European Review for Medical and Pharmacological Sciences 2022; 26: 8879-8886

Oral supplementation of α-lipoic acid (ALA), magnesium, vitamin B6 and vitamin D stabilizes cervical changes in women presenting risk factors for preterm birth

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Abstract. – OBJECTIVE: Recently, the PTB risk has been related to the objective measurement of cervical length (CL), since a CL of less than 25 mm is an accurate predictor of increased risk of PTB. Primary prevention of preterm labor is based on the early identification of symptoms and on pharmacological treatments with tocolytic drugs for inhibition of uterine contractions that are associated with a shortening of the cervix. Unfortunately, most of these drugs have important side effects.

PATIENTS AND METHODS: This study aimed to evaluate whether the administration of a combination of oral α-lipoic acid (ALA), magnesium, vitamin B6 and vitamin D to pregnant women presenting risk factors for PTB could reduce the rate of cervical shortening at 19-22 weeks of gestational age.

RESULTS: A total of 122 women attending the first-trimester aneuploidy screening at 11-14 weeks of pregnancy and presenting risk factors for PTB were included in the study. Cervical length significantly decreased in the control group compared with the treatment group (-3.86 ± 1.97 *vs.* 1.50 ± 1.26; *p*=0.02). Although the rate of preterm birth did not significantly decrease (9.5% *vs.* 5.1%), admission for threatened PTB was statistically reduced in the treatment group compared with the control group (3.4% *vs.* 14.3%).

CONCLUSIONS: Oral supplementation of ALA, magnesium, vitamin B6 and vitamin D significantly counteracted cervix shortening in pregnant women presenting risk factors for PTB.

Key Words:
Oral α-lipoic acid, Preterm birth (PTB), Cervical length, Threatened PTB.

Introduction

The World Health Organization (WHO) defines preterm birth (PTB) as delivery before 37 completed weeks of gestation, and as the major global obstetric health problem that affects 5-18% of pregnancies worldwide. PTB is the leading cause of perinatal mortality in children under 5 years of age[1,2] and represents a large economic burden due to the initial neonatal intensive care unit costs and the longer-term use of health and social care services[3]. Preterm birth incidence is higher in underdeveloped countries (11.8%) compared with developed countries (9.3%)[4]. It is medically induced in 20% of maternal or fetal indications, such as preeclampsia, gestational diabetes, or intrauterine growth restriction. If not medically induced, approximately 25% of cases are caused by preterm rupture of membranes (pPROM); 30% of cases are caused by inflammation and infection, while 45% of cases are considered spontaneous with intact membranes[5]. Recently, cervical length (CL) shortening[6] has become a clinical marker of PTB risk[7,8]. Although still controversial, the National Institute of Child Health and Human Development Maternal-Fetal Medicine Unit Network uses a cutoff of 25 mm to define a short cervix at 22 to 24 weeks of gestation in both low- and high-risk pregnancies[9]. Nevertheless, different studies state that maternal characteristics - age, gestational age, and race - differently influence CL throughout gestation, so a reference range might be more useful instead of a single cut-off value[10]. Transvaginal ultrasonography (TVU) represents the gold standard to measure CL in pregnancy[11,12]. It is safe since it does not increase the risk of ascending infections in patients with preterm premature rupture of membranes (pPROM)[13,14] and has more advantages compared with digital examination methods, in which several factors (e.g., maternal obesity, cervix position, and shadowing from the fetal presenting part) can

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affect the correct prediction of PTB[12,15,16]. A second-trimester universal transvaginal ultrasound screening program for CL has been introduced to reduce the incidence of PTB and admission for threatened PTB in women without a history of spontaneous PTB[15,17]. Due to the different PTB causes, strategies to prevent PTB are complicated, and novel diagnostic and therapeutic approaches are necessary. The objective of this pilot study was to evaluate whether α-lipoic acid (ALA), in association with magnesium, vitamin B6 and vitamin D, could reduce the rate of the short cervix at 19-22 weeks of gestational age and consequently reduce the occurrence of preterm delivery in pregnant women presenting risk factor for PTB[18-20].

Patients and Methods

This is a monocentric open label randomized controlled pilot study. Pregnant women attending the first-trimester aneuploidy screening at 11-14 weeks of pregnancy were recruited among the National Health System (NHS) clinics and enrolled at the Obstetrics and Gynecology Unit of the Policlinic Hospital of Modena, Italy. Women with similar baseline characteristics - age between 18 and 41 years – and presenting at least one of the risk factors for PTB - body mass index (BMI) < 18 kg/m^2 or ≥ 30 kg/m^2, nulliparity, previous history of preterm birth, uterine myoma, hypertensive disorders either chronic or induced by the pregnancy - were eligible for the study. Subjects were excluded if they presented at least one of the following characteristics: twin pregnancies, preterm premature rupture of membranes (pPROM), cervical dilation ≥ 1 cm at the first trimester, or if under progesterone therapy. The principal investigator and sub-investigators obtained written informed consent from all patients at the beginning of the study before assigning eligible women either to the treatment group (a combination of ALA 600 mg, magnesium 450 mg, vitamin B 6 2.6 mg, and vitamin D 50 mcg - DAV®, Lo.Li. Pharma s.r.l, Rome, Italy, 2 tablets daily until delivery), or to the control group (women were followed with standard practice until delivery without any additional supplementation). The randomization was obtained using a computer-generated allocation in a 1:1 ratio. Permuted block randomization was performed. The adherence of participants to the protocol was assessed through telephone calls every two weeks. After baseline measurements at 11-14 weeks, enrolled women underwent ultrasound CL measurement and a general health status check around 19-22 weeks of gestation to evaluate the changes in cervical length between the first and the second trimester. In general, as standard guidelines report, avoiding excessive fatigue was equally advised to both groups. In case of complications for other maternal and fetal reasons, preeclampsia, intrauterine growth restriction (IUGR) or fetal malformations, subjects were excluded from the study. No other complications during the gestation were observed and recorded.

Statistical Analysis

Stata 16.1 (StataCorp. 2016 College Station, TX, USA) was used to analyze data. Statistical tests were used to compare demographic data and maternal-fetal outcomes in the two study groups: the treatment group or the control group (standard practice). Comparisons between the two groups were made using the *t*-student test for continuous variables and the chi-square test for categorical ones. The percentage of CL variation (% CL variation) was calculated as: [(2nd CL-1st CL) *100]/ 1st CL. Continuous data are reported as mean ± standard deviation (SD). Categorical data are reported as the absolute and percentage frequencies. All probability values were 2-tailed, and a *p*-value of < 0.05 was considered statistically significant. Based on a recent study that investigated the efficacy of vaginal alpha-lipoic acid in preventing cervical shortening (Grandi et al[22]), we calculated that 54 patients for each group are necessary to detect a statistical significance of 15% difference in the mean of the cervical length between groups (α-error = 0.05 and power 80%).

Results

A total of 122 pregnant women, between 11 - 14 weeks of gestation, aged 18-41 years, presenting at least one risk factor for PTB - nulliparity, previous PTB, uterine myomas, hypertensive disorders either chronic or induced by the pregnancy - were enrolled in the study. At the beginning of the study, two women from the treatment group resulted non-compliant and were, therefore, included in the control group. A total of 59 women were allocated to the treatment group (DAV®) and 63 to the control group in a 1:1 ratio, as shown in Figure 1. The baseline characteristics of women included in the study and the risk factors for preterm birth were comparable between groups without any statistically significant difference (as shown in Tables I and II, respectively).

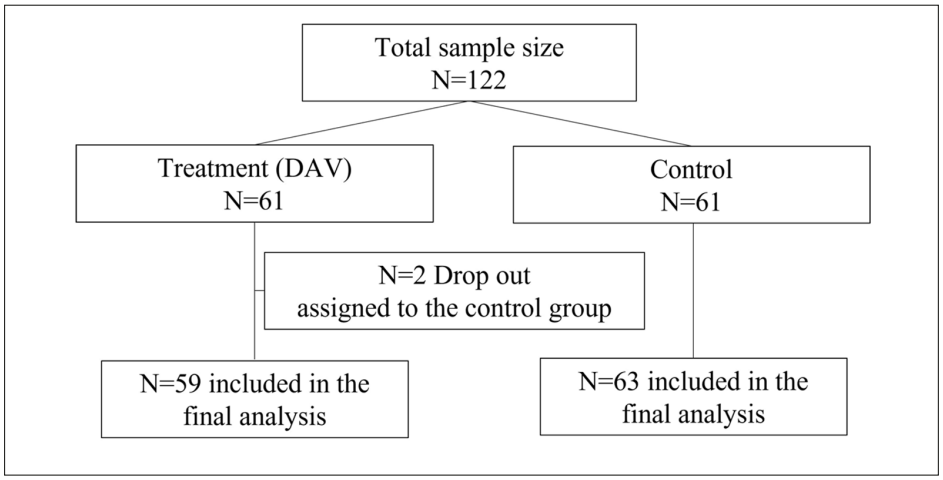

Figure 1. The flow chart of the study.

Changes in Cervical Length Between First and Second Trimester

The % of women presenting a CL ≤ 3.0 cm at the 2nd trimester was similar between groups, treatment: 6 (10.2%) *vs.* control: 6 (9.5%, p = 0.90). Overall, the CL remained unchanged between the 1st and 2nd trimester in the treatment group, while it significantly decreased in the control group (p = 0.02). Moreover, the control group experienced a significant cervical reduction %, compared to the treatment group (p = 0.02) as shown in Table III.

Table I. Baseline features of subjects randomized for the two different groups. Continuous variables are reported as mean ± standard deviation. Dichotomous variables are reported as numbers and ratios (%).

	Treatment group (N=59)	Control group (N=63)	*p*-value
Mean Maternal age (mean)	32.67 ± 4.16	33.7 ± 4.77	0.21
Maternal education level			
Low (≤ 8 years)	11 (18.6%)	19 (30.1%)	0.25
Medium (9 – 13 years)	18 (30.5%)	20 (31.7%)	
High (> 9 years)	30 (50.8%)	24 (38.1%)	
Ethnicity			
Caucasian	50 (84.7%)	53 (84.1%)	
Sub-Saharian	2 (3.4%)	2 (3.2%)	
Arabic	3 (5.1%)	3 (4.7%)	0.19
Asiatic	2 (3.4%)	3 (4.7%)	
Hispanic	1 (1.7%)	2 (3.2%)	
Other	1 (1.7%)	0 (0.0%)	
Smoking habits	6 (10.2%)	12 (19.0%)	0.16
Occupation			
Unemployed	2 (3.4%)	4 (6.3%)	
Housewife	15 (25.4%)	16 (25.0%)	0.95
Employed	40 (67.8%)	41 (65.1%)	
Freelancer	2 (3.4%)	2 (3.2%)	
BMI classes:			
Underweight	2 (3.4%)	6 (9.5%)	
Normal weight	42 (71.2%)	34 (53.9%)	0.22
Overweight	13 (22.0%)	20 (31.7%)	
Obese	2 (3.4%)	3 (4.7%)	
Pregnancy conception:			
Spontaneous	58 (98.3%)	61 (96.8%)	
ART	1 (1.7%)	2 (3.2 %)	0.11

BMI, body mass index.

Table II. Risk factors for Preterm Birth. Variables are reported as numbers and ratios (%).

	Treatment group (N=59)	Control group (N=63)	*p*-value
Nulliparity	49 (83.0%)	49 (77.8%)	0.23
Previous PTB	4 (6.7%)	3 (4.7%)	0.29
Uterine fibromatosis	2 (3.4%)	3 (4.7%)	0.70
Previous cervical surgery	4 (6.8%)	8 (12.7%)	0.27

PTB, preterm birth.

Pregnancy Outcomes

Admission for threatened PTB was significantly higher in the control group compared to the treatment group ($p = 0.03$) (Table IV). The difference in the PTB rate was not statistically significant, but the events observed in the control group were twice as many compared to the treatment group (treatment: 3 (5.1%) *vs.* control: 6 (9.5%), $p = 0.34$). Pregnancy outcomes are reported in Table IV. The mean birth weight, and the rates of small-for-gestational-age (SGA) and large-for-gestational-age (LGA) were similar in both groups. Low 5-minutes Apgar scores and neonatal intensive-care-unit admission (NICU) were slightly higher in the control group compared to the treatment group although they did not reach statistical significance. The mean gestational age at delivery and the vaginal or cesarean section were the same in both groups.

Discussion

This study demonstrated that oral ALA associated with magnesium, vitamin B6, and vitamin D reduce the cervix shortening rate between the first and the second trimester in pregnant women presenting risk factors for PTB. A population of 122 pregnant women, similar for what concerns baseline characteristics (age, education level, ethnicity, smoking habits, occupation, BMI, or pregnancy conception) and with at least one risk factor for preterm birth (nulliparity, previous PTB, uterine fibromatosis and previous cervical surgery), were enrolled and treated with oral ALA, magnesium, vitamin B6 and vitamin D. CL remained unchanged in the treatment group, while it significantly decreased in the control group ($p = 0.02$). Such observation is in line with previous pilot studies, in which ALA administration (vaginal/oral) to pregnant women stabilized cervical length compared to controls[21,22]. In addition, our results highlighted for the first time that ALA, when associated with magnesium, vitamin B6 and vitamin D, also significantly reduced hospital admissions for threatened PTB, compared with the control group (3.4% *vs.* 14.3%; $p = 0.03$). While the rate of PTB remained consistent with the national incidence in the control group (9.5%), it decreased in the treatment arm although it did not reach statistical significance (9.5% *vs*. 5.1%). The small sample size could be one of the limitations of this study, and it could be the reason why differences in PTB rate were not significant between groups. Among 6 women experiencing PTB in the control group, 3 people had a cervical length ≤ 3 cm at the 2nd trimester; on the contrary none of those who experienced PTB in the treatment group, had a cervical length ≤ 3 cm. In addition, our results showed that the combination of ALA, magnesium, vitamin B6, and vitamin D did not affect neonatal outcomes, such as birth weight, the rate of SGA and LGA, or NICU admission. Other tocolytic drugs (β-mimetics, oxytocin receptor antagonists, calcium channel blockers), that are generally used to inhibit myometrial contractility for the acute treatment of threat-

Table III. Cervical length changes between the first and second trimester according to study groups. The mean of the percentage of cervical length (CL) variation was calculated as [(2nd CL-1st CL) *100]/1st CL. Significant differences between the two groups are indicated.

	Treatment group (N=59)		*p*-value	Control group (N=63)		*p*-value
	1st trim (11-13w)	2nd trim (19-22w)		1st trim (11-13w)	2nd trim (19-22w)	
CL (cm)	3.92 ± 0.57	3.95 ± 0.49	0.58	**3.96 ± 0.54**	**3.78 ± 0.65**	**0.02**
Mean % CL variation	**1.50 ± 1.26**			**-3.86 ± 1.97**		**0.02**
CL 2nd trim ≤ 3.0 (cm)	6 (10.2%)			6 (9.5%)		0.90

Table IV. Pregnancy outcomes according to the treatments.

	Treatment group (N=59)	Control group (N=63)	*p* value
Admission for threatened PTB	**2 (3.4%)**	**9 (14.3%)**	**0.03**
Preterm Birth	3 (5.1%)	6 (9.5%)	0.34
Delivery Vaginal Cesarean Section	53 (89.8%) 6 (10.2%)	54 (85.7%) 9 (14.3%)	0.49
Mean GA at birth (weeks)	39.2 ± 0.4	39.4 ± 1.7	0.70
Mean birthweight (g)	3293.6 ± 486.7	3236.6 ± 493.5	0.52
Male	34 (60.7%)	34 (54.0%)	0.46
NICU admission	1 (3.2%)	4 (6.3%)	0.34
pH < 7.2	2 (6.4%)	6 (9.5%)	0.21
Apgar 5' < 7	0 (0.0%)	1 (1.6%)	0.24
SGA	8 (13.5%)	8 (12.7%)	0.83
LGA	4 (6.7%)	4 (6.3%)	0.88

Continuous variables are reported as mean ± standard deviation. Dichotomous variables are reported as numbers and ratios (%). Significant differences between the two groups are indicated. Abbreviation: PTB, preterm birth; GA, gestational age; NICU, Neonatal Intensive Care Unit SGA, small-for-gestational-age; LGA, large-for-gestational-age.

ened preterm labor, despite their action on delaying delivery, and temporary inhibiting uterine contractions, fail to show benefits for neonatal survival and disability. None of the drugs has consistently demonstrated secondary benefits to improve perinatal outcomes[23]. Moreover, their administration is usually recommended only in case of high risk of PTB and their use in routine practice is not recommended due to their several side effects, both for the mother and the fetus[24]. Vaginal progesterone or intramuscular 17 α-hydroxyprogesterone caproate is the standard therapeutic approach to prevent PTB in women with a history of PTB and/or short CL[25]. Despite its wide use and its role in physiologic pregnancy to suppress pro-labor genes, when administered by oral route progesterone may entail various systemic side effects (such as mood swings, headache, dyspepsia, abdominal pain, diarrhea, nausea, and sleep disorders)[26]. Therefore, nowadays vaginal administration would be the best option to use, due to the better concentrations that reach the uterus for the "first uterine pass effect. Nevertheless, some controversial results still exist, and the progesterone issue is considered "a never-ending story", as recently reported in a multicenter, double-blind, randomized, placebo-controlled trial, in which a maintenance treatment of 200 mg of daily vaginal progesterone capsules in women discharged home after an episode of arrested preterm labor did not significantly reduce the rate of preterm delivery[27]. PTB is associated with an inflammatory process that induces morphological changes in the uterine cervix[18]. For this reason, the appropriate tocolytic therapies should be associated with interventions able to counteract morphological changes in the uterine cervix. Unfortunately, most of the first-line tocolytic drugs have important side effects[28] and their efficacy is still controversial[27]. ALA is an endogenous fatty acid with antioxidant and anti-inflammatory properties, which counteracts free radical release during the inflammatory process and exerts a normalizing effect against the alterations of the cervix and vaginal tissues that occur during PTB[29]. ALA reduces the incidence of spontaneous contractions[30] by decreasing the expression of two enzymes involved in PTB, the nuclear factor-kappa B (NF-KB)[31] and the metalloproteinase-9 (MMP-9)[32]. Moreover, ALA is safe for the mother and the fetus; even with limited pharmacovigilance studies about the topic, a recent retrospective study evidenced that no adverse events occurred in 610 pregnant women treated with ALA[33]. Magnesium is an oligo-element that has a pivotal role in several chemical reactions in the body. During periods of extreme physical or psychological stress, magnesium is consumed by the body. Magnesium deficiency contributes to several health problems including pregnancy disturbances, and clinical evidence showed that preterm labor is associated with a significant decrease in serum magnesium levels[34,35]. Its tocolytic action is still not well characterized, but at the pharmacological concentration (5mmol/L), magnesium inhibits con-

tractile response in pregnant human myometrial strips from pregnant women with a pattern that is consistent with both extra and intracellular mechanisms[36]. Vitamin B6 (or pyridoxine), is a water-soluble compound found in animals and plants that generally acts as coenzymes in different metabolic reactions. It preserves nerve functions, counteracts tiredness and fatigue, sustains the functioning of the immune system, regulates hormonal activity and psychological functions[37], and reduces nausea in pregnancy[38]. Moreover, non-randomized studies associated preconception vitamin B6 status with PTB[39]. Vitamin D is a fat-soluble hormone with classical effects in regulating calcium and phosphate homeostasis. Vitamin D supports the function of the immune system and inhibits inflammation by restraining inflammatory cytokines including TNF-α, IFN-γ, and IL-6 while promoting the release of antimicrobial peptide cathelicidin in the placenta[40]. Scientific studies highlighted an association between adequate levels of vitamin D during pregnancy and favorable maternal and fetal-neonatal outcomes[41]. Moreover, vitamin D is defined appropriately as a steroid hormone with progesterone-like activity[42]. It contributes to the maintenance of muscular function, thus preserving uterine quiescence. By supplementing pregnant women with vitamin D in single or multiple administrations, serum 25-hydroxyvitamin D increases, and the risk of preeclampsia, low birth weight, and preterm birth decreases[43].

Conclusions

Avoiding the shortening of the uterine cervix between the first and the second trimester of pregnancy may be a promising strategy to prevent PTB risk since a strong inverse correlation exists between CL and PTB. Most tocolytic drugs although delaying delivery for a short period should be used under strict control due to several side effects for the mother and the fetus. Our results highlight that the association of ALA, magnesium, vitamin B6 and vitamin D, is safe for both mothers and newborns, and stabilizes CL between the first and the second trimester, with respect to the control group. Importantly, treatment with this association significantly reduced admission for threatened PTB in the treatment group compared with the control group, and even though the rate of PTB was not significantly different, twice the number of events was observed in the control group *vs.* the treatment group.

Conflicts of Interest

The authors declare no conflict of interest.

Informed Consent

Written informed consent was obtained from all subjects involved in the study.

Authors' Contributions

Conceptualization, D.M. and I.N.; data curation, M.G.I., L.B., F.M. and F.F.; formal analysis, D.M.; investigation and methodology M.G.I.; L.B.; F.M.; F.F.; project administration, D.M. and I.N.; writing—original draft, D.M.; writing and editing, D.M., I.N. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Ethics Approval

This study was performed in accordance with the Declaration of Helsinki and received IRB approval from the Ethics Committee of Modena, Area Vasta Emilia Nord (AVEN) (reference number 58/2019; clinicalstrial.gov NCT03952533 registered on 16/05/2019).

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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