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Original Article

Dentistry Section

Assessing the Changes in Cariogram Generated Risk Profiles following the use of Casein Phosphopeptide-Amorphous Calcium Phosphate Chewing Gums

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ABSTRACT

Introduction: Caries risk assessment is an essential element in the prevention of the disease. Targeted preventive strategies customised for each individual is economically beneficial and rational. Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) is the preferred anticariogenic agent among non fluoridated preventive agents.

Aim: To assess the changes in cariogram generated risk profiles following the use of CPP-ACP chewing gums.

Materials and Methods: This longitudinal study was conducted at Sri Venkateswara Dental College and Hospital, Tamil Nadu, India, for a period of 14 days, January, 2017. CPP-ACP chewing gums were distributed to a group of 10 healthy individuals of the age group 18-25 years. They were instructed to use the gums for a period of 14 days. Details required for constructing a cariogram were obtained via questionnaires, interview, oral examination and salivary analysis. Two cariograms were constructed for each individual using the details collected before and after usage of the gums. The percentage change in each sector was tabulated and interpreted.

Results: There was a visible increase in the percentage of chances to avoid caries sector (green colour) in all cariograms. The percentage increase ranged from 3-24%. A significant reduction of the susceptibility sector (light blue colour) with difference ranging from 3-12% and bacteria sector (red colour) with difference range from 3-8% was seen. Circumstances and diet sectors (yellow and dark blue colours) showed negligible changes.

Conclusion: The CPP-ACP is an effective preventive intervention that can alter the cariogram of an individual by increasing the green colour sector area.

Keywords: Cariogran, Dental caries, Prevention

INTRODUCTION

Cariology has traversed through various paths and is now witnessing a paradigm shift towards preventive approach. The evolution of treatment strategy for dental caries can be viewed under three phases namely extractive, restorative and preventive phases [1]. The trajectories of caries experience from childhood to adulthood encompassing the functional, social and financial encumbrance warrants a risk assessment based caries prevention and management [2]. Identifying the risk factors for an individual aids in carrying out a correctly targeted, clinically effective and economically beneficial preventive therapy [3,4]. Numerous approaches have been suggested and implemented towards prevention of dental caries [5-9]. Currently, calcium based phosphate system like CPP-ACP is preferred among non fluoride preventive agents that promote remineralisation [10].

Dairy products are one of the food groups that have been recognised as anticariogenic agents [11,12]. Studies have shown that rinsing the mouth with milk for 30 seconds despite refraining from brushing for three days produces a very negligible drop in pH. This property is specifically attributed to the casein, calcium and phosphate present in milk and milk products. The enamel rapidly absorbs casein fractions and enhances the resistance against acid attacks. These casein products are known to alter the adherence of the Streptococcus mutans which is the predominant microorganism convincingly associated with dental caries [13,14]. Furthermore, micelles of casein modulate the composition of the plaque microflora such that it results in the reduction of their cariogenic potential [15]. The CPP is obtained from the tryptic digestion of casein followed by its aggregation with calcium phosphate [16]. The CPP bonds with nanoclusters of ACP via the phosphoseryl residues present in the former to work as an effective remineralising agent [17].

Cariogram is a relatively easy to use and plausibly reliable caries risk assessment tool. It consists of 10 criterions to assess the role of various risk factors for dental caries [18]. This computer based software gives an objective value for each factor and presents the patient's caries risk profile as a pie chart with five domains [19]. The five sectors of the pie chart include diet, bacteria, susceptibility, chance of avoiding new caries and circumstances. Each sector represents the combined values of the risk factors falling under that domain. The green coloured sector representing the actual chance to avoid new cavities is determined by subtracting the share taken by all the other remaining risk factors on the pie chart. Risk stratification level of an individual is inversely proportional to the percentage of the green sector on the cariogram [20]. Thus, the cariogram provides the benefit of understanding to what extent each risk factor can affect the overall risk profile. This aids in designing a customised risk assessment based preventive therapy for each individual [21].

Exploration of the available literature related to cariogram and CPP-ACP reveals that no study has evaluated CPP-ACP as a caries preventive agent based on cariogram outcome. One study done by Patil B et al., has used the cariogram model to evaluate a prevention program for caries among mentally challenged children [22]. Numerous studies have been done to assess the caries risk using cariogram model; however, there is a scarcity in literature when it comes to using the tool for assessing a caries preventive strategy [23-27]. The present study employs cariogram as an evaluator for CPP-ACP's anticariogenic action. Thus, the aim of the present

study is to assess the changes in cariogram generated risk profiles following the use of CPP-ACP chewing gums.

MATERIALS AND METHODS

This longitudinal study was conducted at Sri Venkateswara Dental College and Hospital, Tamil Nadu, India, for a period of 14 days in January, 2017. The study was designed as a longitudinal field trial with before and after comparisons to assess the changes in the caries risk profiles following the use of CPP-ACP chewing gums. The protocol was approved by the ethics committee of the Institutional Review Board. The study was registered with the Clinical Trial Registry of India (CTRI/2017/06/008827). This study was initiated as a pilot study and hence, the sample size was not calculated and the duration of the study was kept short. After elaborating the purpose of the study, written informed consent was obtained from all the subjects.

Inclusion and Exclusion Criteria

Ten healthy individuals falling in the age group of 18-25 years with no related general diseases were invited to participate in the study. The subjects were recruited from the hostel of Sri Venkateswara Dental College and Hospital and hence, subjects with similar diet pattern and fluoride program were included in the study.

Regular users of chewing gums and those who were allergic to these gums, individuals suffering from systemic illness, individuals under antibiotic cover and those who had taken antibiotics in the last two weeks were excluded from the study. In order to prevent confounding errors due to intake of antibiotics or other factors, every subject was continuously monitored throughout the study period. All subjects were asked to report immediately on the occurrence of any side effects. It was decided that under such circumstances the concerned subject will be excluded from the study.

Proforma for Recording Cariogram Scores

A proforma was designed to document all the information required for constructing the cariogram model [20]. The cariogram consists of 10 criterions spread under four categories each represented by a coloured sector. In addition, there is a fifth sector that is obtained by subtracting all the other four sectors from the pie diagram called the 'chance to avoid caries' which is given the green colour. Demographic details and details about fluoride usage, medical history, diet contents and frequency were recorded by taking a detailed case history. Each of these criteria was then allotted scores based on the key provided in the cariogram manual (Bratthall et al., cariogram Manual 2004, Internet version 2.01) [20]. All oral examinations were then carried out by a single investigator.

Recording Caries Status and Plaque Levels

Decayed, Missing, Filled Surface index (DMFS), WHO 1989 criteria was used to record caries experience. A plane mouth mirror and sickle ended probe was used to record the index. The Silness and Loe index was used for assessing the plaque amount. A plane mouth mirror no. #5 was alone used for this index. The DMFS and plaque scores were calculated and the corresponding cariogram scores were then entered in the proforma.

Sample Collection and Salivary Flow Rate

Unstimulated salivary samples were collected in a 5 mL sterile container to evaluate the salivary flow rate, buffer capacity and *Streptococcus mutans* levels. The subjects were asked to be seated upright in a relaxed position and collect their saliva in the given sterile container. The time taken for them to collect 5 mL of saliva was noted and the salivary flow rate per minute was calculated.

Salivary pH and Buffer Capacity

The pH and buffering capacity were identified using a hand held pH meter with digital display (Digital pH meter, MIFA systems pvt ltd, Ahmadabad, Gujarat, India). Standard pH pellets of pH 4.0 and 7.0 were used for the calibration of the pH sensitive electrode. The test samples were then titrated with 250 µL of lactic acid (pH 3, 1.5 mM). The pH values of the titrated samples were then noted using the same pH meter.

Microbial Analysis

For microbial analysis the salivary samples were diluted and vortex mixed. Aliquots of 0.1 mL from the diluted samples were inoculated on the surface of mitis salivarius agar base (Himedia, Mumbai, Maharashtra, India). The petri plates were then incubated for 48 hours at 37° C with 3% CO_2. The colony forming units were counted manually after identifying the organisms based on their colony morphology.

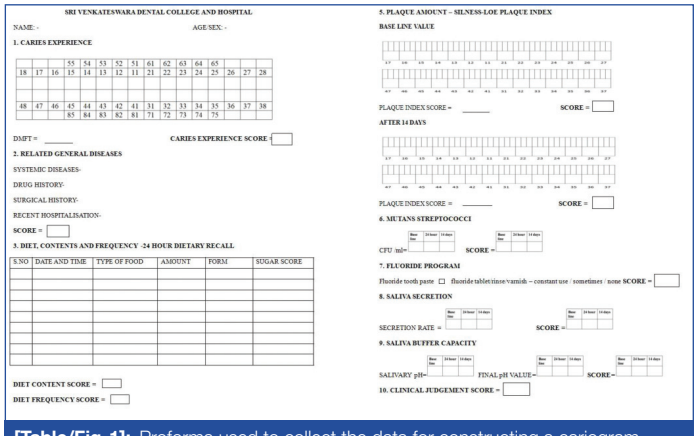
Constructing the Cariogram

All data required for constructing a cariogram were first obtained from all the subjects for baseline. The scores obtained for the 10 criterions based on the key given in the cariogram manual were entered into the cariogram software and the pie chart was constructed. All subjects were given a clinical judgment score of one as there was no disparity between the investigator's opinion and the cariogram. At the start of the study 10 cariograms were constructed based on baseline values.

Once the baseline cariograms were built, CPP-ACP chewing gums (Recaldent, Nihon Kraft foods limited, Tokyo, Japan) were distributed to the subjects. The subjects were instructed to follow similar oral hygiene measures during the entire study period. They were directed to use the gums thrice daily 15 minutes after each mealtime for a period of 14 days. Again at the end of 14 days, fresh set of data were collected pertaining to salivary analysis for flow rate, buffer capacity and *Streptococcus mutans* level and the plaque scoring alone. These scores were utilised to construct the new cariogram for all the 10 subjects. Thus, two cariograms were constructed for each subject with the values obtained before and after usage of CPP-ACP chewing gums. The percentage changes in each sector were noted and tabulated using Microsoft Excel 2007 and interpreted.

RESULTS

All subjects completed the study with good compliance and no unpleasant incidents were reported. The [Table/Fig-1] shows proforma used to collect the data for constructing a cariogram. The [Table/Fig-2] shows the cariogram models constructed for one of the study subjects at baseline and after 14 days. The [Table/Fig-3] gives information on excluded subjects from the study and the flow diagram of the longitudinal study. The [Table/Fig-4] shows the gender distribution and mean age of the subjects. The [Table/Fig-5] shows the data collected for the cariogram at baseline and after 14 days. Changes in values are markedly seen only in *Streptococcus mutans* count and salivary buffer capacity.


SRI VENKATESWARA DENTAL COLLEGE AND HOSPITAL

NAME:- AGE/SEX:-

1. CARIES EXPERIENCE

DMFT = CARIES EXPERIENCE SCORE =

2. RELATED GENERAL DISEASES

SYSTEMIC DISEASES-

DRUG HISTORY-

SURGICAL HISTORY-

RECENT HOSPITALISATION-

SCORE =

3. DIET, CONTENTS AND FREQUENCY -24 HOUR DIETARY RECALL

S.NO	DATE AND TIME	TYPE OF FOOD	AMOUNT	FORM	SUGAR SCORE

DIET CONTENT SCORE =

DIET FREQUENCY SCORE =

5. PLAQUE AMOUNT – SILNESS-LOE PLAQUE INDEX

BASE LINE VALUE

PLAQUE INDEX SCORE = SCORE =

AFTER 14 DAYS

PLAQUE INDEX SCORE = SCORE =

6. MUTANS STREPTOCOCCI

CFU /ml= SCORE =

7. FLUORIDE PROGRAM

Fluoride tooth paste ☐ fluoride tablet/rinse/varnish – constant use / sometimes / none SCORE =

8. SALIVA SECRETION

SECRETION RATE = SCORE =

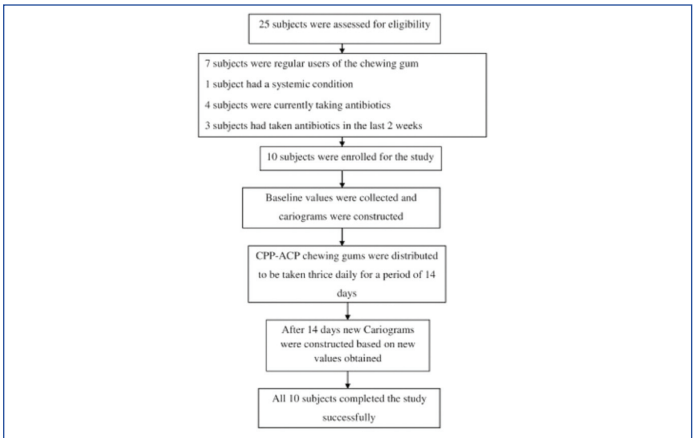
9. SALIVA BUFFER CAPACITY

SALIVARY pH= FINAL pH VALUE = SCORE =

10. CLINICAL JUDGEMENT SCORE =

[Table/Fig-1]: Proforma used to collect the data for constructing a cariogram.

[Table/Fig-2]: Cariogram models constructed for one of the study subjects at baseline and after 14 days.



[Table/Fig-3]: Information on excluded subjects from the study and the flow diagram of the longitudinal study.

Gender (no. of persons in each gender)	Male	5	Total number of subjects=10
	Female	5	
Mean age (in years)	Male	21.2	
	Female	21.1	

[Table/Fig-4]: Gender distribution and mean age of the subjects.

The [Table/Fig-6] shows the change in percentage of green sector of all the subjects before and after the intervention. Results of the field trial show an increase in the percentage of green sector after 14 days in all the subjects. The central tendency value of the green sector has improved from 53.9% to 67.1% after the use of CPP-ACP chewing gums. This indicates that CPP-ACP is an effective caries protective agent. The magnitude of difference in green sector percentage among the subjects ranges from 4% to 24% with a mean value of 13.2%.

Subject	Green sector (%)		Difference (%)
	At baseline	After 14 days	
1	69	73	4
2	48	57	9
3	81	90	9
4	75	85	10
5	54	75	21
6	81	90	9
7	21	45	24
8	75	88	13
9	25	49	24
10	10	19	9

[Table/Fig-6]: Change in percentage of green sector before and after the use of CPP-ACP chewing gum.

The [Table/Fig-7] shows the change in percentage of the four sectors on comparing the pre and post intervention cariogram models. A mean difference of 6.2% is seen under the light blue sector. A dip in the percentage of light blue sector from 18.5% to 13.3% is seen. This sector shows the maximum range of difference when compared to the other three sectors indicating that CPP-ACP improves the salivary flow rate and buffering capacity. Light blue sector is subsequently followed by the red sector with a mean

Subjects	Caries experience	Related general diseases	Diet content	Diet frequency	Plaque amount		*Streptococcus mutans*		Fluoride program	Saliva secretion	Buffer capacity		Clinical judgment score
					Baseline	After 14 days	Baseline	Afrer 14 days			Baseline	After 14 days	
2	0	0	1	2	0	0	3	3	2	0	3	2	1
4	1	0	1	1	1	0	3	3	2	0	3	2	1
6	0	0	1	0	0	0	3	2	2	0	2	1	1
8	0	0	1	1	0	0	3	2	2	0	3	2	1
10	1	0	1	0	0	0	3	2	2	0	3	1	1
12	0	0	1	0	0	0	3	2	2	0	2	1	1
14	3	0	2	2	0	0	3	2	2	0	3	2	1
16	0	0	1	1	0	0	3	2	2	0	3	1	1
18	1	0	2	2	0	0	3	2	2	0	3	2	1
20	13	0	2	1	1	0	3	3	2	0	3	2	1

[Table/Fig-5]: Score given for each parameter based on the data collected to construct the cariograms at baseline and after 14 days.

Subject	Light blue sector (%)		Difference (%)	Red sector (%)		Difference (%)	Dark blue sector (%)		Difference (%)	Yellow sector (%)		Difference (%)
	At baseline	After 14 days		At baseline	After 14 days		At baseline	After 14 days		At baseline	After 14 days	
1	12	9	3	9	9	0	9	9	0	1	0	1
2	19	15	4	19	15	4	10	10	0	3	3	0
3	8	4	4	8	4	4	3	2	1	0	0	0
4	10	6	4	8	4	4	5	4	1	2	1	1
5	21	9	12	16	9	7	5	4	1	3	3	0
6	8	4	4	8	4	4	3	2	1	0	0	0
7	24	15	9	18	10	8	24	20	4	12	10	2
8	10	4	6	8	4	4	5	4	1	2	0	2
9	26	16	10	19	11	8	26	21	5	4	3	1
10	57	51	6	15	12	3	11	12	-1	7	8	-1

[Table/Fig-7]: Change in percentage of other four sectors before and after the use of CPP-ACP chewing gums.

difference value of 4.6% signifying the antimicrobial action and plaque reduction potential of CPP-ACP. The yellow sector and dark blue sector show insignificant alterations from baseline values.

DISCUSSION

Primary prevention is a vital concept in comprehensive dental care. Prevention of dental caries not only improves oral health but also the systemic health and the quality of life of an individual [28]. Before starting with a preventive program, the first imperative step is to assess the risk of the individual rather than proceeding with the routine prevention therapy. Assessing the risk factors will provide information for generating preventive strategies that result in successful work division and economic effectiveness [29]. Four models are available to assess the caries risk which includes Cariogram, Caries Management By Risk Assessment (CAMBRA), American Dental Association (ADA), and American Academy of Paediatric Dentistry (AAPD). Out of these systems, cariogram is found to be quick, inexpensive and acceptable tool [30]. Cariogram is an expedite tool that acts as both a prediction model and risk model for dental caries [21]. Apart from prophesying occurrence of dental caries, this tool can be used to educate and motivate the patient. The simple pie chart representation of the risk profile is easily appreciated by the patient. The cariogram can also be used to prescribe targeted preventive programs [18]. An expansion of the green area in the pie chart proclaims the success of the preventive intervention [20]. Also, an increase in green sector means a decrease in the percentage of another sector or combination of other sectors. This further aids in interpreting the action of the intervention on the risk factor. Hence, in this study the changes in the cariogram outcome following the use of CPP-ACP chewing gums have been assessed.

The CPP-ACP compounds have remarkable potential to act as caries preventive agents [31]. The compound can be delivered in the form of chewing gums, tooth pastes, topical applications, etc [10]. CPP-ACP chewing gums are sugar free and also stimulate salivary flow. Both these factors summated with the benefits of calcium phosphate system in CPP-ACP chewing gums give it the credence of being a caries preventive agent [32]. The amount of CPP-ACP by weight present in the chewing gum is 10.0 mg. Yengopal V and Mickenautsch S, in this systematic review and meta analysis has concluded that a significantly higher level of remineralisation effect is observed after exposure to 10.0 mg of CPP-ACP [33]. Hence, in the present study CPP-ACP was used in the form of chewing gums. The time interval of 5-20 minutes after each meal is when the pH plummets rapidly. In order to offset this pH drop effectively, the gums should be chewed within 20 minutes following each meal [34,35]. Hence, in this study the subjects were asked to follow the same.

Multiple studies have suggested CPP-ACP as a caries preventive agent [36-38]. This statement is in congruence with the result of the present study. The improvement in the caries risk profile after 14 days as indicated by the increase in green sector confirms the anticariogenic effectiveness of CPP-ACP. This seeming increase in the percentage of green sector is because of the actual decrease in percentage of the other sectors. Light blue sector seems to be the most altered sector followed by the red sector while the dark blue and yellow sectors showed very negligible changes.

The light blue sector (represents susceptibility) is based on a combination of fluoride program, salivary buffer and flow rate [20]. Since, the subjects were instructed to follow similar oral hygiene measures the alteration in the susceptibility sector can be attributed to the change in salivary characteristics. Studies done by Harris NO et al., and Iijima Y et al., have shown that the use of chewing gums for 20 minutes triples the salivary flow rate [39,40]. Furthermore studies have shown that CPP-ACP can enhance the buffering ability of saliva with the calcium and phosphate ion system [33]. Karlinsey RL and Mackey AC stated that a single CPP molecule can accommodate up to 25 calcium ions, 15 phosphate ions and five fluoride ions [41]. The CPP-ACP gets incorporated into the plaque and releases its cargo of ions to maintain a supersaturated state with respect to enamel. As the pH declines the bonding between CPP and ACP weakens thereby creating a pool of free calcium and phosphate ions which enhances remineralisation at the crucial time suppressing the demineralisation due to the low pH [42].

The red sector representing bacteria is calculated from weighted values of amount of plaque and *Streptococcus mutans* [20]. The change seen in the red sector is due to the antibacterial action of CPP-ACP on *Streptococcus mutans*. CPP-ACP destroys the calcium bridges among the bacteria and between bacteria and pellicle by competitive uptake of calcium in the plaque [43,44]. Rose RK, in an in vivo study concluded that CPP-ACP binds to calcium with an affinity which is twice as that of bacterial cells to calcium [44]. The Silness and Loe index used for plaque assessment in the cariogram simply objectifies the unmitigated presence of plaque. Thus, the quantity of plaque is taken into consideration and not the quality. Moreover, anticalculus action of CPP-ACP has been mentioned in the literatures but there is no evidence stating the reduction in plaque amounts due to CPP-ACP action [43]. The effect of CPP-ACP on plaque reduction remains questionable as CPP-ACP is known to reduce cariogenicity of the plaque by diminishing the adherence of *Streptococcus mutans* and *Streptococcus sobrinus* to plaque biofilm [45].

Circumstances (yellow sector) comprises of past caries experience, related general diseases [20]. Results of the study show that changes in this sector is almost nil as dental caries take time periods longer than 14 days to occur. Similarly, the systemic health conditions do not sway morbidly in a short duration and hence no significant alteration is seen in yellow sector. Diet content and frequency constitute the diet domain (dark blue) which also shows negligible changes as no change in diet pattern was advised for the subjects to follow [20]. A visible increase is seen in the green sector within a span of 14 days due to the use of CPP-ACP, however, long term clinical trials with a bigger sample size should be done to declare the validity of the preventive intervention.

LIMITATION

The short time duration and fewer subjects stand as a limitation for the present study.

CONCLUSION

Within the limitations of the present study, it can be concluded that the caries risk profile of an individual improves after the use of CPP-ACP chewing gums. The idea of implementing multiple preventive strategies for the same individual based on their risk profile should be taken into consideration in the future. This might help in pushing the patients to low risk categories. Risk assessment is woven deeply into the fabric of preventive dentistry. If prevention is the new paradigm then risk assessment tool should be a mandatory instrument in the dentists' armamentarium.

Risk based prevention should be preferred to routine prevention as it will be more rationale and specific for each individual. Building a cariogram for each subject will allow the clinician to assess the risk based needs for dental caries while simultaneously assessing the anticariogenic effectiveness of the preventive intervention.

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