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Program and Abstracts

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Dental Calculus as an Information Source of Long-term Macronutrients Intake

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Objectives: The current dietary assessment still has many constraints, particularly related to the objectivity of data gathering. The dental calculus, which is usually considered as a medical waste of dental treatment, turns out to be a provider of abundant oral information. The objective of this study was to obtain information about the long-term intake of macronutrients from dental calculus content.

Methods: This study is a descriptive study with a cross-sectional study design. The data used in this study were obtained from the assessment of carbohydrate, protein, and fat content of dental calculus. There were three groups of dental calculus samples. The first group was treated with the Anthron method to assess the carbohydrate content. The second group was treated with the Soxhlet extraction method. The third group was treated with SDS-PAGE. Twenty samples of dental calculus were taken using quota sampling method, each of which, was taken from the medical waste from the patient who got dental calculus cleaning treatment at Maranatha Dental Hospital.

Results: The average concentration of carbohydrates from all dental calculus samples is 0.0246 ppm. The average fat level from dental calculus is 0.05754%. The average concentration of protein from all dental calculus samples is 1.9834 mg/ml.

Conclusions: Carbohydrates, proteins, and fats can be examined from dental calculus; therefore, dental calculus can be an information source of long-term macronutrients intake.

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ABSTRACT

Introduction: The current dietary assessment still has many constraints, particularly related to the objectivity of data gathering. The dental calculus, which is usually considered as a medical waste of dental treatment, turns out to be a provider of an abundant oral information. The objective of this study was to obtain information about long-term intake of macronutrient from dental calculus content. **Method:** This study is a descriptive study with cross sectional study design. The data used in this study were obtained from the assessment of carbohydrate, protein and fat content of dental calculus. There were three groups of dental calculus samples. The first group was treated with Anthron method to assess the carbohydrate content. The second group was treated with Soxhlet extraction method. The third group was treated with SDS-PAGE. Twenty samples of dental calculus were taken using quota sampling method, each of which, was taken from the medical waste from the patient who got dental calculus cleaning treatment at Maranatha Dental Hospital. **Results:** The average concentration of carbohydrate from all dental calculus samples is 0.0246 ppm. The average of fat level from dental calculus is 0.05754%. The average concentration of protein from all dental calculus samples is 1.9834 mg/ml. **Conclusion:** Carbohydrates, proteins and fats can be examined from dental calculus.
Keywords: dental calculus, macronutrient, carbohydrate, protein, fat

INTRODUCTION

Nutrition plays a significant role in various metabolic processes because the human body needs adequate intake of nutrients continuously, in order to carry out the metabolism process optimally. The current dietary assessment still has many constraints, particularly related to the objectivity of data gathering tools such as food frequency questionnaires (FFQ), multiple-day food records and 24-hour dietary recalls.[1,2] The most common constraint is the respondents do not always remember all food that they have consumed, or do not know the specific content of the food (eg sandwich content) and it is difficult to determine an accurate portion size. In short, the respondents generally do not report all food intake.[1]

The limitation of the questionnaire-based assessments is only evident when sufficiently valid and accurate food intake measurement is required.[3] This limitation can be overcome by using dietary biomarkers, which can assess food consumption objectively and which has a lower bias than the self-reported assessment. The need for dietary biomarkers is recognized by medical institutions as a knowledge gap that requires future research.[1]

The potential of dietary biomarkers is needed to obtain an objective assessment of nutritional intake so as to improve the reliability of the study. The ultimate goal is to derive a statistical model that incorporates nutritional data that are based on biomarkers with those that are based on self-reported questionnaires.[4] Dietary biomarkers are not without limitations. High cost and invasive levels are important factors to consider, therefore non-invasive, inexpensive and specific source of dietary biomarkers is very much needed.[1] Dietary biomarkers can be categorized into short-term, medium term and long term.[5] Short term dietary intake indicates the dietary intake of previous few hours / days, medium term dietary intake indicates the dietary intake of previous several weeks, long term dietary intake indicates the dietary intake of previous several months / years. Reviews from all scientific articles from Feb 2001 until Sep 2012 show that long-term dietary biomarkers can be obtained from blood, plasma and adipose tissue.[1] The dietary biomarkers can be obtained through biochemical measurements of nutrients content from biological fluid and biological tissue as well as levels of excretion of nutrients or their metabolites.[5]

Dental calculus from fossil of ancient human teeth can be analysed to obtain data about the types of food as well as the cooking and processing technique.[6,7] Another study has also shown evidence that ancient human consumed milk

based on their dental calculus content.[8] Because of its location within the mouth, the dental calculus offers a direct path to the material that has inhaled or ingested.[6]

Dental calculus is a complex, mineralized bacterial biofilm formed on the surfaces of teeth, principally from dental plaque but also with additional contributions from saliva and gingival crevicular fluid. Biofilm formation begins when salivary proteins is deposited as a thin film on the surface of the teeth, forming the acquired enamel pellicle (AEP). During life, the AEP serves as a primary barrier and defensive layer between the calcium phosphate mineral of the enamel and bacterial and dietary acids.[9] Dental calculus mainly composed of calcium phosphate mineral salts that are stored between and within the remnants of previously active microorganisms. The dental plaque covers the mineralized dental calculus. The level and location of dental calculus formations are specific for each population and are influenced by oral hygiene habits, access to professional care, diet, age, ethnic origin, time since last dental cleaning, systemic disease and prescription drug use.[5]

Bacteria in dental plaque are related closely to the host. They use endogenous nutrients such as saliva and glycoprotein proteins such as mucin for their growth. The Bacteria also produce a little acid and help to remove exogenous microorganisms.[6] Dental calculus is also an important source of microbiome information, which can provide an accurate information about the evolution of microbiome, diet and human health. Various diseases including obesity are found to be associated with changes in human microbiome.[8]

Dental calculus, both supragingival and subgingival are present in most adults worldwide. Dental calculus is formed by bacteria and calcium phosphate salts that combine to form calculus. Subgingival calculus is useful for analysis as it accumulates and endures indefinitely if it is not mechanically removed.[6]

There are two different categories of nutrients: the macronutrient and micronutrient. The macronutrient which include carbohydrate, protein and fat is a nutrient that is needed by human body in a large amount.[10] The macronutrient content in food can be trapped inside the dental calculus. The dental calculus, which is usually considered as a medical waste of dental treatment, turns out to be a provider of an abundant oral information of long-term oral microbiotics, as well as food and environmental debris information.[9]

Based on the above description, the authors intend to conduct a research to assess the content of carbohydrates, proteins and fats contained in dental calculus, so that it can be a source of long-term dietary patterns information. The aim of this study is to obtain information about long-term intake of macronutrient from dental calculus content.

METHODS

This study is a descriptive study with cross sectional study design. The data used in this study were obtained from the assessment of carbohydrate, protein and fat content of dental calculus. The dental calculus sample was taken from the medical waste from the patient who got dental calculus cleaning treatment at Maranatha Dental Hospital.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects / patients were approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia (No.784/UN6.C.10/PN/2017). Written informed consent was obtained from all subjects / patients.

Twenty samples of dental calculus were taken using quota sampling method and were then divided into 3 groups. The first group consisted of 5 dental calculus samples which were treated with Anthron method to assess the carbohydrate content. The second group consisted of 5 dental calculus samples which were treated with Soxhlet extraction method. The third group consisted of 10 dental calculus samples which were divided further into 2 smaller groups of 5 samples, each of which, was assessed with Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) method and spectrophotometer using a different preparation technique.

The Dental calculus sample was taken from patients with several criteria as follow: the patients had never done dental calculus cleaning treatment before, the patients age was between 17 and 65 years. All dental calculus samples were treated in the central laboratory of Universitas Padjadjaran.

Carbohydrate Assessment

Each of the dental calculus samples were weighed to determine the initial weight and then those samples were smoothed and dissolved into 80% ethanol. The resulting solution was then filtered by using cotton and then the pH level of the filtered result was measured using pH meter. CaCO3 was added until the pH level was above 8. Afterwards, the solution was heated at 100^{o}C for 30 minutes. The solution volume was maintained by adding some water while it was heated. Afterwards the solution was filtered for the second time using a filter paper and then was rinsed with aquades. Thereafter the solution was reheated at 85oC to evaporate the ethanol. After that the solution was moved into the measuring flask and was added with saturated Pb Acetate until the solution became clear (about 3-5 ml), then it was vortexed for several minutes. Then Natrium Oxalate was added to settle all the Pb. After that the solution was refiltered once again.

Meanwhile the Anthron solution was prepared by dissolving 0,01 gr Anthron in 10 ml of 95-97% H2SO4. Then the anthron solution was poured into the flask that contained research sample. The solution was heated at 100ºC for 12 minutes. The colour of the solution which had carbohydrate content would change when it was heated. The concentration of carbohydrate in the solution was measured using spectrophotometer.

Fat Assessment

The fat content in dental calculus was assessed using soxhlet extraction method. Each of the dental calculus samples was weighed to determine the initial weight and then those samples were smoothed and placed into rolls of filter paper and then the samples were placed into the soxhlet equipment. The condenser was placed on top of it. The fat flask that had been heated in the oven was weighed and was filled with hexan at the bottom of it. Afterward the extraction process was run for more than 5 hours. Then the flask was heated at 105ºC overnight to evaporate the solvent. After that the flask was cooled in desiccator and was again weighed. The weight that resulted was calculated with the initial weight of the sample so that the fat concentration in the sample was obtained.

Protein Assessment

The protein was assessed using SDS-PAGE method and spectrophotometer. The 5 samples in first group were smoothed using speedmill PLUS and then it was dissolved in PBS and was filtered using whatman no.4 + Desalting coloumn 5000. While the 5 samples in second group were smoothed manually using mortar and then it was dissolved in SDS lysis buffer and wasn't filtered.

Afterwards the solution was centrifuged in 4500 RPM for 15 minutes. The supernatant was taken and then it was assessed using spectrophotometer to measure the protein content. The supernatant from both groups were also assessed using SDS-PAGE. The supernatant of the first group is taken as much as 10 µl which was then added with 10 µl loading sample buffer. While the supernatant of the second group was divided further into 3 groups that had different volumes. The first group contained 15 µl of the sample which was then added with 15 µl loading sample buffer. The second group contained 10 µl of sample which was added with 2 µl loading sample buffer. The third group contained 20 µl sample that was added with 2 µl of loading sample buffer. All samples from all groups were incubated at 100ºC for 5 minutes.

Meanwhile the SDS-PAGE gel was prepared and was placed into the electrophoresis equipment. Samples of 10-15 µl were placed in each well of the SDS-PAGE gel. A protein ladder of 5 µl was placed into the first well from the left and the protein positive control of 10ul was placed into the last well. The electrophoresis was run in 80-100V for 2 hours.

The next step of the treatment was protein transfer / Blotting using membrane nitrocellulose or Polyvinylidene fluoride (PVDF). The gel that had been treated in electrophoresis was arranged in Mini Blot Module. Afterwards it was treated again in electrophoresis in 42V for 1 hour. The resulted membrane of first group was coloured using silver stain, while the second group was coloured using Ponceau Stain.

RESULTS

The first group consisted of 5 dental calculus samples which were treated with Anthron method to assess the carbohydrate content. The result of carbohydrate content in dental calculus are shown in Table 1. All samples were treated equally according to the method that has been described previously.

Based on examination results that is showed in the Table 1, it was found that carbohydrates can be detected from all samples of dental calculus. The concentration of carbohydrate in the solution was measured using spectrophotometer. The average concentration of carbohydrate from all dental calculus samples in this study is 0.0246 ppm.

Table 1. Carbohydrate content

Sample	Sample's Weight (mg)	Initial concentration of the sample (ppm)	The concentration of carbohydrate (ppm)	Dilution Factor	carbohydrate Level (%)
1	354.3	354300	0.014	1	0.000003951
2	85.4	427000	0.010	1	0.000002342
3	34.2	28500	0.021	1	0.000075406
4	42	35000	0.026	1	0.000074581
5	60	50000	0.052	1	0.000103994

The second group consisted of 5 dental calculus samples which were treated with Soxhlet method to assess the fat content. The result of fat content in dental calculus are shown in Table 2. All samples were treated equally according to the method that has been described previously. Based on examination results that is showed in the Table 2, it was found that fat can be detected from all samples of dental calculus. The level of fat in the solution was measured by calculates the percentage of weight of fat obtained, with the initial weight of the sample. The average of fat level from dental calculus in this study is 0.05754 %.

Table 2. Fat Content

Sample	Weight of sample (mg)	Weight of Fat (mg)	Fat Lavel (%)
6	50	0.855	0.0171
7	50	1.61	0.0322
8	63	2.6964	0.0428
9	48.6	1.99746	0.0411
10	109.4	16.9023	0.1545

The third group consisted of 10 dental calculus samples which were assessed using SDS-PAGE method and spectrophotometer. The result of protein content in dental calculus are shown in Table 3. All samples were treated according to the method that has been described previously.

Based on examination results that is showed in the Table 3, it was found that protein can be detected from all samples of dental calculus. The concentration of protein in the solution was measured using spectrophotometer. The average concentration of protein from dental calculus samples in group 1 this study is 0.748 mg/ml. The average concentration of protein from dental calculus samples in group 2 this study is 3.2188 mg/ml. The average concentration of protein from all dental calculus samples in this study is 1.9834 mg/ml.

Table 3. Protein Content

Group	Sample	Dilution agent	Filtration	Weight of Sample (mg/ml)	Smoothing technique	Concentration of protein (mg/ml)
1	11	PBS	whatman no.4. + Desalting coloumn 5000	25	SpeedMill PLUS	0.9
	12	PBS	whatman no.4. + Desalting coloumn 5000	25	SpeedMill PLUS	0.86
	13	PBS	whatman no.4. + Desalting coloumn 5000	25	SpeedMill PLUS	0.7
	14	PBS	whatman no.4. + Desalting coloumn 5000	25	SpeedMill PLUS	0.65
	15	PBS	whatman no.4. + Desalting coloumn 5000	25	SpeedMill PLUS	0.63
2	16	lysis buffer	non	50	Manual	11.4
	17	lysis buffer	non	13.5	Manual	2.058
	18	lysis buffer	non	50.3	Manual	1.509
	19	lysis buffer	non	23.8	Manual	0.781
	20	lysis buffer	non	21	Manual	0.346

The Samples from third group were also examined using SDS-PAGE electrophoresis then they were transferred / blotted on PVDF and finally they were stained using Ponceau staining. The bands that were appeared on the staining result are showed in fig.1

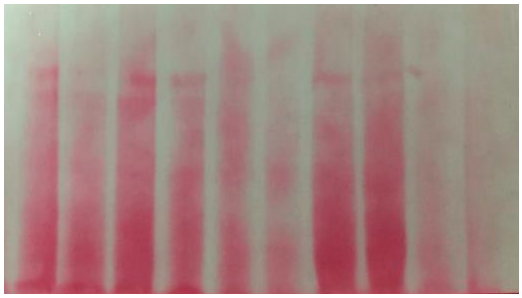

Figure 1. Ponceau Staining of Dental Calculus Samples

DISCUSIONS

FFQ, food diaries and 24 h recall methods represent the most commonly used dietary assessment tools in human studies on nutrition and health, but food intake biomarkers are assumed to provide a more objective reflection of intake. Unfortunately, very few of these biomarkers are sufficiently validated.[11]

Dental calculus, a mineralized form of dental plaque that serves as a long-term reservoir of dietary biomolecules and microfossils.[12] Nearly ubiquitous in archaeological populations and sourced directly from the oral cavity, dental calculus presents a unique opportunity to access primary evidence of ancient diets at an individual level.[12] Dietary reconstructions based on plant microfossils, such as starch grains and phytoliths, also have been useful in increasing our understanding of past human populations.[13] The development of dental calculus is a dynamic process that starts with a non-mineralized biofilm which eventually calcifies. Non-mineralized dental biofilm entraps particles from the oral cavity, including large amounts of oral bacteria, human proteins, viruses and food remnants, and preserves their DNA.[14]

Dental plaque is a dense mass of bacteria also known as biofilm that is tightly adherent to the tooth surface. Bacterial attachment to the tooth is mediated by receptors in the thin layer salivary coating of the tooth surface, termed the acquired pellicle. The pellicle and plaque matrix are composed of host-derived and bacterial products. Bacteria in the dental plaque have a close relationship with the host, they use endogenous nutrients such as saliva and glycoprotein proteins such as mucin for their growth, from which there is little acid production and their presence helps remove exogenous microorganisms (colonization resistance).[15]

The composition of dental plaque formed in the presence of sucrose or glucose and fructose and its relation to cariogenicity was evaluated and the results suggested that the high cariogenicity of dental plaque formed in the presence of sucrose.[16]

Dental calculus is indeed a stable, long-term reservoir of proteins as previously reported, but further systematic studies are needed to identify mechanisms associated with protein entrapment and survival in dental calculus.[17]

The mineralized matrix of dental calculus is of high physical hardness and durability, preserving organic microscopic debris and biomolecules. Frequently found on skeletal material, calculus has been described as “one of the richest known sources of ancient biomolecules in the archaeological record”,9 preserving molecular evidence of oral bacteria, the human host, as well as consumed foodstuffs, all of which can be directly tied to the individual.[9,17,18]

CONCLUSION

This study has succeeded in examining the macronutrient content of dental calculus in humans. The conclusion of this study is carbohydrate, protein and fat can be assessed from dental calculus. The relationship between macronutrient content on dental calculus with macronutrient intake examination using questionnaire and also the assessment to determine which type of carbohydrate, protein and fat that was trapped in dental calculus will be done in the further studies.

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All authors have made substantive contribution to this study and/or manuscript, and all have reviewed the final paper prior to its submission.

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Conflict of interest

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REFERENCES

1. Hedrick VE, Dietrich AM, Estrabrooks PA, et al. Dietary Biomarkers: Advances, Limitations and Future Directions. *Nutritional Journal* 2012; 11: 1–14.
2. Kowalkowska J, Slowinska MA, Slowinski D, et al. Comparison of a Full Food-Frequency Questionnaire with The Three-Day Unweighted Food Records in Young Polish Adult Women: Implications for Dietary Assessment. *Nutrients* 2013; 5: 2747–2776.
3. Corella D, Ordovas JM. Biomarkers: Background, Classification and Guidelines for Applications in Nutritional Epidemiology. *Nutricion Hospitalaria* 2015; 31: 177–188.
4. Prentice RL, Sugar E, Wang CY, et al. Research Strategies and The Use of Nutrient Biomarkers in Studies of Diet and Chronic Disease. *Public Health Nutrition* 2002; 5: 977–984.

5. Gibson RS. *Principles of Nutritional Assessment*. 2nd ed. New York: Oxford University Press, 2005.
6. Buckley S, Usai D, Jakob T, et al. Dental Calculus Reveals Unique Insights into Food Items, Cooking and Plant Processing in Prehistoric Central Sudan. *PLOS ONE* 2014; 9: 1–10.
7. White DJ. Dental Calculus: Recent Insights into Occurrence, Formation, Prevention, Removal and Oral Health Effects of Supragingival and Subgingival Deposits. *European Journal of Oral Sciences* 1997; 105: 508–522.
8. Kaufman E, Lamster IB. The Diagnostic Applications of Saliva— A Review. *Critical Reviews in Oral Biology And Mediciine* 2002; 13: 197–212.
9. Warinner C, Speller C, Collins MJ. A New Era in Palaeomicrobiology: Prospects for Ancient Dental Calculus as a Long-Term Record of The Human Oral Microbiome. *Philosophical Transaction Royal Society B* 2014; 370: 1–11.
10. Fahmida U, Dillon DHS. *Handbook Nutritional Assessment*. 2nd ed. Jakarta: SEAMEO RECFON UI, 2011.
11. Brouwer-Brolsma EM, Brennan L, Drevon CA, et al. Combining traditional dietary assessment methods with novel metabolomics techniques: present efforts by the Food Biomarker Alliance. *Proceedings of the Nutrition Society* 2017; 76: 619–627.
12. Warinner C, Hendy J, Speller C, et al. Direct Evidence of Milk Consumption From Ancient Human Dental Calculus. *Scientific Reports* 2014; 4: 1–6.
13. Henry AG, Piperno DR. Using plant microfossils from dental calculus to recover human diet: a case study from Tell al-Raqā'i, Syria. *Journal of Archaeological Science* 2008; 35: 1943–1950.
14. Ackali A, Lang NP. Dental calculus: the calcified biofilm and its role in disease development. *Periodontology 2000* 2018; 76: 109–115.
15. Lamont RJ, Burne RA, Lantz MS, et al. *Oral Microbiology and Immunology*. Washington DC: ASM Press, 2006.
16. Cury J., Rebelo MA., Del Bel Cury AA, et al. Biochemical Composition and Cariogenicity of Dental Plaque Formed in the Presence of Sucrose or Glucose and Fructose. *Caries Research* 2000; 34: 491–497.
17. Mackie M, Hendy J, Lowe AD, et al. Preservation of the metaproteome: variability of protein preservation in ancient dental calculus. *Science & Technology of Archaeological Research* 2017; 3: 74–86.
18. Weyrich LS, Dobney K, Cooper A. Ancient DNA Analysis of Dental Calculus. *Journal of Human Evolution* 2015; 79: 119–124.