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Original Research Article

Prevalence of ecosapentaenoic acid and docosahexaenoic acid deficiency in pregnant women: a prospective observational study

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ABSTRACT

Background: Docosahexaenoic acid (DHA), ecosapentaenoic acid (EPA) and alpha lineoloic acid (α-LA) are essential fatty acids of the long chain polyunsaturated fatty acid (LC-PUFA) or omega-3 fatty acid. These fatty acids are not synthesized by our body. Diets rich in LC-PUFA reduces the incidence of - preeclampsia, intrauterine growth restriction, preterm delivery, in utero fetal death and placental abruption. Objectives of the study were: to estimate the prevalence of EPA and DHA deficiency in pregnant women by omega-3 index test (O3I), and also to correlate blood levels of EPA and DHA with various socio demographic parameters.
Methods: All enrolled subjects were assessed with a pre-defined performa for their demographic and socio-economic characteristics, personal history, obstetric history, body mass index (BMI), education, diet and blood samples were tested for O3I.
Results: Result of the study indicated that 98% of pregnant women had EPA and DHA deficiency as indicated by O3I levels. This was regardless of age, parity, BMI, and socio economic status. Women consuming vegetarian diet had statistically significant lower mean and median levels of O3I as compared to the women having a non-vegetarian diet, p value <0.001, 0.004 respectively. In our entire cohort of 150 women, only 2 non-vegetarian women had sufficient O3I.
Conclusions: 98% of pregnant women across various socio economic classes had EPA and DHA deficiency as measured by O3I levels. Vegetarians had statistically significant lower levels of O3I as compared to the non-vegetarians.

Keywords: Ecosapentaenoic acid, Docosahexaenoic acid, Omega 3 index

INTRODUCTION

Docosahexaenoic acid (DHA), ecosapentaenoic acid (EPA) and alpha lineoloic acid (α-LA) are essential fatty acids of the long chain polyunsaturated fatty acid (LC-PUFA) or omega- 3 fatty acid.[1] These LC-PUFAs form essential components of phospholipids present in all our tissues and are actively involved in the functional regulation of cellular and subcellular membranes.[2-4] These fatty acids are not synthesized by our body and hence have to be included in our diet.[2,3]

Reports, mainly observational, suggest that levels of LC-PUFA in blood have a great impact on the mother and fetus during pregnancy. In women with deficient levels of EPA and DHA, diets rich in LC-PUFA reduces the incidence of deep placentation disorders such as - preeclampsia, intrauterine growth restriction, and preterm delivery, in utero fetal death and placental abruption.[5-7] DHA and EPA are known precursors of various resolvins that control the duration and magnitude of inflammatory response, their low levels are also associated with sleep disturbance and postpartum depression in the mother.[8]

Lower levels of maternal LC-PUFA have short as well as long term consequences on the fetus. Cross talk between the mother and fetus during pregnancy decides the level of DHA in the fetus which is responsible for maturation of the gut microbe in the developing fetus. Numerous studies from the Western World have linked the deficiency of EPA and DHA levels in pregnancy to various diseases in infants, early and late childhood, adulthood and later in life *viz.* allergy, asthma, psychomotor, visual, cognitive development, metabolic disorders causing hypertension and cardiovascular diseases.[9-15]

Omega -3 index (O3I) which is a sum of EPA + DHA content in the red blood cell membranes is a biomarker of n-3 fatty acids status and is expressed as mgm/100 mg of total fatty acids identified.[16] Defined optimal levels of O3I published and accepted globally is >8% in general population, which is supposed to be disease protective.[17]

Maternal DHA concentration in pregnancy is reduced by 50% due to decreased intake as well as increased blood volume and enhanced placental and fetal requirements. During the 1st trimester the fetus is consuming 50 to 70 mgm a day of omega-3 fatty acid DHA. Babies accrue DHA in their central nervous system until about 18 months of age.[18]

Across the globe pregnancy specific levels of DHA is not yet defined, though supplementation of 200 mgm-400 mgm of DHA is recommended by US food and drug administration guidelines and Norwegian directorate of health during pregnancy and lactation to improve the fetal outcome with respect to various illnesses from infancy to adulthood.[12]

Due to lack of data on the deficiency in Indian women, obstetricians differ in their approach towards assessing and supplementing EPA and DHA in pregnancy.

With this in background in mind we undertook this study with following aims and objectives: to estimate the prevalence of EPA and DHA deficiency in pregnant women by omega-3 index (O3I), and also to correlate blood levels of EPA and DHA with various socio demographic parameters.

METHODS

Study type

It was a prospective observational study.

Study place

The study was conducted at the antenatal OPD of Sir Gangaram Hospital, New Delhi, from June 2020 to December 2021.

This study was conducted on all pregnant women between 16-26 weeks of gestation, attending the antennal OPD of Sir Gangaram hospital who consented to the study and were not taking EPA and DHA prior to enrolment in the study. Ethical clearance was obtained from the board before conducting the study.

Methodology

Dried blood sample was collected using an Omega health test kit.

Sampling

After taking due consent and explaining the procedure to the patient in the language that she understood patient was asked to wash her hands thoroughly with soap and water and be seated comfortably in a chair. She was instructed to hold her arm down for 20 seconds to increase the blood flow to the fingers. The tip of the finger was wiped with alcohol swab provided and a single use lancet was used to collect the blood sample as a blood drop. The centre of the filter paper on dry blood spot kit was touched to the blood drop so that it spreads by capillary action. It is important that 3 drops of blood each is collected on both the circles on filter paper. The dried blood spot (DBS) card was placed on a flat surface and blood spot was allowed to dry for 30 minutes. The DBS card was put in a pre -addressed aluminium pouch and sent to the biochemistry lab within 3 days (Figure 1).

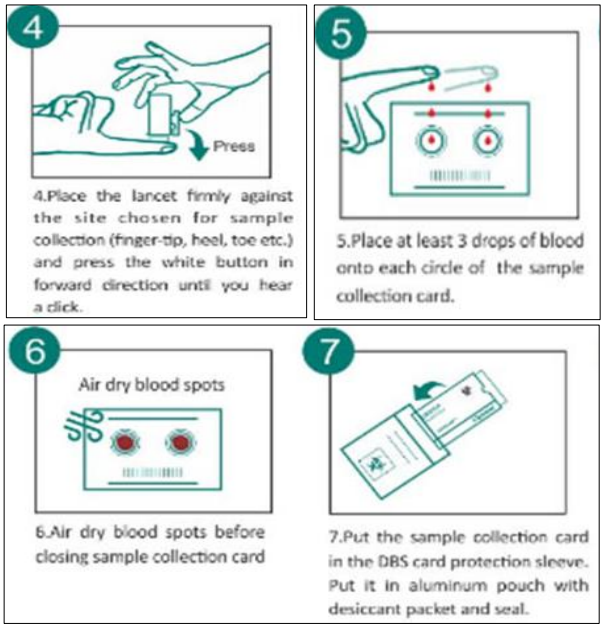

Figure 1: Sample collection on DBS card.

Extraction

In the laboratory testing for EPA +DHA levels, 3 discs of size 4.5 mm of the dried blood spot sample was punched out and placed in a 2 ml glass vial with boron-trifluoride (BF3) and methanolic 14% was added. N-hexane was added to the vial and capped well. The sample was

vortexed for 30 minutes and then incubated in dry bath block at 100 ^{0}C for 10 minutes. The vial was removed and allowed to cool for 5 minutes.

After adding HPLC grade water to the vial, it was recapped and vortexed for 30 seconds. Then the sample was centrifuged for 3 minutes at 3000 rpm at room temperature. The top layer (hexane) was placed in another glass vial and finally the sample was analysed in gas chromatography (GC-FID 2010 Plus) detector (Figure 2).

Figure 2: Gas chromatography (GC-FID 2010 Plus) detector.

Fatty acid methyl ester (FAME) peaks are identified by comparing their retention times with those of known standards (38 FAME MIX). Desired FAME peaks are qualified by calculating the area % under the peak using GC solution software. The concentration of individual fatty acid was expressed as percentage of total area under the peaks.

Test reports were sent to the patient as well as the treating obstetricians by mail as follows (Figure 3). EPA DHA levels were correlated with age, parity, BMI, and socio economic status.

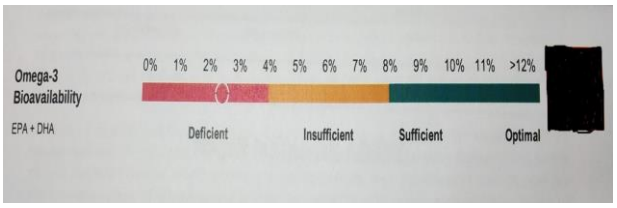

Figure 3: Test card.

Sample size

The sample size was calculated, to estimate the prevalence of deficiency of EPA+DHA among women of child bearing age, which has been reported as 70%.[19] The minimum required sample size with a margin of error of 5% and a confidence level of 95% was found to be 323.

Since this study was time bound we planned to study 150 subjects. We recruited 154 subjects and excluded 4 as they were consuming DHA (Figure 4).

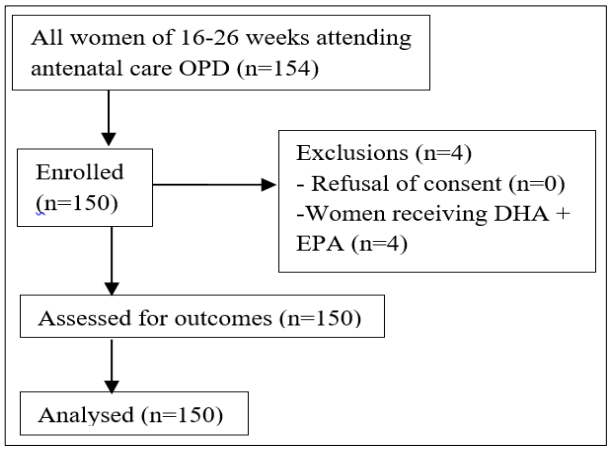

Figure 4: Flow chart.

Following formula was used for calculating the sample size, where Z(1-α/2) =1.96 for 95% confidence interval, p=0.70 (expected proportion), and d=0.05 (margin of error/precision).

$$n \geq Z_2 1 - \alpha/2 \times p(1-p)/d^2$$

Statistical methodology

Prevalence was estimated in percentage/proportion with 95% confidence interval. Other demographic details were expressed as mean±SD or median and IQR for numeric data. Other nominal/categorical data was expressed as percentage/proportion/frequency. Excel and statistical package for the social sciences (SPSS) were used for data recording and analysis.

RESULTS

Total 150 women were assessed.

One hundred and forty-eight (98.67%) of women had inadequate O3I (deficient-28%, insufficient-70.67%) and only 2 women (1.33%) had sufficient levels of O3I (Table 1).

O3I did not vary across the various age groups (p=0.36) (Table 2).

No significant difference in O3I was seen as per BMI of the subjects (p=0.36) (Table 3).

O3I did not differ across various socio-economic strata (p=0.12) (Table 4).

The mean and median value of O3I in women with vegetarian diet was 4.31% and 4.82% respectively, while the mean and median value in women with non-vegetarian diet was 4.7% and 4.7% respectively, which was significantly more than women with vegetarian diet (p<0.001, p=0.004) (Table 5).

Table 1: Incidence of RBC omega 3 index.

Total number (n=150)	Deficient (0-≤4%)	Insufficient (>4-≤8%)	Sufficient (>8-≤12%)	Optimal (>12%)
n (%)	42 (28%)	106 (70.67%)	2 (1.33%)	0 (0%)
Total	Total % of subjects with inadequate levels=148 (98.67%)		Total % of subjects with adequate levels=2 (1.33%)	

Data expressed as n (%)

Table 2: Distribution of patients according to the age.

Age (years)	n	Total % of subjects with inadequate levels	Deficient	Insufficient	Sufficient
18 to ≤25	23	23 (100)	3 (13.04)	20 (86.96)	0
>25 to ≤35	118	116 (98.31)	35 (29.66)	81 (68.64)	2 (1.69)
>35 to ≤45	9	9 (100)	3 (33.33)	6 (66.67)	0

Data expressed as n (%)

Table 3: Distribution of patients according to the BMI.

BMI (kg/m²)	n	Total % of subjects with inadequate levels	Deficient	Insufficient	Sufficient
<18.5 (underweight)	15	15 (100)	5 (33.33)	10 (66.67)	0
≥18.5 to ≤24.9 (normal weight)	56	54 (96.42)	15 (26.78)	39 (69.64)	2 (3.57)
≥25 to ≤29.9 (over weight)	55	55 (100)	13 (23.63)	42 (76.36)	0
≥30 to ≤40 (obese)	24	24 (100)	9 (37.5)	15 (62.5)	0

Data expressed as n (%)

Table 4: Distribution of patients according to the socio economic class (modified Kuppuswamy classification-2020).

Socio economic class (total no=150)	n	Total % of subjects with inadequate levels	Deficient	Insufficient	Sufficient
Upper (class I)	27	25 (92.59)	13 (48.14)	12 (44.44)	2 (7.40)
Upper middle (class II)	52	52 (100)	11 (21.15)	41(78.85)	0
Lower middle (class III)	45	45 (100)	13 (28.89)	32 (71.11)	0
Upper lower (class IV)	21	21 (100)	3 (14.28)	18 (85.71)	0
Lower (class V)	5	5 (100)	2 (40)	3 (60)	0

Data expressed as n (%)

Table 5: Diet pattern and O3I.

Parameters	Vegetarian (%)	Non-vegetarian (%)	P value
Mean O3I %	4.31%±0.89	4.82%±1.05	<0.001
Median O3I %	4.4 (3.8-4.9)	4.7 (4.1-53.3)	0.004
Inadequate O3I	71/71 (100)	77/79 (96.4)	0.029
Deficient	27 (38.03)	15 (18.99)	-
Insufficient	44 (61.97)	62 (78.48)	-
Sufficient	0 (0)	2 (2.53)	-

Data expressed as n (%)

In our entire cohort of 150 women, only 2 women had sufficient O3I and both consumed non-vegetarian diet. In brief, result of this pilot study has indicated that 98% of pregnant women had EPA and DHA deficiency as indicated by O3I levels. This was regardless of age, parity, BMI, and socioeconomic status. Women consuming non-vegetarian diets had significantly higher O3I as compared to women consuming vegetarian diet.

DISCUSSION

DHA and EPA, both long-chain polyunsaturated fatty acids (LC-PUFA), play various essential roles in human health. Due to continuous fetal DHA accretion, the levels of maternal DHA during pre-conception, pregnancy, and lactation is important.

Inadequate dietary consumption of DHA and eicosapentaenoic acid impairs the optimal growth of the feto-placental unit and is a cause of risks of cognitive decline, inflammatory disease, cardiovascular disease, behavioural changes, and mental stress in later life.[2-4] Modern refined diets are mostly deficient in n-3 PUFAs leading to decreased organ function that may predispose individuals to an increased risk of diseases. Western diets are also deficient in omega-3 fatty acids, and have excessive amounts of omega-6 fatty acids. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio, as is found in today's Western diets promotes the pathogenesis of many diseases including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a low omega-6/omega-3 ratio) has beneficial health effects.

Across the globe pregnancy specific levels of DHA is not yet defined, though Supplementation of 200 mgm-400mgm of DHA is recommended by the US food and drug administration and the Norwegian directorate of health during pregnancy and lactation.[12] Recommendation made by the International Society for the Study of Fatty Acids and Lipids for North Americans is (300 mg/day).[12] World Health Organization (WHO) recommends taking 200–500 mg of EPA+DHA during pregnancy.[20]

Studies conducted around the world to assess the association between intakes of DHA during pregnancy or lactation and neurodevelopment outcomes in childhood have been inconsistent. There is a paucity of data on the potential benefits of maternal DHA supplementation in infants, especially in the Indian population. Study named DHANI (maternal DHA supplementation and offspring neurodevelopment in India) was conducted in India, and concluded that supplementing 400 mg/d DHA (vs. placebo) to mothers in pregnancy and lactation did not prove beneficial in the neurodevelopment of the offspring at 12 months of age.[21]

Deeper insights into maternal dietary patterns, young child feeding practices, home environment, and the interactions amongst these factors are warranted to understand what shapes early neurodevelopment.

A review article by Basak from India highlights the importance of maternal intake of DHA for the infant's brain development structurally and functionally. It further suggests that insufficient intake of n-3 PUFA may lead to DHA deficiency states that could affect the offspring's metabolic phenotypes by altering placental structure and function, fetal adiposity, body fat distribution, energy utilization, musculoskeletal growth, signaling between brain-adipose, epigenome stability, and inflammation.[22]

Federation of Obstetric Gynaecological Society of India's (FOGSI) TOG on nutrition supplementation in pregnancy also acknowledges role of DHA in infant's physical and neurodevelopment but presently neither it is recommending the measurement of EPA and DHA levels nor its routine supplementation during pregnancy.[23]

Globally, four discrete colours have been assigned according to the level of EPA and DHA in the red blood cells (Figure 3). Majority of the countries fall into the inadequate zone depicted as red, orange and grey. A very thin population falls into yellow and green zone of adequate levels. Whole of India falls in the red zone. American data reflects that 70% of the women in the reproductive age group have deficient levels with less than 5% of RBC DHA.[17] Data from the west also suggests that non-pregnant population not taking n-3 fatty acid supplements have an O3I ranging from 4 to 5% only.[15]

Out of total number of 150 study subjects, 148 (98.67%) had inadequate levels of EPA and DHA as measured by omega 3 index (Table 1). This is in accordance with the global data.[17,19]

There was no correlation of O3I levels with maternal age, BMI and socio-economic status (Tables 2-4). To the best of our knowledge, no study has specifically addressed the issue of deficiency of EPA and DHA levels with maternal age. Study by Young et al indicates obese women to have significantly lower levels of O3I as compared to normal weight women.[24] Like our study Christian have also reported that O3I levels are similar amongst all socio-economic classes.[16]

The mean and median value of O3I in women consuming vegetarian diet was 4.31% and 4.82% respectively, while the mean and median value in women with non-vegetarian diet was 4.7% and 4.7% respectively, which was significantly more than women with vegetarian diet ($p<0.001$, $p=0.004$). In our entire cohort of 150 women, only 2 women had sufficient O3I and both consumed non-vegetarian diet (Table 5). This is in accordance with a study by Saunders where vegetarian diets have significantly lower amounts of EPA and DHA (measured by O3I) as compared to the non-vegetarian diet.[25]

Limitations

Major limitation of our study was small sample size. Since it was a time bound study we did not follow up the women for obstetric outcomes. New born follow up was not done.

CONCLUSION

DHA, EPA and alpha lineoloic acid (α-LA) are essential fatty acids of the long chain polyunsaturated fatty acid

(LC-PUFA) or omega- 3 fatty acid. These fatty acids are not synthesized by our body and hence have to be included in our diet. Diets rich in LC-PUFA reduce the incidence of deep placentation disorders such as - preeclampsia, intrauterine growth restriction, and preterm delivery, in utero fetal death and placental abruption.

Result of this pilot study has indicated that 98% of pregnant women had EPA and DHA deficiency as indicated by O3I levels. This was regardless of age, parity, BMI and socio-economic status. Women consuming vegetarian diet had statistically significant lower mean and median levels of O3I as compared to those having non-vegetarian diet.

Larger data especially from India will be required for establishing and documenting the role of deficient levels of O3I on the maternal health and fetal outcomes. This will help us form guidelines for supplementation of EPA and DHA in Indian pregnant women.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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