Article type: *Original Article*

Salivary Omega6/ Omega 3 Fatty Acid Ratio And Dental Caries-Less is More

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Abstract

Background: Omega 3 and omega 6 are essential polyunsaturated fatty acids (PUFA) which cannot be synthesized in the body and needs to be obtained from dietary sources.The omega 6-omega 3 fatty acids ratio is important for human health, hence appropriate amounts of both these fatty acids should be considered for dietary recommendations.. A ratio ranging between 2:1 to 4: 1 (Omega 6: Omega 3) has been stated to be the proper ratio by a summary of several nutritional estimation studies However the relation to dental caries which is an infectious disease with multi-factorial etiology and is attributed to be the major reason for loss of tooth in children & adolescents, has not yet been explored. **Aim**: To assess and compare the salivary omega 6/omega 3 fatty acid ratio in children with varying levels of dental caries. **Methodology**: A total of 205 children aged 7-14 years were selected for the study. The children were grouped based on ICDAS-II classification system into Group I (Study group- 102 children) and Group II (Control = 103 children). Saliva samples were collectedand subjected to Gas Chromatography to quantify all the fatty acids. The mean omega 6/omega 3 ratio was compared among the two groups. The statistical analysis was done by one-way ANOVA. **Results**: On comparing the mean fatty acids ratio among the study and control groups,no statistically significant differences (p=0.313) was noted. **Conclusion:**No statistically significant difference was observed on comparing the ratio of omega 6 to omega 3 fatty acids in the 2 groups.

Keywords: *Omega 3, Omega 6, Saliva, Dental caries*

Introduction

Nutrition, being a part of systemic health of an individual, plays an important role in maintaining equilibrium between health and disease status. [1]These dietary nutrients have an impact on oral health status and can contribute as a major factor in improving oral inflammation and diseased conditions, thus enhancing the overall well-being of an individual. [2,3]Omega 6 and omega 3 are essential polyunsaturated fatty acids (PUFA) which cannot be synthesized in the body and needs to be obtained from dietary sources. [4] Omega 3 is a combination of alpha-linoleic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The main sources of omega 3 fatty acids are fish (seafood - salmon, mackerel, tuna) and fish oil

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beside the plant sources including soybean oil, canola oil, walnut and the seeds of flax, chia. [5,6]Omega 6 is a combination of linolenic acid (LA), dihomogamma linoleic acid (DGLA), docosapentaenoic acid (DPA), gamma linoleic acid (GLA) and Arachidonic acid (AA). The major sources of omega 6 fatty acids are meat, egg yolk and vegetable oils. [7]

Both these polyunsaturated fatty acids are needed for growth and repair, and they play multiple roles in cell membrane structure, lipid metabolism, blood clotting, blood pressure, and in controlling inflammation.[8]Literature states that these fatty acids also have an anti-inflammatory, anti-oxidant and antimicrobial activity[9,10] When compared to normal quantities in which omega 6 fatty acids are biologically active, larger quantities are toxic to health. [11]

The omega 6-omega 3 fatty acids ratio is important for human health, hence appropriate amounts of both these fatty acids should be considered for dietary recommendations.[12] A ratio ranging between 2:1 to 4: 1 (Omega 6: Omega 3) has been stated to the proper ratio by a summary of several nutritional estimation studies .[8] When compared to the olden times of Paleolithic period, where a normal balance was seen in between omega 3 and 6 fatty acids, the present day human life has resulted in a drastic change in the diets of individuals which has consequently resulted in a paradigm shift in the amount and type of several antioxidants including omega 3 and 6 fatty acids and the same has been reported in several studies.[13-17]

Present day diet is rich in prepared and fast foods, which results in excessive amounts of omega 6 PUFA while lacking beneficial amounts of omega 3, thus resulting in a very high omega 6/omega 3 ratio (15:1), which can predispose to cardiovascular diseases, cancer, diabetes, neurodegenerative diseases. [18]

However the relation to dental caries which is an infectious disease with multi-factorial etiology and is attributed to be the major reason for loss of tooth in children & adolescents, has not yet been explored.[19,20]

The amount of fatty acid of a person can be studied by analyzing various biological components namely, saliva, serum and adipose tissue. [18] However the potential of saliva to estimate the levels of PUFA remains unexplored. The use of saliva is considered to be more effective owing to the fact that the collection of saliva is a non- invasive procedure when compared to others which require invasive methods to acquire the specimens. The other added advantages being less cumbersome equipment required and ease of storage.

This study is done with the hypothises that if the ratio of omega 6 omega 3 fatty acids are in the normal range there will be a decrease in dental caries levels and other inflammatory diseases.

Since there are no studies that correlates the ratio of omega 6/omega 3 in saliva and its relationship with dental caries, there is a need for this study.

Method

Source of Data: An observational (case control) study was conducted. A total of 205 children, both male and female (102 study and 103 control) aged between 7-14 years were selected after conducting a screening camp at Aided Holy Angels' Higher primary school, Thokkottu, Mangalore. Informed consent was obtained from the parents and school authority and assent was obtained from the children. Ethical clearance was obtained from the institutional ethical committee.

Inclusion Criteria:Children within the age group of 7-14 years with 3 or more carious teeth (ICDAS-II Code 3-6) and children who are willing to participate in the study with parental consent were included in the study group and children within the same age groups as controls. (ICDAS-II Code 0,1,2)

Exclusion Criteria:Children who have systemic diseases, children under any medications and children who are not willing to participate in the study and without parental consent.

Methodology

The children were grouped based on ICDAS-II system for measuring dental caries .

Group 1: Caries Group - 102 children with 3 or more carious teeth classified as Codes 3,4,5,6 under ICDAS – II

Group 2: Control group – 103 children with carious teeth classified as Codes 0,1,2 under ICDAS – II

Method of collection of saliva - 5 ml of unstimulated saliva was collected within a 10 minute period between 9 am and 11 am to minimize any possible effects of diurnal variation. Saliva samples were collected by passive drooling, in which subjects were asked to sit in a quiet environment in the "coachman position" and expectorate for 5 minutes in to sterile containers.

Saliva samples were transported to Central research laboratory, KSHEMA within 30 minutes and centrifuged at 4000 rpm for 15 min at 4 degree Celsius.The supernatants were stored at -80^0 C. Gas Chromatography was done to quantify all the fatty acids.

Statistical Analysis: Descriptive and analytical statistics were done. The data is represented in mean and standard deviation. The normality of continuous data was analysed by Shapiro-Wilk test. As the data followed normal distribution, parametric tests were used to analyse the data. The independent sample t-test and one way ANOVA tests were used to check mean differences wherever appropriate. The level of significance was kept at $p<0.05$.

SPSS (Statistical Package for Social Sciences) Version 24.0 (IBM Corporation, Chicago, USA) was used for carrying out statistical analysis.

Results

Out of the 205 salivary samples collected, omega 3 and omega 6 fatty acid levels could be estimated from only 60 (30%) and 72 (36%) samples respectively. Salivary omega 6/omega 3 fatty acid ratio was calculated only for those children were both these fatty acids were obtained. It was 12(11.76%) for Group 1 and 24 (23.3%) for Group 2. Due to this reason the remaining samples were not considered and the calculations are solely based on these samples.

Table 1: Comparison of mean omega fatty acids ratio between the two groups

Groups	N	Mean	S.D.	S.E.	M.D.	95% C.I.	t-value	P-value#
Group 1(Study)	12	0.75	0.37	0.10	-1.18	-2.72-0.34	-1.573	0.125
Group 2 (Control)	24	1.94	2.58	0.52				

#P-value derived from independent sample t-test

The mean fatty acids ratio (omega 6/omega 3) between the two groups was compared. It was found that there was NO statistical significant difference in mean fatty acids ratio (p=0.840) between the caries and control groups. [Table 1]

Discussion

Omega 3 and omega 6 PUFA have been widely studied for their beneficial effects on human health, mainly the brain, eye, cardiovascular system, and general human growth. Recent reports have noted that PUFA could improve oral health since they possess antioxidant, anti-inflammatory and antibacterial activities. However, their utilization as antimicrobial agents has not been widely appreciated.[21]

Several studies evaluating the level of PUFA in serum are available in literature proving that fatty acid estimation is possible in serum.[22] However, a standardization regarding the normal values has not been made. Serum estimation of fatty acid is a relatively invasive procedure that requires blood sample collection from the patient. If a suitable alternative procedure that is non-invasive is available, it would lead to ease in estimation of levels of PUFA. Since many of the

serum biomarkers are also present in saliva, it could be a suitable alternative for PUFA estimation as well.

To the best of our knowledge, this is the first clinical study using saliva for estimation of omega 6/omega 3 ratio and its correlation with dental caries. So, the present study was conducted to find whether such a correlation existed, and if present, to observe its relationship to dental caries. We expect it to open a new avenue for caries control.

Out of the 205 salivary samples collected, omega 3 and omega 6 fatty acid levels could be estimated from only 60 (30%) and 72 (36%) samples respectively. Based on these results, it is evident that not all children in our study showed the presence of omega 3 or omega 6 fatty acids in saliva. Still lesser number of children showed the presence of both omega 3 and omega 6 fatty acids in saliva. Salivary omega 3 and omega 6 fatty acids were both present in 12 children (11.76%) belonging to Group 1 and 24 children (23.3%) from Group 2. This shows that the presence of both these fatty acids could relate to the severity of caries in the children included in our study.

Studies have shown that omega 3 and omega 6 polyunsaturated fatty acids have anti-bacterial activity which could be attributed to the resemblance with the bipolar membrane of the bacterial cell wall due to having both a hydrophilic head and a hydrophobic tail. This similarity suggests that the target of these fatty acids could be the cellular membrane because fatty acids could possibly penetrate into the cell membrane of the bacteria disrupting normal cell[23]

A direct comparison could not be made since no studies have been performed on dental caries and omega fatty acids.

The normal ratio of serum omega fatty acids (omega 6/omega 3) is reported to be between 2:1 to 4:1.[19]Although several studies have evaluated fatty acid profiles in saliva, studies on omega fatty acids levels have not been carried out. So a comparison with standard normal ratio could not be done in our study. However when the mean salivary fatty acids ratio (omega 6: omega 3) between the study and control groups were compared, it was found that there was no statistical significant difference. This could be due to the insufficient number of children exhibiting both omega 3 and omega 6 salivary fatty acids, for calculation of ratio in the different groups. These values cannot be relied upon to form any definite conclusions.

Conclusion and Future Recommendations:

1) With the available estimation methods, the omega 6 and omega 3 fatty acids could not be detected in the saliva of all the subjects in our study probably due to their insignificant levels in saliva. Tools with better precision are required for accurate estimation of omega 3 and omega 6 fatty acids.

2) Similar studies with larger sample size are required to establish the role of omega 3 and omega 6 fatty acids in dental caries definitively.

3) Once standardisation of estimation method and normative values are established, longitudinal studies and clinical trials using salivary omega 3 and omega 6 fatty acids may be undertaken.

Ethical Clearance: Taken from Instituitional Ethical committee (NITTE Deemed to be) University

Source of Funding : Self

Conflict of Interest: Nil

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