ISRCTN41631598 https://doi.org/10.1186/ISRCTN41631598

Examination of caries activity of children by investigating the dental plaque and saliva

Submission date
08/07/2019

Recruitment status
No longer recruiting

Registration date
12/07/2019

Overall study status
Completed

Last Edited
26/11/2021

Condition category
Digestive System

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[X] Results
[_] Individual participant data

Plain English summary of protocol

Background and study aims
Arginine and urea play an important role in balancing the pH of dental biofilm and prevention of dental caries. The reduced alkalinogenic potential in dental biofilm is associated with increased caries risk. It has been reported that dental biofilms of the caries-free subjects have a more alkali-generating potential. The main sources of alkaline generation in dental plaque and saliva are hydrolysis of urea by urease enzyme and arginine metabolism by the arginine deiminase (ADS) system. This study aimed to investigate the urease and ADS activity in dental plaque and saliva in both caries-free and caries-active children and to analyze the effect of dental treatment on enzyme activity levels and to evaluate potential use of these enzyme levels as dental caries risk indicators.

Who can participate?
The following inclusion criteria were applied in the selection of the children: Whom were volunteered for the study, being aged between 4 and 6 years old, with unerupted first permanent molars, no history of any systemic disease, no history of antibiotic use within the recent three months, no use of oral care product containing arginine, no history of a periodontal disease, not any applied fixed or removable retainers.

What does the study involve?
Totally 40 children aged between 4-6 years old were examined and salivary flow rate, pH, plaque and gingival index scores were determined. Saliva and supra-gingival plaque samples were obtained from 20 caries-free (CF) children and 20 caries-active (CA) children before and after dental treatment in high caries risk group. Urease and ADS activities were measured in saliva and plaque samples by quantification of ammonia produced from arginine and urea at baseline, at the end of and 3 months after treatment using a spectrophotometric assay. In conclusion, ADS and urease enzyme in saliva and dental plaque are positively correlated with dental health. ADS and urease enzyme levels may be used as novel indicators in detection of caries risk.

What are the possible benefits and risks of participating?
Direct benefits of the study were having detailed information about your ora-dental health, caries risk factors and preventive strategies. There were no any direct risks fro the patients participating in the study.

Where is the study run from?
The study was performed at Istanbul University, Faculty of Dentistry, Department of Pedodontics and the experiments were carried out at Istanbul University, Faculty of Dentistry, Department of Biochemistry.

When is the study starting and how long is it expected to run for?
This study was carried out between the dates 2 nd January 2017 to 13th December 2017.

Who is funding the study?
The present work was supported by the Scientific Research Projects of Istanbul University. Project No.23611.

Who is the main contact?
Assoc. Prof. Arzu Pınar-Erdem
aperdem@gmail.com

Contact information

Type(s)
Scientific

Contact name
Prof Arzu Pinar Erdem

ORCID ID
https://orcid.org/0000-0002-3940-4761

Contact details
Address Istanbul University, Faculty of Dentistry, Department of Pedodontics, Capa
Istanbul
Türkiye
34093
+905325431453
apinar@istanbul.edu.tr

Additional identifiers

Clinical Trials Information System (CTIS)
Nil known

ClinicalTrials.gov (NCT)
Nil known

Protocol serial number
Project No.23611

Study information

Scientific Title

Investigation Of Urease And Arginine Deiminase Activity In Dental Plaque And Saliva of Children

Acronym
N/A

Study objectives
The hypothesis of the study was that arginine deiminase and urease enzyme levels in saliva and dental plaque are correlated with dental caries and that they are available to use as dental caries risk indicators.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Approved 16/11/2016, the Ethics Committee of Clinical Researches of Istanbul University, Dentistry Faculty (T.C. İSTANBUL ÜNİVERSİTESİ DİŞ HEKİMLİĞİ FAKÜLTESİ ETİK KURULU, ÇAPA-FATİH/İSTANBUL-TURKEY, 34093; disheketikkurul@istanbul.edu.tr; 02124142020/30326), ref: 134.

Study design
Single centre, observational cross-sectional study.

Primary study design
Observational

Study type(s)
Prevention

Health condition(s) or problem(s) studied
Dental caries

Interventions
Oral and dental health evaluation was performed by an experienced pediatric dentist using a dental mirror, dental explorer and a World Health Organization periodontal probe. In the clinical examination of the patients; dmf(t), plaque index (Silness Loe plaque index) and gingival index (Loe and Silness) values were determined and unstimulated salivary flow rate and buffering capacity (BC) were calculated. The saliva sample was collected before the dental examination, and participants were asked to refrain from eating, drinking, toothbrushing, and rinsing their mouths for at least 8 h before sample collection which was performed between 09:00 and 10:00 a.m.

The dental caries risk groups of the patients were determined based on an overall evaluation of the patients involving medical history, clinical examination and performed analysis and study groups were established. The children were assigned into two groups as high (study /caries active group) and low (control/caries free group) dental caries risk groups according to the Caries Risk Assessment Tool determined by American Academy of Pediatric Assessment (AAPD) for an age range of 0 to 5 years which was revised in 2014.

Treatment protocols were regulated and applied in both groups in accordance with Dental Caries Prevention Protocol of AAPD. Daily twice toothbrushing with fluoride-containing toothpaste was recommended for the low dental caries risk group (500 ppm) whereas

professionally applied topical fluoride supplements were also provided (at the end of and 3 months after treatment) in addition to toothbrushing in the high dental caries risk group. Both of the groups were informed about a non-cariogenic diet.

Saliva and plaque samples were obtained from high and low caries risk groups to investigate the relationship of arginine deiminase and urease enzyme activities in saliva and dental plaque with dental caries. dmf(t), plaque index, gingival index, unstimulated salivary flow rate and buffering capacity values were assessed. After obtaining dental plaque and saliva samples; routine clinical appointments were scheduled. Oral hygiene and non-cariogenic diet recommendations were performed. All the clinical and laboratory evaluations were performed initially in both groups and they were repeated at the end of and 3 months after treatment to assess the effect of treatment protocol applied to the pediatric patients in the high dental caries risk group.

Sample Collection and Preparation
The parents of the pediatric patients were informed about the procedure and signed informed consents were received. The sampling was performed between 9:00 and 10:00 a.m. Whole unstimulated saliva samples were collected by expectorating at least 2 ml saliva into a plastic 50 ml conical sterile tube. The collected saliva samples were divided into two centrifuge tubes. Sterile periodontal curettes (4/5 Gracey, Dentsply Sirona, New York, USA) were used for the collection of the supragingival plaques. The plaque samples obtained from the buccal surfaces of incisors and molars were transferred into the centrifuge tubes containing 250 l 10 mM K2HPO4 solution (pH 7).

All samples were immediately transferred to the laboratory and stored at -80C until analysis time. All samples were placed in a +4C one day before the analysis. Prior to analysis, dental plaque and saliva samples were dispersed by external sonication for two cycles of 15 seconds each, the samples were maintained on ice between two sonication intervals. Plaque samples were first washed with 10 mM Tris–maleate (pH 7.0) buffer and then were re-suspended in the same buffer solution of 500 µl.

Biochemical assays: ADS and urease activity levels
ADS and urease activities were measured in saliva and plaque samples by quantification of ammonia produced from arginine and urea at baseline, at the end of and 3 months after treatment using a spectrophotometric assay.

Measuring ADS Activity
ADS activity was measured by monitoring the production of ammonia from arginine as previously described. Briefly, 25 µl plaque and 5 µl saliva samples were incubated in a mixture containing 50 mM arginine-HCl and 0,5 mM Tris maleate buffer (pH 6,0) for 90 min at 37 ° C. After the incubation period, the ammonia produced was measured with the Nessler’s reagent (Sigma-Aldrich, 72190) via spectrophotometry at 425 nm (Thermo Scientific Multiskan Go, Wilmington, USA). Standard curves were constructed using solutions of varying concentrations of ammonium sulfate between 10µM and 100µM.

Meanwhile, protein content in each sample was determined by bicinchoninic acid (BCA) technique. A standard curve was created using standard concentrations of bovine serum albumin (BSA) between 0,1 and 10 mg/ml. ADS activities were expressed as µmol ammonia produced per min and were normalized to mg of protein (µmol/min/mg).

Measurement of Urease Activity
Urease activity assay kit (Sigma Aldrich, Taufkirchen, Germany) was used to measure urease activity in saliva and plaque samples. Urea is hydrolyzed to NH3 and CO2 by a urease-catalyzed

reaction. The ammonia is determined by the Berthelot method resulting in colorimetric product measure at 670 nm (Thermo Scientific Multiskan Go, Wilmington, USA.), proportionate to the urease activity present in the sample. Standard curves were constructed using solutions of varying concentrations of ammonia chloride between 0 µM and 50µM. Urease activities were expressed as µmol ammonia produced per min and were normalized to mg of protein (µmol/min /mg).

The total duration of treatment and follow-up for all study arms: 2nd Jan- 11th September 2017

Oral and dental examinations of the patients who applied to Istanbul University, Faculty of Dentistry, Department of Pedodontics were performed daily and the patients who recruit the inclusion criteria were directed to the investigator. The investigator (experienced pediatric dentist) performed detailed oral examinations, recorded the data, collected the samples and the samples and the filled forms were coded numerically and experimental tests were performed blindly without any identification. At follow-up controls, new coded forms were filled and the control samples were recoded again and tested in the same manner.

Intervention Type
Other

Primary outcome(s)
Urease and ADS activities are measured using a spectrophotometric assay at baseline, at the end of and 3 months after dental treatment using a spectrophotometric assay.

Key secondary outcome(s))
1. Dental caries prevalence is evaluated using (dmf(t) at the end of and 3 months after treatment.
2..Oral hygiene is evaluated using the Silness Loe plaque index at the end of and 3 months after treatment.
3. Periodontal health is evaluated using the Loe and Silness gingival index at the end of and 3 months after treatment.
4. Saliva activity is evaluated using unstimulated salivary flow rate at the end of and 3 months after treatment.
5. Saliva activity is evaluated using buffering capacity (BC)) at the end of and 3 months after treatment.

Completion date
13/12/2017

Eligibility

Key inclusion criteria
1. Aged between 4 and 6 years old.
2. Unerupted first permanent molars.
3. No history of any systemic disease.
4. No history of antibiotic use within the recent three months.
5. No use of oral care product containing arginine.
6. No history of periodontal disease.
7. Not any applied fixed or removable retainers.

Participant type(s)

Healthy volunteer

Healthy volunteers allowed
No

Age group
Child

Lower age limit
4 years

Upper age limit
6 years

Sex
All

Total final enrolment
40

Key exclusion criteria
N/A

Date of first enrolment
02/01/2017

Date of final enrolment
13/09/2017

Locations

Countries of recruitment
Türkiye

Study participating centre
ARZU PINAR ERDEM
Address Istanbul University, Faculty of Dentistry, Department of Pedodontics, Capa
ISTANBUL
Türkiye
34093

Sponsor information

Organisation
Scientific Research Projects of Istanbul University.

ROR
https://ror.org/03a5qrr21

Funder(s)

Funder type
University/education

Funder Name
Istanbul Üniversitesi

Alternative Name(s)
Istanbul University

Funding Body Type
Private sector organisation

Funding Body Subtype
Universities (academic only)

Location
Türkiye

Results and Publications

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study are/will be available upon request from Assoc. Prof. Arzu Pınar-Erdem (aperdem@gmail.com). Data is available in excel format after the publication of one of the SCI-expanded Journals for one year after publication. Volunteered informed consents forms were obtained from the parents at the beginning of the study. According to the Helsinki Declaration, the identities of the patients and the parents participating in the study will be kept confidential. The raw data will be shared with the investigators researching a similar hypothesis.

IPD sharing plan summary
Available on request

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article		06/02/2019	26/11/2021	Yes	No