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Original Article

Estimation of salivary omega-6 and omega-3 fatty acid levels in children and its relationship to varying levels of dental caries

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Abstract

Omega 3 and omega 6 are essential polyunsaturated fatty acids (PUFA) that must be obtained from dietary sources. Dental caries is an infectious disease with multi-factorial etiology and is the major reason for tooth loss in children & adolescents. The aim was to assess and compare the levels of salivary omega-6 and omega-3 fatty acids in children with varying levels of dental caries. A number of 205 children aged 7–14 years were selected and grouped based on the ICDAS-II classification system into Group I (Active caries), Group II (Arrested caries) and Group III (Controls). Saliva samples were collected and subjected to Gas Chromatography to quantify all the fatty acids. Group III had the highest mean omega-3 fatty acids value, followed by Group I and Group II. Group II had the highest mean omega-6 fatty acids value, followed by Group III. Group I had the least mean of Omega-6. The highest levels of omega-3 fatty acids were observed in the control group, the lowest in the arrested caries group, and the omega-6 levels were the highest in the arrested caries group and the lowest in the active caries group.

Keywords: nutrition, polyunsaturated fatty acids, health, disease, saliva, dental caries.

Introduction

As stated by WHO, nutrition is the science of food and its relationship to health [1, 2]. Adequate intake of vitamins, proteins, essential fatty acids and micronutrients constitutes a nutritious diet [3]. These dietary nutrients improve the well-being of an individual by improving diseased conditions and oral inflammation [4, 5]. Omega-3 and omega-6 are essential polyunsaturated fatty acids (PUFA) that cannot be synthesized in the body and need to be obtained from dietary sources [6]. 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, besides the plant sources including soybean oil, canola oil, walnut and the seeds of flax and chia [7, 8]. Omega 6 is a combination of linolenic acid (LA), dihomo-gamma-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 [9].

Omega-3 and omega-6 PUFA play multiple roles in cell membrane structure, blood clotting, lipid metabolism and blood pressure and are needed for growth and repair and in controlling inflammation [10]. Studies have shown that omega-3 and omega-6 fatty acids have antioxidant, anti-inflammatory, and antimicrobial activity [11, 12]. Larger quantities of omega-6 fatty acids are toxic to health and biologically active [13].

When compared to the olden times of the Paleolithic period, where a normal balance was seen between omega-3 and 6 fatty acids, 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 [14–18].

Present day diet is rich in prepared and fast foods, resulting in excessive amounts of omega 6 PUFA while

Table 1: Comparison of mean omega-3 fatty acids between the two groups.

Groups	N	Mean	S.D.	S.E.	M.D.	95% C.I.	t-value	P-value #
Group 1 and 2 (Active & arrested)	27	5.92	4.25	0.81	-4.05	-6.55–1.56	-3.261	0.002 †
Group 3 (Control)	33	9.98	5.19	0.90	-	-	-	-

Note: # – P-value derived from independent sample t-test; † – significant at p<0.05.

lacking beneficial amounts of omega-3, thus resulting in a very high omega-6/omega-3 ratio (15:1), that can predispose to cancer and cardiovascular disease, diabetes and neurodegenerative diseases [19].

However, the relation to dental caries, an infectious disease with multi-factorial etiology, is attributed to the major reason for tooth loss in children & adolescents, which has not yet been explored [20, 21]

The amount of fatty acid in a person can be studied by analyzing various biological components, namely, saliva, serum and adipose tissue [19]. However, the potential of saliva to estimate the levels of PUFA remains unexplored. The use of saliva is considered more effective because the collection of saliva is a non-invasive procedure compared to others, which require invasive methods to acquire the specimens. The other added advantages are less cumbersome equipment required and ease of storage.

Since there are no studies on the levels of omega-3 and omega-6 fatty acids in saliva and also its correlation with varying levels of dental caries, it paves a need for research in this field. Therefore, the present study was an effort to assess the omega-3 and omega-6 fatty acid levels in saliva with varying levels of dental caries.

Material and methods

An observational (case-control) study was conducted. A total of 205 male and female children (102 study, of which 50 with arrested caries, 52 with active carious lesions 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.

Children with 3 or more active/arrested carious teeth (ICDAS-II Code 3-6) and those 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). Children suffering from systemic diseases, under any medications

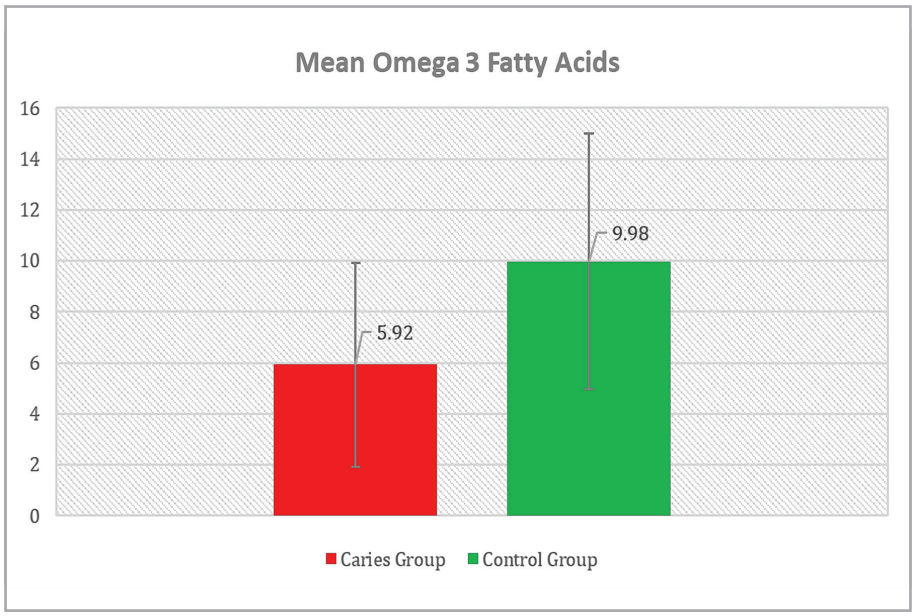

Figure 1: Comparison of mean omega-3 fatty acids between the two groups. The error bar represents the standard deviation.

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Table 2: Comparison of mean omega-3 fatty acids among the three groups.

Groups	N	Mean	S.D.	S.E.	Min.	Max	F-value	P-value #
Caries Active (Group 1)	16	6.06	4.11	1.02	0.42	17.33	5.243	0.008 †
Caries Arrested (Group 2)	11	5.72	4.65	1.40	0.52	10.78	-	-
Control (Group 3)	33	9.98	5.19	0.90	0.66	18.38	-	-

Note: # – P-value derived from one-way ANOVA test; † –significant at p<0.05.

and not willing to participate in the study without parental consent were excluded from the study.

Methodology

The children were grouped based on the ICDAS-II system for measuring dental caries: Group 1: Active caries Group – 52 children with 3 or more active carious teeth classified as Codes 3,4,5,6 under the ICDAS – II; Group 2: Arrested caries Group – 50 children with 3 or more arrested carious teeth classified as Codes 3,4,5,6 under the ICDAS – II; Group 3: Control group – 103 children with carious teeth classified as Codes 0,1,2 under the ICDAS – II.

Method of collection of saliva – 5 ml of unstimulated saliva was collected within 10 minutes between 9 a.m. and 11 a.m. 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 into sterile containers.

Saliva samples were transported to the central research laboratory, KSHEMA, within 30 minutes and centrifuged at 4000 rpm for 15 min at 4˚C. The supernatants were stored at -800 Celsius. 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 Shapiro-Wilk test analyzed the normality of continuous data. As the data followed a normal distribution, parametric tests were used to analyze 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

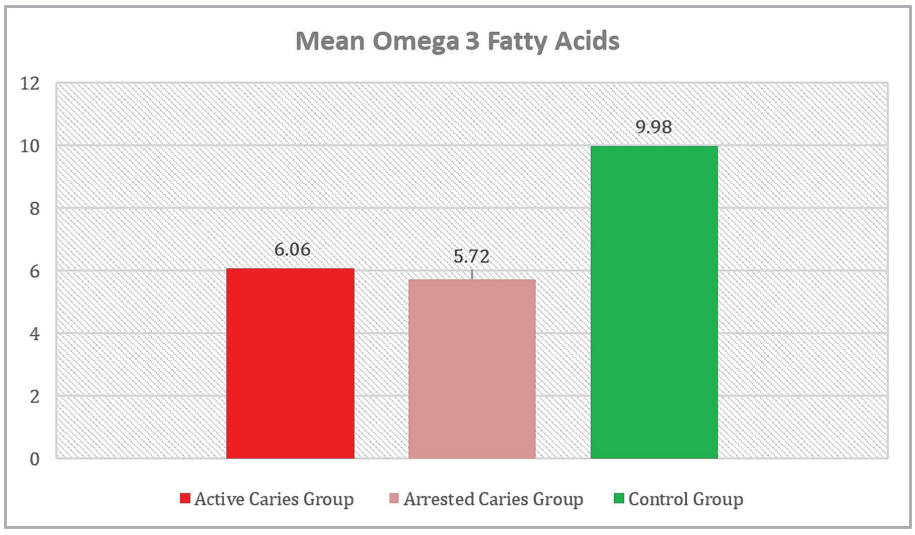

Figure 2: Comparison of mean omega-3 fatty acids among the three groups.

Table 3: Comparison of mean omega-6 fatty acids between the two groups.

Groups	N	Mean	S.D.	S.E.	M.D.	95% C.I.	t-value	P-value #
Groups 1 & 2 (Active & Arrested)	33	6.88	6.52	1.13	-0.25	-2.79-2.28	-0.202	0.840
Group 3 (Control)	39	7.14	4.19	0.67	-	-	-	-

Note: # – P-value derived from independent sample t-test.

only 60 (30%) and 72 (36%) samples, respectively. Due to this reason, the remaining samples were not considered and the calculations are solely based on these available samples.

The mean omega-3 fatty acids between the study and control groups were compared. It was found that there was a statistically significant difference in mean omega-3 fatty acids (p=0.002) between caries and control groups. Those patients who had caries had significantly lower levels of omega-3 fatty acids than controls (Table 1, Figure 1).

The mean omega-3 fatty acids were compared among the three groups. The analysis done by one-way ANOVA showed statistically significant differences (p=0.008) in omega-3 fatty acids. The control group had the highest mean value of omega-3 fatty acids (9.98±5.19), followed by the active caries group (6.06±4.11) and arrested caries group (5.72±4.65) (Table 2, Figure 2).

The mean omega-6 fatty acids between the two groups were compared. It was found that there was no statistically significant difference in mean omega-6 fatty acids (p=0.840) between caries and control groups (Table 3, Figure 3).

The mean omega-6 fatty acids were compared among the three groups. The analysis done by one-way ANOVA showed statistically significant differences (p=0.003) in omega-6 fatty acids. The arrested caries group had the highest mean value of omega-6 fatty acids (9.84±4.96), followed by the control group (7.14±4.19). The active caries group had the least mean omega-6 fatty acids (3.74±6.64) (Table 4, Figure 4).

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 antimicrobial agents use has yet to be widely appreciated [22].

Several studies in literature prove that fatty acid estimation is possible in serum [23]. However, a standardization regarding the normal values has yet to be made. Serum estimation of fatty acid is a relatively invasive

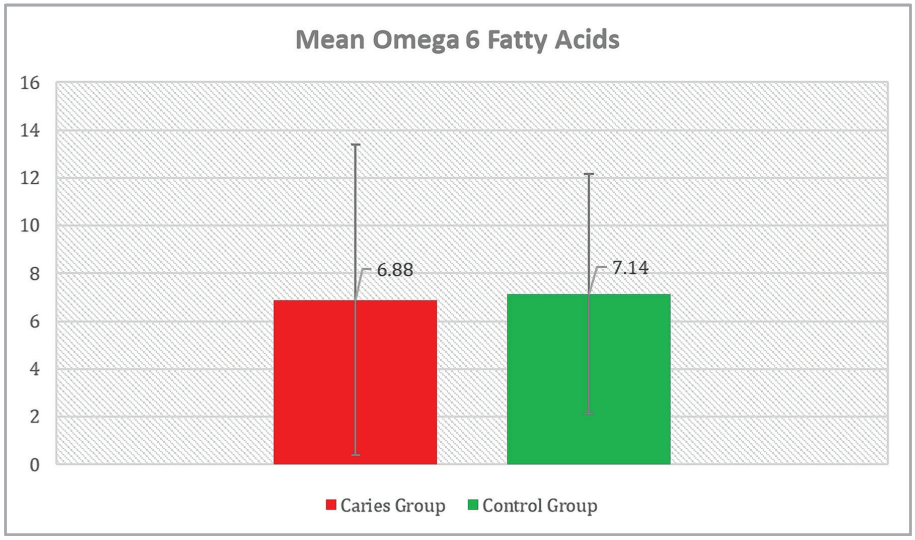

Figure 3: Comparison of mean omega-6 fatty acids between the two groups. The error bar represents the standard deviation.

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Table 4: Comparison of mean omega-6 fatty acids among the three groups.

Groups	N	Mean	S.D.	S.E.	Min.	Max	F-value	P-value #
Caries Active (Group 1)	16	3.74	6.64	1.66	0.37	25.09	6.159	0.003 †
Caries Arrested (Group 2)	17	9.84	4.96	1.20	0.50	22.11	-	-
Control (Group 3)	39	7.14	4.19	0.67	2.01	14.62	-	-

Note: # – P-value derived from one-way ANOVA test; † – significant at p<0.05.

procedure that requires blood sample collection from the patient. Since many of the serum biomarkers are also present in saliva, it could be considered a suitable non-invasive alternative for PUFA estimation.

To the best of our knowledge, this is the first clinical study using saliva to estimate PUFA and its correlation with the severity of 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 assessing caries activity.

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. Where it was present, omega-6 showed more correlation to caries. Still, fewer children showed the presence of both omega-3 and omega-6 fatty acids in saliva. Both salivary omega-3 and omega-6 fatty acids were present in 4 children (7.6%) belonging to Group 1, 8 (16%) children belonging to Group 2 and 24 children (23.3%) from Group 3. This shows that the presence of both these fatty acids could relate to the severity of caries in the children included in our study.

On comparing the omega-3 fatty acid levels between the two groups (study and control groups), a statistically significant difference (p=0.002) was found. Patients with more severe caries lesions were found to have significantly lower levels (mean=5.92) of omega-3 fatty acids than controls (mean=9.98). This could be attributed to the lower antibacterial activity of these fatty acids against caries-causing pathogens, which resulted in a higher incidence of dental caries in the study groups. These results were similar to the findings of C.B Huang and J.L Ebersole [24].

When the mean omega-3 fatty acid levels were compared among the control and the study subgroups, there was a statistically significant difference (p=0.008). The control group had the highest mean value of omega-3 fatty acids (9.98±5.19), followed by the active caries group (6.06±4.11) and arrested caries group (5.72±4.65). This indicates that omega-3 fatty acid levels are higher in control subjects when compared to study subjects. A direct comparison could not be made since no studies have been performed on dental caries and

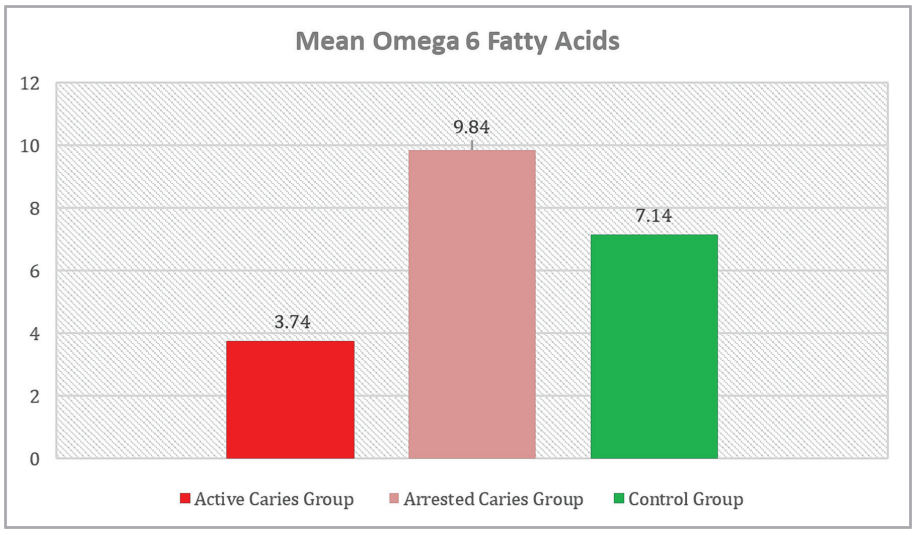

Figure 4: Comparison of mean omega-6 fatty acids among the three groups.

omega fatty acids. However, in a study done by Campan *et al.*, it was seen that individuals with healthy periodontal status were found to have higher omega-3 fatty acid levels compared to subjects with periodontitis [25].

Considering the fact that both caries and periodontitis are oral inflammatory diseases, we stipulate that in the presence of inflammation, omega-3 fatty acid levels decrease in saliva, thereby indicating its protective role against the inflammatory process. The order of decrease in levels of omega-3 fatty acids could be attributed to the fact that in a healthy state, there is definite protection from inflammation, whereas, in the presence of active carious lesions, the inflammatory process is ongoing. With appropriate measures during this active inflammatory process, inflammation reduction and even reversal could be possible. However, in arrested carious lesions, the lesion neither progresses nor regresses and remains static. This could explain the lower levels of salivary omega-3 fatty acids in this subgroup.

When the salivary omega-6 fatty acids were compared between the two groups (study and control groups), it was found that there was no statistically significant difference (p=0.840). Since literature evidence states that omega-6 fatty acids levels are increased in disease states [13], one would expect a rise in levels of omega-6 fatty acids with an increase in the severity of dental caries. This was not observed in our study. A possible reason could be because of an insufficient number of children detected with the presence of salivary omega-6 fatty acids in our study.

When the mean omega-6 fatty acids were compared among the control and study subgroups, there were statistically significant differences (p=0.003) in omega-6 fatty acids. The omega-6 fatty acids levels were found to be highest in the arrested caries subgroup and lowest in the active caries subgroup, respectively. This observation is contrary to that seen in omega-3 fatty acid levels. This indicates that there is an inverse relationship between the severity of dental caries and omega-6 fatty acid levels in saliva.

Conclusions

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. There was a statistically significant difference in the salivary omega-3 levels among the 3 groups, based on levels of caries activity. The highest levels were observed in the control group and the lowest were in the arrested caries subgroup. There was a statistically significant difference in the salivary omega-6 levels among the 3 groups, based on levels of caries activity. The highest levels were observed in the arrested caries subgroup and the lowest were in the active caries subgroup.

Conflict of interest

The authors declare no conflict of interest.

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