exposure estimate can only be interpreted as suggestive of a causal link, rather than proof of one. To prove causation, the ideal research design would be that of a controlled cohort study in which children would be followed for a period, throughout which their exposure to sugarcane and relevant confounding factors, would be assessed at regular intervals. In this way, the exposures could be better quantified.

The explanation of the effect of sugarcane on caries incidence is problematic. Both organic and inorganic phosphates have been investigated in this regard. A review of more than 100 experiments showed conclusively that the addition of inorganic phosphates to cariogenic diets reduced the caries experience in animals (23). However, in a human clinical trial in which sweets supplemented with dicalcium phosphate were provided to school children over a three year period, no reduction in caries incidence was observed (24). The potential
effect of the organic phosphate, calcium sucrose phosphate, already referred
to, is to raise both calcium and phosphate levels in plaque and saliva and its
anti-cariogenic effectiveness is reportedly due to its ability to adsorb firmly to
enamel surfaces and thus prevent its dissolution. In this regard, phytate,
derived from unrefined cereals was identified as being more active than
calcium sucrose phosphate (25). Wilson and Ashley (26) have reported on
studies that have shown a strong relationship between levels of calcium and
phosphate in plaque and caries incidence in adolescents. The increased
concentration of calcium and phosphate in saliva reduces the likelihood of
enamel dissolution as pH falls (27). However, more recently, in a 2-year
cohort study of children aged 12 years, Pearce et al (28) demonstrated that
while plaque calcium and phosphate were highly correlated with each other,
when a set of confounding variables were taken into account, only calcium,
and not phosphate, was significantly associated with a caries incidence of
three or more DMFS.

On the other hand, the Indian diet is rich in milk, yoghurt, and cheese. In
1975, Rugg-Gunn reported that eating cheese after a sugar-containing snack
raises plaque pH back to a safe level within 5 minutes (29). It has been
proposed that this effect could be due to the stimulation of salivary flow which
causes pH rise, or to the raising of calcium concentrations in plaque, or to
increasing alkaline substances in plaque, or finally, due to the adsorption of a
protective protein, such as casein, on the enamel surface, thereby physically
slowing the dental caries process (29, 30).
In summary, the lower caries experience observed in the sugarcane group may be due to the protective effect of an organic phosphate, such as calcium sucrose phosphate, however, as already noted, the chewing of sugarcane stimulates saliva production, the rinsing effect of which reduces the potential for the sucrose to depress pH. The effect of chewing on saliva stimulation may be the protective factor, since the pH fall associated with the use of sucrose-sweetened chewing gum is neither deep nor long lasting as compared with the fall which follows a sucrose exposure alone (31).

A low caries experience was observed in both groups of children, despite their very high consumption of added sugar; sugar which is added, two spoonfuls at a time, to beverages including milk and yoghurt. In addition, for good measure, a spoonful of sugar is eaten by itself frequently, at the end of a meal. These observations indicate that our understanding of the relationship between caries and refined carbohydrates remains rudimentary.
Conclusion

It is concluded that the lower caries experience of Punjabi children, who chew large quantities of sugarcane daily during a period of about six months per year, is supportive of the hypothesis that raw sugarcane is associated with a caries protective effect. The protective factor may be intrinsic to the sugarcane such as an organic phosphate, or extrinsic, such as a saliva factor that is associated with vigorous chewing.

Acknowledgements

We are grateful to the school principals, parents, and children for their co-operation during the survey, and we thank the water engineers for data on the fluoride concentration in the water supplies in the survey regions. We are grateful to Dr Karen Byth for her advice on the statistical analysis.
References


Appendices

Appendix A: Correspondence with the District Education Officer seeking permission for conducting the survey.

Appendix B: Letter of approval from the Human Research Ethics Committee, The University of Sydney, including, the Participant Information Sheet (English and Punjabi), the Parent/Guardian Consent Form (English and Punjabi), and the Need for Dental Care Form (English and Punjabi).

Appendix C: Clinical Examination Form.

Appendix D: Questionnaire (English and Punjabi).

Appendix E: Details of the multiple variable logistic regression analysis.

Appendix F: List of schools surveyed in both the sugarcane and the non-sugarcane growing areas.

Appendix G: Map of Amritsar (Punjab).
Appendix A.

Correspondence with the District Education Officer seeking permission for conducting the survey.
1 November 2004

The District Education Officer
Amritsar
Punjab
INDIA

Dear Sir

Proposed dental survey of 12-year-olds in Amritsar District

I am the director of the Master of Dental Science (MDSc) program in the discipline of Community Oral Health and Epidemiology, Faculty of Dentistry, University of Sydney. Most candidates enrolled in this program are international students. During Year 2 of the MDSc program, international students conduct a research project in their country of origin.

I have a student from India, Dr Amandeep Singh, who graduated in dentistry at the Magadh University, Bihar, and he is registered to practice dentistry with the Punjab Dental Council.

He is planning to conduct a research project in Amritsar District, Punjab. This will entall a dental survey of children aged 12 years attending schools in Amritsar District. The survey will comprise (1) a brief dental examination of the children which will be conducted according to World Health Organisation protocols, and (2) a short questionnaire to be completed by the children - this questionnaire will collect information on sugar intake including raw sugar cane chewing.

Altogether we plan to survey 200 children living in the sugar cane belt and 200 children living in other locations where there are no sugar cane plantations. It is anticipated that this survey will take place during the months of March and April 2005. Our plan is to survey children aged 12 years, however, for practical purposes it may be more convenient to survey whole school classes of children in which the majority of children would be aged 12, that is, we would examine children aged 11, 12, and 13.

I would be grateful if your advice on three matters. Firstly, what is the process for obtaining permission for Dr Singh to conduct this proposed survey. Secondly, what are the requirements in relation to obtaining consent from the school principals and the parents of the children. Thirdly, would you please advise the school grade or school class that would be likely to have mainly 12-year-olds.

Thank you for your help and I look forward to hearing from you shortly.

Yours faithfully,

Wendell Evans
Associate Professor
Office of District Education Officer (E/E) Amritsar

To

All Block Elementary Education Officer
District Amritsar

Letter No. G-1 / 2004 / 10055 to 82
Dated: 8-11-2004

Subject: Regarding Dental Survey of Children in Amritsar District

Inform all School Heads that Dr. Amandeep Jee will visit their schools in connection with Dental Survey of Children's. Please extend full cooperation to him. Treat it as most urgent.

1. A copy of forwarded to Wendell Evans, Associate Professor.
The University of Sydney, Australia

2. A copy is forwarded to Dr. Amandeep Singh, Westmead Center for Oral Health,
Westmead Australia, NSW 2145

Sd/-
District Education Officer
Elementary Education
Amritsar

Attested to be correct translation of document produced before me

Sd/-
District Education Officer
Elementary Education
Amritsar
सिल्प प्रमाण (संपत्ति) अदालत (प्रम. म.) शैक्षिक

==

निदेश:
भवन यादव, विभागीय प्रमाण, अदालत सिल्प प्रमाण।

टाइप: जी-1/2004- 10.55-82

संस्करण: शैक्षिक प्रमाण संख्या हड़कन्त जो दूसरे से मात्र लिखा है।

नाम: मुख्य संदर्भ से संबंधित है प्रमाण जो संस्करण से दूसरे से केवल मात्र लिखी गई है।

शैक्षिक प्रमाण संख्या हड़कन्त जो दूसरे से मात्र लिखी गई है।

A copy is forwarded to Wendell Evans, Associate P:

The University of Sydney, Australia.

2. A copy is forwarded to Dr. Amandeep Singh, Westmead Centre for Oral Health, Westmead Hospital, Australia. NSW-

 Distt. Education Officer (E.F.)
 Amritsar.
Appendix B.

Letter of approval from the Human Research Ethics Committee, The University of Sydney, including, the Participant Information Sheet (English and Punjabi), the Parent/Guardian Consent Form (English and Punjabi), and the Need for Dental Care Form (English and Punjabi).
24 May 2005

Associate Professor W Evans
Community Oral Health and Epidemiology
Faculty of Dentistry
Westmead Centre for Oral Health
Westmead Hospital
C24

Dear Professor Evans

Thank you for your correspondence dated 29 April 2005 addressing comments made to you by the Committee. After considering the additional information, the Executive Committee approved your protocol entitled "Dental survey of 12-year-olds in India"

Details of the approval are as follows:

Ref No.: 05-2005/1/8029
Completion Date of Project: 31 December 2005
No. of Participants: 200 [per group]
Authorised Personnel: Associate Professor W Evans
Dr A Singh

To comply with the National Statement on Ethical Conduct in Research Involving Humans, and in line with the Human Research Ethics Committee requirements this approval is for a 12-month period. At the end of the approval period, the HREC will approve extensions for a further 12-month, subject to a satisfactory annual report. The HREC will forward to you an Annual Progress Report form, at the end of each 12-month period. Your first report will be due on 31 May 2006.

Conditions of Approval Applicable to all Projects

(1) Modifications to the protocol cannot proceed until such approval is obtained in writing. (Refer to the website www.usyd.edu.au/ethics/human under 'Forms and Guides' for a Modification Form).
(2) The confidentiality and anonymity of all research subjects is maintained at all times, except as required by law.

(3) All research subjects are provided with a Participant Information Sheet and Consent Form, unless otherwise agreed by the Committee.

(4) The Participant Information Sheet and Consent Form are to be on University of Sydney letterhead and include the full title of the research project and telephone contacts for the researchers, unless otherwise agreed by the Committee.

(5) The following statement must appear on the bottom of the Participant Information Sheet. *Any person with concerns or complaints about the conduct of a research study can contact the Manager, Ethics Administration, University of Sydney, on (02) 9351 4811.*

(6) The standard University policy concerning storage of data and tapes should be followed. While temporary storage of data or tapes at the researcher's home or an off-campus site is acceptable during the active transcription phase of the project, permanent storage should be at a secure, University controlled site for a minimum of seven years.

(7) A report and a copy of any published material should be provided at the completion of the Project.

Yours sincerely

[Signature]

Associate Professor J D Watson
Chairman
Human Research Ethics Committee

Encl. Dear "Parent" letter
Participant Information Statement
Parent/Guardian Consent Form
Questionnaire
Dental survey of 12-year-olds

Need for dental care

Dear Parent,

I have examined your child ................................ today and wish to inform you that he/she needs to attend the dental clinic at ................................ for urgent treatment.

Sincerely,

DR Amandeep Singh
Dentist
12 माह दौं टैंसर की सिमायी से धवन से टैंसर की सिमायी के से

भारती यूनियन

माफ़ करें भाजप भुजा

मे इंजन होइ तांतर एल इए से भी टैंसर करते हैं टैंसर के लिए नदी के समय में दर्शन तलवार...

दिन है दिक्कत दिखाये शंकर सभी नहीं है।

अपने नी द चुकिंदार

उप अभियान भर्ती

जेऩ्टेमप्ट

Attested to be true copy of English:

Sub Divisional Engineer,
Ph. VI & S/Div.8.
Dental survey of 12-year-olds

Participant Information Statement

1. What is this study about?
   Sugar that is added to food and drinks causes tooth decay. However, it is not certain that natural sugar in food and in sugar cane causes tooth decay. This study will check on tooth decay in 12-year-olds and find out if eating sugar cane causes tooth decay.

2. Who is carrying out the study?
   This study is been conducted by DR. Amandeep Singh who is an Indian dentist and now training to be a dental specialist. His training is supervised by Associate Professor Wendell Evans at the University of Sydney, Australia.

3. What does the study involve?
   This study involves a dental examination of your child conducted according to proper standards. DR Singh will also ask questions about your child's sugar eating habits.

4. How much time will the study take?
   It will take not more then 5 minutes to complete everything.

5. Can I withdraw from study?
   Yes, you can withdraw anytime during study without penalty.

6. What if I require further information?
   Please contact Dr. Amandeep Singh by telephone (9814129121) or by mail at House No 235 Sector 7 Panchkula, Haryana.

Any person with concerns or complaints about the conduct of a research study can contact the Manager for Ethics Administration, University of Sydney on (612) 9351 4811.
1. दिए दिशितांक एकस दिने चाहे जाने?
दिए दिशितांक एक्स दिम ऊटी बीटी ना जाने हैं एं से टूटंग हैं बीटा न ठनाट अच्छे निर्देशांक चीत नाम दिशितांक एक्स उर्म बीटी ना दिम घरे हैं।
2. दिए दिशितांक एक्स बेटा बन बिदा जाने?
दिए दिशितांक एक्स जा. भागलधी गिरिज बन बिदा जाने से ने दिए बाड़ी शरीर से उदाहरण ने अच्छे निर्देशांक भागलधी दिशि टूटंग दी बिसेंग भुजानी बन बिदा है।
3. दिम दिशितांक एक्स दिम आर दी गुप्त है?
दिम दिशितांक एक्स दिम आर दी शरीर ने न नाट घरे जुटर हैं डूंगर से सैकड़ा दे टूटंग ही दिमितांक घाये मगर मुम्बत नह।
4. दिए दिशितांक एक्स दिम निदु मांग देंदी है?
दिए दिशितांक एक्स बन भिडा दे प्लेट मो दिम बीटी ना मंडली है।
5. नी मे दिए दिशितांक एक्स हैं भ्रूं हां भ्रूं जाने?
नी, नी मे दिए दिशितांक एक्स हैं आद सी मे बिसेंग बिमे नामागे हैं जां मंडली है।
6. नौल मे दिए दिशितांक एक्स घरे डूंग गिरिज बन बिदा जाने उं मे दी है?
म. आदलधी गिरिज दूंग टूटिंग सवा 98141-24121 दे संवाद दे सं बिसेंग राख दिम घुंटे डूंग हांवर रेखा 235, नौलल 7, झंकडा, धीरक दे प्लेट बिदां नां मंडली है।

नौल मे दूंग एक्स दिम घरे बेटी निवासित इंडे उं एक्स वेश्मय देन एक्स आदलधितंत्रस्म, जुरितांकिटी जा मीती, हैं दूंग (612) 93514811

Executive Engineer,
Pb. W/S. & Sewerage Division,
AMRITSAR.
Dental survey of 12-year-olds

Parent / Guardian Consent Form

I, permit ................................................, who is aged ........ Years, to participate in the Dental Survey of 12-year-olds.

In giving consent I acknowledge that:

1. I have received the Participant Information Statement and that I have read and understood the information given.

2. Dr. Amandeep Singh has given me the opportunity to discuss this information and to ask questions about the project, and they have been answered to my satisfaction.

3. I understand that I can withdraw my child from study at any time without penalty now or in future.

4. I understand that if I have any further questions relating to my child’s participation in this survey I may contact Dr. Amandeep Singh who will be happy to answer them.

Signature of parent/guardian ................................................

Date ......................................
12 मस्त की रंगें दी शिविरीयाँ घरे येते
बाह्यिक सात शान्तिमाद दी मृत्यु दिन हटवा

में दिना, मृत्यु दिन का वर घरे…………… 12 मस्त की रंगें दी शिविरीयाँ घरे
येना दिन दिन के मवर दिते।

हे दिका मृत्यु दिका दिका दिका दिका दिका मवर दिता दिता मवर दिता दिता मवर दिता दिता मवर दिता
1 में दिना देशद कुल जेनाला हो मुक्ता 12 मस्त की रंगें दी शिविरीयाँ घरे येते दिना हो मुक्ता
पुष्पत वर दिका हो। दिना हो दिका उदय धर इती दिका दिता।

2 ए. आचार्यसिद्ध डे में दिना देशद कुल योग देशद में दिना देशद दिता दिता दिता दिता दिता दिता
देशद में देशद में देशद में देशद में देशद में देशद में देशद
देशद दिता दिता हो दिता दिता दिता दिता दिता दिता दिता दिता दिता दिता दिता दिता

3 में दिना मवर दिता दिता दिता दिता दिता दिता दिता दिता दिता
रर आयर दिता रर आयर मवर मवर मवर मवर

4 में मवर दिता दिता दिता दिता दिता दिता दिता दिता दिता दिता दिता
मवर मवर मवर मवर मवर मवर मवर मवर मवर मवर मवर

वास्तव माना दिना अथवा अवरीिप उपरान्त
अजी………………………………………………………………………………………
Appendix C.

Clinical Examination Form.
Dental survey of 12-year-olds

Clinical examination form

<table>
<thead>
<tr>
<th>Name</th>
<th>Sex</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Duplicate

<table>
<thead>
<tr>
<th>Status</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Status</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

STATUS
0 = Sound
1 = Decayed (Decayed = D3)
F =Filled
M = Missing (Missing = missing due to caries)
T = Trauma (Trauma = a broken tooth due to trauma or a filled tooth due to trauma)
8 = Unerupted

TREATMENT
0 = None
F = Filling
X = Extraction
Appendix D.

Questionnaire (English and Punjabi).
Dental survey of 12-year-olds

Questionnaire

1. What did you eat or drink before breakfast?
   1) Milk  2) Tea  3) Water  4) none
   5) Other (please specify) ...........

2. How many spoons of sugar did you put in your milk/tea?
   1) 1  2) 2  3) 3 or more  4) 0

3. What did you drink for breakfast?
   1) Milk  2) Tea  3) Juice  4) Lassi
   5) Others (please specify)

4. How much spoons of sugar you put in your milk/tea?
   1) 1  2) 2  3) 3 or more  4) 0

5. What did you eat for breakfast?
   1) Chapatti  2) Bread  3) Nothing  4) Others (please specify)

6. If you had curd/lassi did you put sugar in it?
   1) Yes  2) no

7. How many spoons of sugar you put?
   1) 0  2) 1  3) 2  4) 3 or more

8. What did you have in your Tiffin?
   1) Bread  2) Sweet roti  3) None  4) others (please specify)....

9. What did you put on bread?
   1) Jam  2) butter  3) Butter with sugar  4) others (please specify)....

10. What did you have for dinner?
    1) Rice  2) Chapatti  3) Bread  4) others (please specify)....
11. What did you drink for dinner?
   1) Lassi  2) Water  3) Fizzy drinks  4) Others (please specify)...

12. Do you usually eat anything sweet after dinner?
   1) Yes   2) no

13. What do you usually have?
   1) Sweet dish prepared at home  2) Ice cream  3) Plain sugar  4) Gur

14. How often do you have it?
   1) Everyday  2) Once a week  3) Twice a week  4) Rarely

15. If you have plain sugar/gur how much do you have?
   1) One teaspoon  2) More than 1 teaspoon  3) One handful  4) More than handful

16. How often do you have fizzy drinks like Pepsi/coke?
   1) At least once a day  2) Every alternate day
   3) At least once a week  4) Once a month

17. Did you also have other sweet drinks during day except sweet lassi or coke?
   1) Yes   2) No

18. How often do you have it?
   1) Once a day  2) Every alternate day
   3) Once a week  4) Once a month

19. How many sweets do you have in a day?
   1) 2   2) 4   3) 6   4) 10

20. How often do you have them?
   1) Once a day  2) Once a week
   3) Whenever you feel like  4) Once a month

21. How many sticks of sugarcane do you usually chew?
   1) 1   2) 2   3) 3   4) 4 or more

22. How many pinglee's do you have from one sugarcane at 1 time?
   1) 1   2) 2   3) 3   4) Whole sugarcane

23. How many times do you brush your teeth in a day?
   1) Less than once a day  2) Less than twice a day
   3) Less than once a week  4) Less than once a month
24. What did you put on your toothbrush?

1) Colgate  
2) Pepsodent  
3) Close up  
4) Tooth powder  
5) Others (please specify)......
1. इसी समय 3 पहिला दूसरा घर पहुचे ना पीटे े?
1) घंटा 2) घर 3) घरी 4) बेहद घरी
5) घेंट घेंट (टिमाना नाम) ...

2. इसी अपने घर से घर लिए लिये जमी घर पहुंचे ी?
1) 1 2) 2 3) 3 4) 0

3. इसी घरिए इसी के पहुंचे े?
1) घंटा 2) घर 3) घरी 4) संभी
5) घेंट घेंट (टिमाना नाम) ...

4. इसी अपने घर से घर लिए लिये जमी घर दे पहुंचे े?
1) 1 2) 2 3) 3 4) 0

5. इसी घरिए इसी के पहुंचे े?
1) घरी 2) घरी 3) घरी 4) घेंट घेंट
5) घेंट घेंट (टिमाना नाम) ...

6. नैसर्गिक इसी घर से नैसर्गिक संपति उ घर लिए लिये घर पहुंचे े?
1) घर 2) घर

7. इसी लिए घर दे पहुंचे े?
1) 0 2) 1 3) 2 4) 3 घर

8. इसी घरिए लिए लिये घर दे पहुंचे े?
1) घरी 2) घरी 3) घरी 4) घरी घरी
4) घेंट घेंट (टिमाना नाम) ...

9. इसी अपने घर घरी दे लिए लिये े?
1) घरी 2) घरी 3) घरी 4) घरी घरी
4) घेंट घेंट (टिमाना नाम) ...

10. इसी घर दे घरि लिए लिये घर े?
1) घरी 2) घरी 3) घरी
4) घेंट घेंट (टिमाना नाम) ...

11. इसी घर दे घरि लिए लिये घर े?
1) घरी 2) घरी 3) घरी 4) घरी घरी
4) घेंट घेंट (टिमाना नाम) ...

12. जरूर इसी घर दे घरि दे घरि अगर अगर घर दे लिए लिये घर े?
1) घरी 2) घरी

13. इसी अपने घर दे घरि घरि घर े?
1) घर घर घरी 2) घरी 3) घरी 4) घरी
12. माफ की बाबा देव दी निदानधीकता गर्खे भेंस पुस्तकारकी

14. की झमी अभाव लिखा घटे ते?
1) उत्तर देख 2) उदबे च दिख रुप 3) दिख पेयकी 4) घुड़ घटे भटी सती

15. मेछब झमी घटेथा न झमु घटे ते न दिखा घटे ते?
1) दिख ती सहात 2) दिख ती निदानधीकता नी गर्खे 3) दिख पेयकी 4) दिख ती निदानधीकता रुपतीका

16. की झमी अभाव दिखा दिखा निदानधीकता रुप दुः दिखा की घटेयो ना छेंगे घटे ते?
1) दिख न दिख 2) दिख दिख घट 3) दिख घट दिख 4) मत्तजी न दिखा

17. की झमी सस्ती ना घट ते दिखा रुप ती निदानधीकता दिखा घटे ते?
1) दिख 2) दिखा

18. की झमी अभाव घटे ते?
1) दिख न दिख 2) दिख दिख घट 3) उदबे न दिख 4) भटी न दिखा

19. दिख दिख दिख दिख घट घट दिखा निदानधीकता दिखा निदानधीकता घट घट?
1) 2 2) 4 3) 6 4) 10

20. की झमी अभाव दिखा दिखा दिखा घट घट घट घट?
1) घट घट दिखा 2) घट घट दिख रुप 3) घट घट दिखा घट घट घट
4) मत्तजी न दिख घट

21. झमी दिखे की कीअभाव घटा ते?
1) 1 2) 2 3) 3 4) 4 ना दिखा

22. दिख ना दिख दिख दिख की की की झमी दिखा निदानधीकता घट घट?
1) 1 2) 2 3) 3 4) 4 ना दिखा

23. झमी दिख दिख दिख दिख घट घट घट घट घट?
1) दिख न घट घट दिख रुप 2) दिख न घट घट दिख रुप 3) घट घट दिख घट घट 4) मत्तजी न घट घट दिख घट

24. झमी अभाव दिख दिख दिख घट घट घट घट?
1) दिख दिख दिख दिख घट घट घट
2) दिख दिख दिख दिख
3) घट घट घट घट
4) मत्तजी न घट घट घट घट
5) दिख दिख दिख (दिखाकी ना दिखा)

Attention to be traced Mary
Appendix E.

Details of the multiple variable logistic regression analysis.
Details of the multiple variable logistic regression analysis.

At step 1, in the backward stepwise selection model, all potential confounders are entered into the regression at once. The analysis examines each confounder in turn, adjusting for the effects of the others. The p value of 0.956 indicates that sugar consumption at breakfast was a non significant predictor of DMFT and also the least significant variable in the model, and was hence dropped from the model at step 1.

The remaining confounders were assessed at step 2 where the p value of 0.848 indicated that the contribution of Tiffin was also a non significant, and the least significant predictor at this step, and hence dropped from the model.

At steps 3 and 4, lollies and sugar consumption at dinner were eliminated, leaving sugarcane group as the only significant predictor of experiencing one or more DMFT. The odds ratio of 0.507 indicates that compared with non-cane group, the children in the sugarcane group were only 51 percent as likely to have one or more DMFT. The confidence limits of this estimate are 0.327 and 0.784.
Table E. Odds ratios from best fitting multiple variable logistic regression model (using the backward stepwise selection method) for dental caries experience. Respective reference categories were: water; and non-sugarcane area. Caries experience was dichotomised as DMFT = 0 or 1+.

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Explanatory variable</th>
<th>Variable category</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast*</td>
<td></td>
<td>Milk</td>
<td>0.905</td>
<td>0.331, 2.473</td>
<td>0.956</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tea</td>
<td>1.098</td>
<td>0.684, 1.763</td>
<td>0.698</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yoghurt</td>
<td>1.171</td>
<td>0.511, 2.682</td>
<td>0.710</td>
</tr>
<tr>
<td>Tiffin</td>
<td></td>
<td>Milk</td>
<td>0.819</td>
<td>0.424, 1.583</td>
<td>0.853</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tea</td>
<td>0.814</td>
<td>0.439, 1.510</td>
<td>0.514</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yoghurt</td>
<td>1.173</td>
<td>0.339, 4.053</td>
<td>0.801</td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
<td>Milk</td>
<td>0.507</td>
<td>0.236, 1.089</td>
<td>0.238</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tea</td>
<td>0.721</td>
<td>0.402, 1.292</td>
<td>0.271</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yoghurt</td>
<td>0.555</td>
<td>0.274, 1.124</td>
<td>0.102</td>
</tr>
<tr>
<td>Lollies</td>
<td>Frequency</td>
<td>1.156</td>
<td>0.730, 1.833</td>
<td>0.536</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td>Sugarcane area</td>
<td>0.502</td>
<td>0.304, 0.829</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>0.698</td>
<td></td>
<td></td>
<td>0.541</td>
</tr>
<tr>
<td>Step 2</td>
<td>Tiffin</td>
<td>Milk</td>
<td>0.827</td>
<td>0.428, 1.595</td>
<td>0.848</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tea</td>
<td>0.817</td>
<td>0.441, 1.513</td>
<td>0.520</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yoghurt</td>
<td>1.203</td>
<td>0.350, 4.139</td>
<td>0.770</td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
<td>Milk</td>
<td>0.512</td>
<td>0.238, 1.099</td>
<td>0.242</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tea</td>
<td>0.712</td>
<td>0.399, 1.270</td>
<td>0.250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yoghurt</td>
<td>0.554</td>
<td>0.275, 1.117</td>
<td>0.099</td>
</tr>
<tr>
<td>Lollies</td>
<td>Frequency</td>
<td>1.153</td>
<td>0.729, 1.826</td>
<td>0.543</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td>Sugarcane area</td>
<td>0.493</td>
<td>0.302, 0.805</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>0.742</td>
<td></td>
<td></td>
<td>0.604</td>
</tr>
<tr>
<td>Step 3</td>
<td>Dinner</td>
<td>Milk</td>
<td>0.506</td>
<td>0.236, 1.086</td>
<td>0.212</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tea</td>
<td>0.698</td>
<td>0.394, 1.236</td>
<td>0.217</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yoghurt</td>
<td>0.545</td>
<td>0.279, 1.065</td>
<td>0.076</td>
</tr>
<tr>
<td>Lollies</td>
<td>Frequency</td>
<td>1.155</td>
<td>0.732, 1.824</td>
<td>0.536</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td>Sugarcane area</td>
<td>0.509</td>
<td>0.318, 0.815</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>0.639</td>
<td></td>
<td></td>
<td>0.375</td>
</tr>
<tr>
<td>Step 4</td>
<td>Dinner</td>
<td>Milk</td>
<td>0.514</td>
<td>0.240, 1.101</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tea</td>
<td>0.702</td>
<td>0.397, 1.243</td>
<td>0.225</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yoghurt</td>
<td>0.549</td>
<td>0.281, 1.073</td>
<td>0.079</td>
</tr>
<tr>
<td>Residence</td>
<td>Sugarcane area</td>
<td>0.5</td>
<td>0.313, 0.797</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>0.833</td>
<td></td>
<td></td>
<td>0.493</td>
</tr>
<tr>
<td>Step 5</td>
<td>Residence</td>
<td>Sugarcane area</td>
<td>0.507</td>
<td>0.327, 0.784</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Constant</td>
<td>0.575</td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>

* Indicates sugar-containing beverages as consumed at breakfast. Similarly for tiffin and dinner.
Appendix F.

List of schools surveyed in both the sugarcane and the non-sugarcane growing areas.
Government elementary schools in the sugarcane belt:

Chhiden
Bhangarpur
Mehta (Boys)
Mehta (Girls)
Saiduke
Chung
Chowk Mehta

Government elementary schools in the non-sugarcane growing area:

Gurinda
Gagarbhana
Phalurimanj
Gausabad
Bhutar Kalan
Bhankarpur
Appendix G.

Map of Amritsar (Punjab).