Effect of vaginally administered DHA fatty acids on pregnancy outcome in high risk pregnancies for preterm delivery: a double blinded randomised controlled trial

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Summary

Objectives: to verify whether vaginally intake of docosahexaenoic acid (DHA), an n-3 long chain polyunsaturated fatty acid, would improve length of gestation and newborn birth weight in high risk pregnancies for preterm delivery.
Methods: this study was a randomized, double-blind, controlled, clinical trial, including women at high risk for preterm delivery. Of 74 eligible women, 31 refused to participate and 34 were enrolled and randomized with equal chance of selection, 22 were assigned to treatment group and 21 were assigned to the control group, and received placebo. One gram of DHA was administered vaginally once a day starting from 21 (1 week of gestation until 37 weeks + 0 day). The primary endpoint was to determine the length of the pregnancy and secondary endpoint the newborn weight.

Results: gestational age at delivery was 38.6 (SD, 1.05) weeks in the docosahexaenoic acid group and 37.6 (SD, 0.84) weeks in the placebo group (P =0.007). For women who completed the treatment and delivered at term there was a statistically difference of the weights in the two groups [3082.1 (SD, 293) gr cases *vs* 2699.3 (SD, 150) gr controls P <0.0001].
Conclusion: in high risk patients for preterm delivery, the vaginal administration of a DHA increases length of gestation and newborn birth weight.

Key words: vaginal DHA, omega-3, preterm delivery, high risk pregnancy, docosahexaenoic acid.

Introduction

Preterm delivery is the leading cause of perinatal mortality and morbidity in industrialized countries, accounting for 28% of neonatal mortality worldwide (1). Mortality and morbidity are inversely related both to gestational age at delivery, and the most severe consequences occur when delivery is preterm (2). The risk of spontaneous preterm delivery is inversely correlated with cervical length: the shorter the cervix the higher the risk of preterm delivery (3, 4). Another important predictor of spontaneous preterm delivery is obstetric history, including a previous spontaneous preterm delivery.
Once the threat of preterm labor is established clinicians may attempt to stop or attenuate uterine contractions using tocolytic drugs. However there is no evidence that tocolytics can prolong pregnancy for sufficient time to improve perinatal outcome (5), rather therapy is used to achieve short-term gains such as administering corticosteroids to improve fetal lung maturity or enabling the transfer of the mother to a tertiary referral centre. Moreover, several tocolytic agents may give rise to adverse effects in the women or the fetus.
Successful strategies to prevent significant numbers of preterm births are most likely to be effective when they are scientifically based, when they address common conditions, and when they are shown to be effective in well-controlled trials. Evidence presented by several research groups suggests that essential fatty acids and their metabolites of both of the linoleic acid (n-6) series and the linolenic acid series (n-3) play important and modifiable roles in prolonging gestation (6-8), delaying the time of spontaneous delivery (6) and reducing the recurrence of preterm delivery (7) in both human and animal studies and improving the neonatal outcome.
Linoleic acid (LA) and linolenic acid (LnA) are required in the diet. Dietary LA serves as the precursor for the n-6 series of polyunsaturated fatty acids (PUFAs) and dietary LnA is the precursor for the n-3 PUFA series.
An n-6:n-3 ratio of approximately 1:1 is considered normal, whereas the current diet ranges from 10:1 to 25:1 (9), fueling concern that today's diet may be insufficient to meet n-3 requirements, particularly for docosahexaenoic acid (DHA, an elongation and desaturation metabolite of LnA). In fact omega-3 fatty acids are essential and can only be obtained from the diet.

Pregnancy is associated with maternal difficulty in coping with high demands for DHA (10, 11) since both n-3 and n-6 fatty acids required for the fetus are by preferential placental transfer and therefore fetus storage depends on maternal intake of these fatty acids (11-14).

The requirements during pregnancy have not been established despite it is demonstrated that omega-3 fatty acids are critical for fetal neurodevelopment and may be important for the timing of gestation, birth weight and inflammation as well. For pregnant women to obtain adequate amount of omega-3 fatty acids, a variety of dietary sources should be consumed.

Even though all of the studies in the body of literature show the effectiveness of omega-3 in high risk pregnancies in preventing or reducing the recurrent risk of preterm delivery, pre-eclampsia and fetal growth improvement (15, 16), there is not a general agreement on the amount of DHA required to achieve these effects (17-19). All studies show in fact a wide range of daily dosage for oral administration (7).

Until today the supplementation in pregnancy is performed only with sources consumed *per os*. Moreover, trials for prevention of premature delivery or low weight baby uses DHA administered orally.

The metabolism and absorption of lipids in the gastrointestinal tract is related to a complex pathway involving liver, pancreatic and gastric enzymes. Hence, we speculated that this mechanism could lead to a loss of concentration in the blood that could causes in turn a low effective concentration.

The purpose of our study is to assess the efficacy of DHA bypassing the gastrointestinal metabolism and reaching directly the cervix.

Methods

Study design and participants

We did a prospective randomised double-blind, controlled, clinical trial, between September 2009 and January 2011, at the Artemisia Medical Centre, in Rome, to determine the effects of vaginally administered DHA on high risk pregnancy outcome.

We enrolled all pregnant women with a viable fetus at high risk of preterm delivery, including women with a history of a previous IUGR, fetal demise or pre-eclampsia.

The women were selected according to the inclusion criteria based on anamnestic data (one or more previous preterm delivery) and/or ultrasonographic findings of cervical incompetence, according to Colombo et al. (Colombo DF, Iams JD. Cervical length and preterm labor. Clin Obstet Gynecol. 2000 Dec; 43(4):735-45).

The sonographic criteria used for cervical incompetence were at least one of 1) cervical length less than 25 mm, 2) the presence of a funnel that accounts for 50% of cervical length, 3) cervical width of 20 mm or more.

We excluded patients who had one or more of the following: 1) a non-viable fetus (before or after randomisation), 2) a history of placental abruption, 3) bleeding episode in the present pregnancy, 4) use (or used) of prostaglandin inhibitors, 5) multiple pregnancy, 6) allergy to fish, 7) regular intake of fish oil, 7) a positive cervical swab for chlamydia, mycoplasma/ureaplasma and bacterial vaginosis infections, 8) major fetal abnormalities. Gestational age was calculated based on the ultrasound scan performed in the first trimester of pregnancy and if not available between 16-18 weeks of gestation.

We obtained written informed consent from each patient. The study was approved by the Local Ethics Committee of the "Artemisia Medical Institute Network" [created according to the guidelines reported in the "Decreto Ministeriale (DM) 15/7/1997", Ministry of Health of Italy] on the 11th April 2009.

Procedures

Eligible women were randomly allocated to either the treatment or control group as soon as they consented to participate.

To randomise participants, we used a customised randomisation programme that generated a random number for each participant, with equal ratio of selection.

Eligible women who were randomly allocated to the treatment group were given vaginally 1 gr of DHA daily from 21 (1 week of gestation until 37 weeks + 0 day. Controls were given a placebo in the same modality).

The modality of application of the ovule was explained by a physician dedicated to the enrollment: the ovule had to be inserted deep in the vagina up to the posterior fornix, before sleeping.

Both the physicians and the women were blinded to the treatment.

Statistical analysis

In order to achieve a difference between the two groups, we will need to recruit and randomize 21 patients per arm (power = 90%, alpha = 0.05 two-sided).

Parametric continuous variables were summarized as means (±SD), non-parametric continuous variables as medians [interquartile range (IQR)] and categorical data as percentages. Categorical variables were analyzed using chi-square or Fisher's exact test and continuous variables were compared using unpaired *t*-test and Mann-Whitney *U*-tests.

No interim analysis was programmed in order to avoid any bias deriving from the early stop of the trial. All study personnel and participants were blinded to treatment assignment for the duration of the study. Only the study statisticians and the data monitoring committee saw unblinded data, but none had any contact with study participants.

Follow-up

All patients were asked to report any complications to the centre. In case of worsening of clinical conditions, the patient left the study starting the standard recommended tocolysis in a tertiary referral centre to delay the latent phase of labour.

Women were followed until delivery. The time of delivery, the weight of the newborn and any other complications occurred during pregnancy were recorded into a database in a SPSS format, as well as the baseline characteristics of both groups. Every woman has been checked a week after the expected day of delivery from a physician not involved in the study.

Pregnancies that worsened and needed hospitalization were excluded from the treatment protocol but were still followed until delivery.

No major neither minor birth defects were observed in both groups.

Role of the funding source

The study sponsor, the Artemisia Foundation in Fetal-Maternal Medical Research had only a role in support the project and study design, no role had in data collection, data analysis, data interpretation, or writing of the report. The corresponding author had final responsibility for the decision to submit for publication.

Results

A total of 74 patients had the eligibility criteria. After exclusion of 31 women (5 not meeting all inclusion criteria, 25 declined
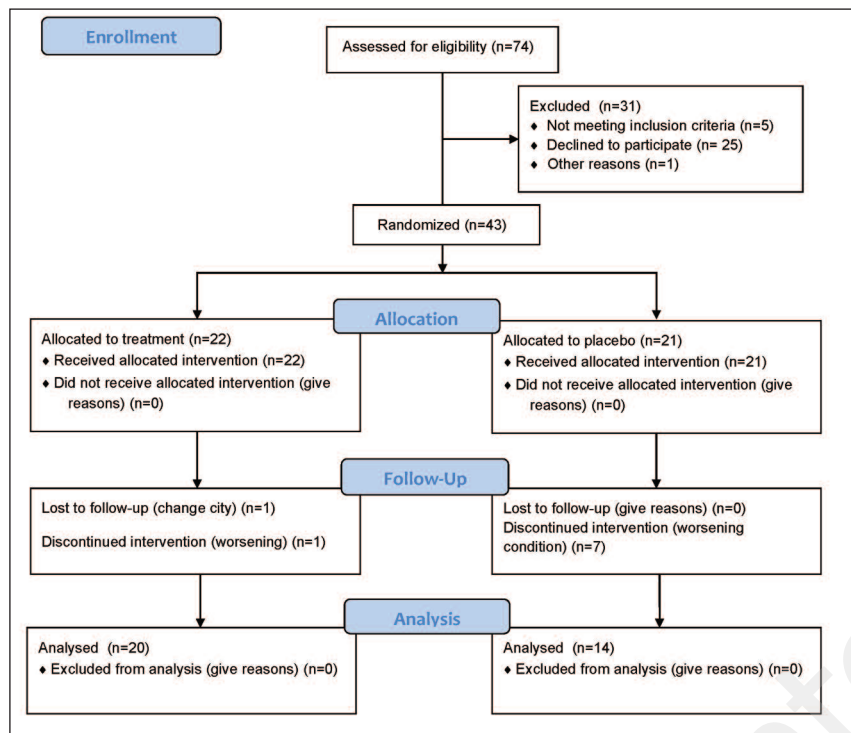
Figure 1 - Consort diagram.

Table 1. Baseline characteristics of the enrolled patients.

	Cases (N=21)	Controls (N=21)	P
Maternalage (years)	32.6+/-4.6	32.2+/-4.8	0.81
BMI (Kg/m²)	23.0+/-2.9	23.2+/-3.4	0.84
Smoking	50%	47.6%	0.87
Cesarean Section	57.1%	47.6%	0.54
Previouspretermdelivery	59.1%	52.4%	0.66
Shortening of cervix	73.6%	76.2%	0.37

Table 2. Gestational age at delivery of the enrolled patients.

	Mean +/- SD	Median	IQ range	Range
Cases (N=21)	38.4 +/- 1.3	38	38-39	35-40
Controls (N=21)	36.5 +/- 1.8	37	35-38	33-40

T Student p <0.0001; Mann-Whitney P <0.0001

Table 3. Gestational age at delivery after excluding patients with worsening of clinical conditions.

	Mean +/- SD	Median	IQ range	Range
Cases (N=20)	38.6 +/- 1.05	38.5	38-39	36-40
Controls (N=14)	37.6 +/- 0.84	37.5	37-38	37-40

T Student p <0.05; Mann-Whitney P <0.01

to participate, 1 other reason), 43 pregnancies were enrolled and randomized as per-protocol. Of these, 22 women were allocated to treatment group and 21 to placebo group.

In the treatment group 1 patient lost to follow-up and 1 patient discontinued intervention for worsening of the clinical conditions. In the placebo group no patient was lost to follow up and 7 patients discontinued intervention for worsening of the clinical conditions.

Baseline characteristics of the enrolled patients are compared in Table 1. The treatment group did not differ significantly from the placebo group in any of the baseline characteristics (Fig. 1).

All the patients still continued to be followed-up including women whose treatment was interrupted for worsening of the clinical conditions.

We firstly conducted a crude analysis in the two groups (N=21 group of treatment, N=21 group of placebo).

Then, after excluding patients that interrupted the treatment for worsening of clinical conditions (1 in the treatment group and 7 in the control group), a per protocol analysis was carried out.

The mean gestational age at delivery between the treatment and placebo groups [respectively 38.4 (SD, 1.3) weeks vs 36.5 (SD, 1.8) weeks, P<0.0001] was statistically different (Tab. 2). This difference persists after excluding for all patients interrupting therapy for worsening conditions [38.6 (SD, 1.05) weeks vs 37.6 (SD, 0.84) weeks, P=0.007] (Tab. 3) .

In order to evaluate the differences on birth weight in both groups, we considered only women who completed the treatment and delivered at term (over 37 weeks): we found a significant statistically difference of the weights in the two groups [3082.1 (SD, 293) gr cases *vs* 2699.3 (SD, 150) gr controls P <0.0001].

Discussion

In this study we demonstrate that vaginally administered DHA during pregnancy, significantly improves both the length of pregnancy and birth weight in high risk pregnancy when compared with controls.

Several randomized controlled trials indicate that omega 3 supplementation can influence the process of parturition, delaying onset of labor, reducing recurrence risk of preterm de-

livery and in animal studies they seem to have a tocolytic effect (Baguma-Nibasheka M, Brenna JT, Nathanielsz PW. Delay of preterm delivery in sheep by omega-3 long-chain polyunsaturates. Biol Reprod 1999 Mar; 60(3):698-701). The exact mechanisms are not well understood but it seems involved the eicosanoid-mediated changes in myometrial contractions and connective tissue remodeling, changes in eicosanoid receptors, or membrane effects of these fatty acids such as alterations in signal transduction pathways or modulation of ion-mediated contractions. In particular, linoleic acid (n-6) series are the parent fatty acids of the prostaglandins, PGF2alpha and PGE2, which are essential to the delivery process. The n-3 fatty acids may reduce the activity of eicosanoid promoters of the parturition process, particularly prostaglandins F and E, and increase the activity of eicosanoids with myometrial-relaxant properties, particularly prostacyclins (15, 20).

We believed that these evident results could be related by a direct effect of prostacylcine deriving from PUFA on the muscle cells and blood vessels of the uterus. Probably, the advantage of a vaginal administration of DHA lies not only in the fact that it is avoided the first-pass effect and therefore a better standardization of the dosage, but there is also a direct local cervical action.

A secondary mechanism could be related to the fact that, an increased intake of omega 3 in proportion to other fatty acids lead to the down-regulation of the synthesis of omega 6 and consequently to a reduced production of PGE2 and PGF2a, with cervical modifications.

It would also increase the production of prostacyclin PGI2 and PGI3, which play a role in relaxing the myometrium. Another possible effect on the duration of pregnancy could be linked to the disorganization of the electrical myometrial much like antiarrhythmic activity exerted by omega 3 derivatives on the heart, which could result in a delay in the onset of rhythmic contractions and regular level myometrial (21) presumably through Ca2+ channels, having similar roles in myometrial contraction, influencing gestational length (22).

This is the first trial evaluating the effect of prophylactic vaginally DHA treatment in preterm delivery in high risk patients, even if it is not aimed at the research of pathogenetic mechanisms underlying the improved outcome after the omega 3 intake.

At first glance, the considerable difference between the number of patients that presented a so evident worsening of the clinical condition that lead to leave the treatment before delivery, clearly suggests an evident protective effect of the DHA therapy.

Conclusion

In conclusion, these results clearly demonstrated that treatment with vaginal DHA determines a significant reduction on the rate of preterm delivery in pregnant women at high risk for preterm delivery and ameliorate the intrauterine fetal growth.

Acknowledgment

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