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Effect of Zinc Supplement on Prevention of PPROM and Improvement of some Pregnancy Outcomes in Pregnant Women with a History of PPROM: A Randomized Double -Blind Controlled Trial

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Abstract

Background: Preterm premature rupture of membrane (PPROM) prior to 37 weeks' gestation is among the most common obstetrics problems, which is associated with prenatal mortality and several maternal and neonatal complications. History of PPROM is a risk factor for recurrence. Zinc has an important effect on the strength of membranes by affecting collagen [tensile] strength - a substance with immunity mechanism and antioxidant properties.

Objectives: This study was conducted to investigate the effect of zinc supplement on the prevention of PPROM and improvement of some pregnancy outcomes in pregnant women with a history of PPROM during the second trimester and the early third trimester.

Methods: In this randomized, double-blind, controlled clinical trial, 108 healthy pregnant women (at gestational age of 16 - 30 weeks) with a history of PPROM and singleton pregnancy were selected by convenience sampling method in the Midwifery Clinic of Shahid Akbarabadi hospital in Tehran, Iran, between 2014 and 2015. They were then divided into two groups of placebo and zinc sulfate tablet (40 mg) recipient using randomized block design. In total, 92 subjects completed the study. The frequency of PPROM was regarded as the primary outcome, and frequency of PROM, average gestational age at birth, average birth weight, and average head circumference were considered as the secondary outcomes. The statistical analysis was based on intent-to-treat principle.

Results: There was no between-groups difference in terms of demographic and pregnancy specifications. Results showed no significant between-groups (zinc versus placebo recipient) difference ($P > 0.05$) in terms of the frequency of preterm pregnancy (22% versus 33.3%), frequency of PPROM (4.9% versus 11.8%), frequency of PROM (14.6% versus 17.6%), average birth weight (3192.17 g versus 3080.52 g), average gestational age at birth (38.2 weeks versus 37.2 weeks), and average head circumference at birth (34.63 cm versus 34.81 cm).

Conclusions: According to the results, daily intake of zinc sulfate (40 mg) by pregnant women with a history of PPROM does not contribute to the prevention of PPROM and PROM and improvement of average gestational age at birth and anthropometric measurements.

Keywords: PPROM, Zinc Sulfate, Supplement, Prevention, Pregnancy, Outcomes

1. Background

The PPROM prior to 37 weeks' gestation is common in 2-5% of singleton pregnancies (1) and 7% -10% of twin pregnancies (2). It is of the most prevalent obstetrics problems, which is associated with preterm pregnancies (30% - 40%), and prenatal mortality (10%), as well as maternal complications, such as placenta abruption and chorioamnionitis (2, 3). The prevalence of PPROM recurrence is somewhere between 21% and 32% (1). History of preterm birth, cigarette-smoking, uterine distention, and the use of some diagnostic methods, such as amniocentesis and bacterial vaginosis are among the risk factors of PPROM (1-4). Reduction in the intake of nutrients, such as thiamin, riboflavin, vitamin A, C, and E, copper, and zinc, is associated with preterm birth and higher risk of PPROM (5). This results from nutrient-deficiency problem that predispose women to develop abnormal collagen structure (6). Since preterm fetal membranes are very strong, the PPROM should be a result of an internal pathology and/or external factors (7). According to the recent evidence, membrane rupture is related to biochemical processes, including disruption of collagen within the extracellular matrix of the amnion and the chorion and apoptosis in the fetal membranes (8).

The disruption of collagen is primarily a result of successive activities of a number of matrix metalloproteinases (MMPs) and tissue-specific inhibitors (9). The MMPs have been identified within human and some other species. They belong to a large multigene family of zinc-dependent, proteolytic enzymes with more than 26 known species,

and specific structure and several properties (10).

Pregnant women are at highest risk of zinc deficiency. Zinc deficiency places women at risk of spontaneous abortion, preterm delivery and labor abnormalities, congenital malformations, low birth weight (LBW), and intrauterine growth retardation (IUGR) (11).

Since the PPROM is associated with maternal and fetal infections, prenatal mortality, and preterm complications, it is essential to study preventive techniques. In addition, regarding the role of oxidative activities in the rupture of membranes, moderating the effect of free radicals of oxygen and nitrogen through supplying antioxidative resources from food or dietary supplements is a PPROM prevention measure. Trace minerals, such as zinc (12), involve in biosynthesis of collagen and stabilization of chorionic and amniotic layers. They also protect the body against oxidative activities (8, 13). According to some studies, plasma zinc concentration is significantly lower in women with PPROM than in controls (14-16). In addition, gestational age at birth is greater in the zinc recipient group (17). Some other studies report positive effects of zinc on the birth weight and head circumference (18-20). However, some studies reject such effects on pregnancy outcomes (21-23). There is scant and contradictory information on the relationship of zinc supplementation with prelabor rupture of the membranes (PROM) and neonatal anthropometric measurements. In addition, the researcher had no access to any study devoted to the role of zinc in PPROM prevention.

2. Objectives

This study was conducted to investigate the effect of zinc supplementation on PPROM and PROM prevention, improvement of average gestational age at birth, and neonatal anthropometric measurements in pregnant women with a history of PPROM.

3. Methods

3.1. Research Design and Participants

This randomized, double-blind, controlled clinical trial with placebo was conducted in Tehran, Iran. The inclusion criteria were a history of PPROM, singleton pregnancy, normal fetus and amniotic fluid, maternal age over 18 years, gestational age between 16 and 30 weeks, normal pregnancy without underlying diseases (cardiac, pulmonary, gastrointestinal, diabetes, epilepsy, blood pressure, etc.), tendency to participate, reading and writing skills, and accessibility by phone. The exclusion criteria were uncertain status of the fetus (abnormal nonstress test), any fetal congenital anomaly, any pregnancy complication (history of preeclampsia, current chronic blood pressure, bleeding, risk of abortion, severe nausea and vomiting, etc.), probability of residential mobility in the next six months, addiction to illicit drugs and alcoholic drinks, receiving another intervention, intake of multivitamin-mineral supplements during pregnancy, and severe vaginal infection (with discharge, itching, swelling, erythema and foul-smelling discharge as its symptoms). The frequency of PPROM was measured as the primary outcome and frequency of PROM, average gestational age at birth, average head circumference, and average birth weight were measured as secondary outcomes.

The sample size was obtained as 49 for each group, according to the study by James et al. (1) and using t-test in Gpower 3.1.2 (Germany), considering the PPROM as research variable, $\alpha = 0.05$, power of 80%, $p_1 = 25\%$, and $p_2 = 5\%$. With regard to the 10% probability of loss to follow-up, 54 subjects were taken for each group.

3.2. Sampling

Sampling was conducted according to the ethical principles of the Declaration of Helsinki between December, 2014 and September 2015, through convenience method. The participants were selected among non-hospitalized patients referred to the Prenatal Clinics during the morning shift of Shahid Akbarabadi hospital, after obtaining the required permissions from the ethics committee of the research deputy of Tabriz University of Medical Sciences under the project no. 9316, and the head of the midwifery clinic of Shahid Akbarabadi hospital (a governmental center affiliated to Tehran University of Medical Sciences and Center's gynecologic), and being registered in IRCT (IRCT201311296709N14). Prior to the sampling, the inclusion and exclusion checklist and demographic questionnaire were completed by the researcher after explaining the research objectives and intervention method to the subjects. Then, the eligibility of the subjects was investigated. To meet the inclusion criteria, we examined the volunteered (with informed consent) healthy multiparous with a history of PPROM, singleton pregnant women (aged over 18 years and gestational age between 16 to 30 weeks), after being explained on the research objectives and methodology. Among 4,320 examined women, 4,197 cases were excluded for ineligibility and 15 eligible subjects for unwillingness to participation. Finally, 108 eligible women were randomly placed in two equal-sized groups (zinc sulfate and placebo recipient).

3.3. Randomization and Intervention

Volunteered subjects were divided in to the zinc sulfate and placebo recipient groups using a random number table based on randomized block design with blocks

of size 4 and 6, after obtaining their informed written consent. According to the integrated maternal health program for pregnant women in Iran (provided by the ministry of health and medical education), each participant received a sealed opaque pocket, containing 84 supplementary tablets, two times during pregnancy (weeks 16^{th} to 20^{th} and 26^{th} to 30^{th}). To ensure the intake of tablets, the old pocket was exchanged for the new one. It is worth mentioning that those included in the study with the week 30^{th} received only one pocket (once) containing 56 supplementary tablets. Pockets were prepared through random allocation sequence (1: 1) by using computerized software of RAS (Random Allocation Software) and randomized blocks with sizes of 4 and 6. These pockets coded from one to 108 by someone other than the researchers. Supplementation was initiated from the 16^{th} to 30^{th} weeks. The pregnant women and researcher had no knowledge of the content of the pockets. The first pocket was given to the first eligible subject. This process continued until all subjects received their pockets. In this way, the first group received zinc sulfate tablet (40 mg) once a day and the second group received placebo once a day, containing microcrystalline cellulose, lactose, and starch (It was recommended to be taken on an empty stomach and with water). The placebo tablets were exactly the same as the zinc sulfate tablets in appearance, color, and weight (provided from Tabriz faculty of pharmacy). The intake of tablets continued until the delivery time. To ensure the regular intake of supplements, a checklist was given to the subjects to record daily intake of tablets and probable complications. These checklists were reviewed at the end of the intervention. In addition, the researcher checked the intake of supplements by asking the subjects every month during the pregnancy and then every two weeks via phone calls, and at every pregnancy care visit in the clinic. Moreover, the symptoms of vaginal infection were controlled at every visit. Researcher and participants were blind in this study.

3.4. Data Collection

Demographic data of eligible participants was collected by using a questionnaire completed by the researcher when they included in the study. The midwifery and obstetric inventory was also completed by the researcher at postpartum period. The questionnaires were evaluated in terms of face validity by 7 - 10 faculty members of Tabriz University of Medical Sciences. The required modifications were made based on their opinions and comments. The reliability of the instrument, used for membrane rupture diagnosis (speculum), was assured through direct observation by the researcher and her assistant using Cohen's kappa coefficient with numerical value of 0.76 at the client level. The midwifery and obstetric inventory was completed at postpartum by the researcher, according to the patient's medical record. The gestational age was calculated from the first day of the mother's last menstrual period. The fetal crown-rump length was measured via medical ultrasound between the 7 and 17 weeks' gestation. The rupture of membrane and/or hospitalization for delivery or any other reason was diagnosed by the subject's phone call, confirmation by the researcher or her assistant using sterile speculum, the presence of fluid in cervical canal, and/or Fern and Nitrazin tests. Obtained data were then recorded. The birth weight of all neonates in both groups was measured and recorded immediately after admission to the neonatal unit, using Seca scale LRSC 021($\pm$ 50). The head circumference of the neonates was also measured using a measuring tape and then, the obtained data were recorded.

3.5. Data Analysis

Data were reported as mean (standard deviation) and frequency (percentage) for quantitative and qualitative variables, respectively. The Kolmogorov-Smirnov test was used to evaluate data normality (Normal distribution of quantitative variables). To compare the qualitative variables between the groups, Chi-square test, trend test, and exact test were used. To compare the quantitative variables between the groups, t-test was used in the case of normal data; otherwise, Mann-Whitney test was applied. Data were analyzed using SPSS21 at significance level of $P < 0.05$.

To investigate the effect of missing data at follow-up on the significance of research objectives, the intent-to-treat analysis was employed with multiple imputations using the Markov chain Monte Carlo (MCMC) approach. The analysis was repeated with new data, and the results were compared to the earlier results in terms of significance. There was no significant difference between the pre-test and post-test data.

4. Results

In total, 108 eligible women included in the study from December 2014 to September 2015. The selected subjects were followed up until April 2016 (i.e. 5 to 6 month post-intervention). Finally, 92 subjects were examined and their data were analyzed. Eight subjects (5 in zinc sulfate recipient and 3 in placebo recipient groups) failed to follow up for unwillingness to continue, three subjects for irregular intake of supplement (in zinc sulfate recipient group), two subjects for diarrhea (in zinc sulfate recipient group), and three subjects for discontinuing the supplementation following the physician order (in zinc sulfate recipient group). Finally, 41 subjects in zinc sulfate recipient

group and 51 in placebo recipient group completed the intervention (Figure 1).

Results showed that both groups were similar in terms of demographic characteristics (mother's age, age at marriage, education level, employment status of spouse, tendency to pregnancy, and body mass index) as well as in midwifery specifications (current and previous labor, cause of C-section, and gravidity). The mean ± standard deviation of the participants' age and age at marriage were 30.5 ± 5.2 and 20.4 ± 4.3 years, respectively, in the zinc sulfate recipient group and 30.4 ± 5.4 and 20.3 ± 4.7 years, respectively, in the placebo recipient group. Education level of the majority of mothers in the zinc sulfate recipient group (46.3%) and placebo recipient group (40.7%) was diploma. In the zinc sulfate recipient group, 44.4% of participants had the BMI between 18.5 and 24.9. In the placebo recipient group, 38.9% of participants had the BMI ≤ 30. In addition, 55.6% of the subjects in the zinc sulfate recipient group and 50% in the placebo recipient group had C-section. The main reason for C-section in the zinc sulfate (86.7%) and placebo (81.5%) recipient groups was having a history of C-section. The other demographic and midwifery information is presented in Tables 1 and 2.

The average gestational age at birth in the zinc sulfate and placebo recipient groups was 38.2 ± 1.2 and 37.2 ± 3.4 weeks, respectively. MD (CI 95%) was 949/0 (-0.07 to 1.97). Among the participants, 22% in the zinc sulfate recipient group and 33.3% in the placebo recipient group went into preterm labor. This between-groups difference was not statistically significant (P = 0.07; Table 3).

The prevalence of the PPROM prior to 37 weeks' gestation was 4.9% in the zinc sulfate recipient group and 11.8% in the placebo recipient group. This between-groups difference in the frequency of PPROM was not statistically significant (P = 0.291; OR (95% CI) = 0.385 (0.073 to 2.016)). Among the participants, 14.6% in the zinc sulfate recipient group and 17.6% in the placebo recipient group had PROM; however, this between-groups difference was not statistically significant (P = 0.697; OR (95% CI) = 0.800(0.259 to 2.467)) (Table 4).

The birth weight of the majority of neonates in the zinc sulfate recipient group (95.1%) and placebo recipient group (88.2%) was higher than 2,500 g (4.9% versus 11.8%). There was no statistically significant difference in the average birth weight (P = 0.492) between the zinc sulfate and placebo recipient groups. The average head circumference in the zinc sulfate and placebo recipient groups was 34.63 ± 1.47 cm and 34.81 ± 1.41 cm, respectively. MD (CI 95%) was -0.175 (-0.78 to 0.43). There was no significant between-groups difference in terms of head circumference at birth (P = 0.572; Table 5).

Although no specific side effect was observed after the intake of the supplement, 3.7% of subjects in the zinc sulfate recipient group discontinued the supplementation for developing diarrhea.

5. Discussion

Results showed that daily intake of zinc sulfate (40 mg) during the second trimester and the early third trimester had no impact on the prevention of PPROM and PROM and improvement of average gestational age at birth and neonatal anthropometric measurements in pregnant women with a history of PPROM. Although the incidence of preterm labor (2.6%) and PPROM, as well as birth weight less than 2,500 g (3.6%) was lower in the zinc sulfate recipient group than the placebo recipient group, these between-group differences were not statistically significant.

In a clinical trial, Danesh et al. (21) examined 84 pregnant women with a history of preterm labor in two groups including zinc sulfate (50 mg) and placebo recipient. They found that although the gestational age and birth weight were higher in the zinc sulfate recipient group, this between-groups difference was not statistically significant. This finding is consistent with the results of the present study, indicating the ineffectiveness of daily intake of zinc sulfate (40 mg) in improving the maternal and neonatal outcomes. Aminisani et al. (22) investigated 196 pregnant women at gestational age of 19 - 20 weeks in the zinc (50 mg) and placebo recipient groups. They found that zinc supplementation had no impact on the incidence of PROM and gestational age at birth, which is consistent with our findings. Jonsson et al. (24) examined 1,206 healthy pregnant women (from gestational age < 20 weeks until labor) by dividing them into two groups of zinc sulfate (44 mg) and placebo recipient, and found no significant between-groups difference with respect to the gestational age at birth and incidence of preterm labor, which is consistent with the present study. Findings of Zahiri et al., Osendrap, Sakia et al., and Laura E. Caulfield et al. (25-27), who compared three zinc recipient groups (15, 30, 15 mg) with a placebo recipient group, showed that zinc supplementation during the second and third trimesters did not offer a significant benefit to gestational age at birth, PROM, and LBW. These findings are consistent with those of the present study. Tamura et al. (23) examined 580 women less than 34 weeks pregnancy and serum zinc level < 50%. Subjects received a supplement containing calcium, folic acid, iron, and vitamin or the same supplement plus zinc. Results did not show any significant relationship between the serum zinc concentration (during the last weeks of the first trimester to the early weeks of the third trimester) and pregnancy outcomes, such as gestational age at birth,

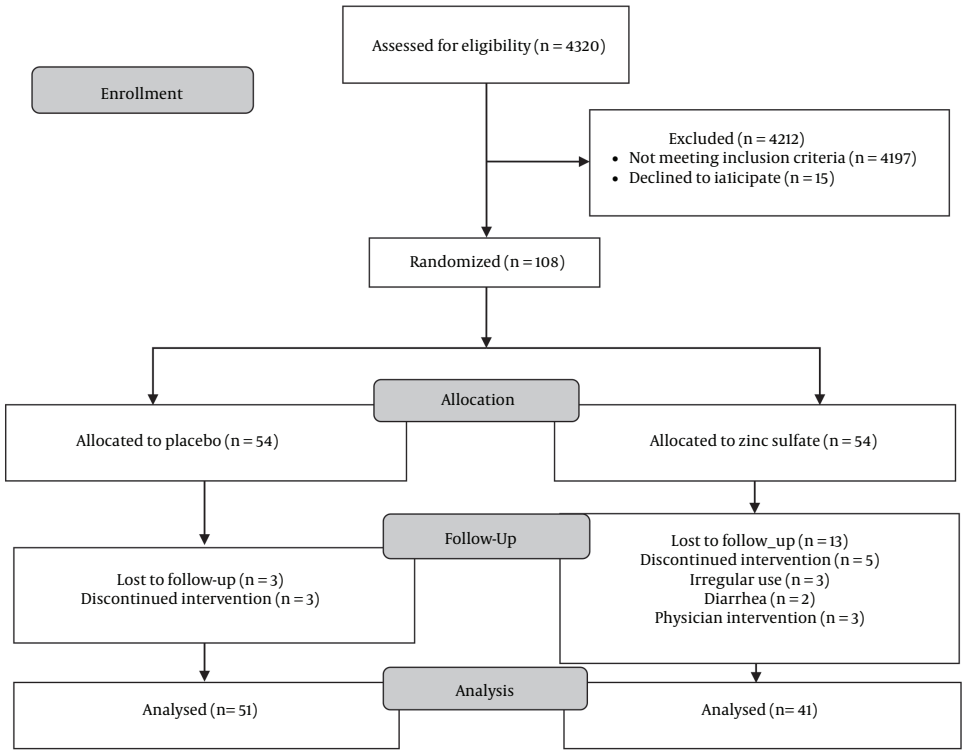

Figure 1. Flow Diagram of the Progress Through the Phases of the Randomized Controlled Trial

preterm labor (less than 37 weeks), premature preterm pregnancy (less than 32 weeks), birth weight, and head circumference. These findings are consistent with ours.

In a clinical trial, Garg et al. (17) investigated two groups of pregnant women: zinc (45 mg) and placebo recipient groups. The mean gestational age at birth and birth weight were higher in the zinc recipient group than placebo recipient group, which is inconsistent with our findings. This difference may be due to the difference in zinc dosage (40 mg in the present study versus 45 mg in the Garg et al. study) and duration of supplementation so that in the their study, subjects included at different trimesters and received the supplements from the first visit until the labor, whereas in the present study, subjects included in the second and early third trimesters, and thus the duration of supplementation was shorter. In addition, Goldenberg et al. (20) investigated two prenatal supplements with and without zinc (25 mg). They found that the average head circumference and birth weight were higher in the zinc recipient group than placebo recipient group, which is inconsistent with our findings. This inconsistency may be due to the difference in administered dosage (25 mg plus other minerals in the study of Robert Goldenberg et al. versus 40 mg zinc sulfate without other minerals in our study), low serum zinc level in the early weeks of pregnancy, or the BMI. Hininger et al. (18) investigated two supplementation groups (with and without zinc; 15 mg). Findings showed that the birth weight was 10% higher in the zinc recipient group than the placebo recipient group. In addition, frequency of LBW was significantly lower in the zinc recipient group. These findings are inconsistent with ours. It seems that these differences are due to the supplementation with zinc plus other nutrients and vitamins in the mentioned study. Aminisani et al. (22) reported significantly higher prevalence of LBW in the placebo recipient group, and lower prevalence of birth weight in the zinc recipient group. In Danesh et al. study (21), the mean head circumference was significantly higher in the zinc recipient group (50 mg), which is inconsistent with our findings. This inconsistency may be due to the difference in the administered zinc dosage (40 versus 50 mg).

Morphological and biochemical data showed that disruption in factors responsible for tensile strength of fetal membranes is the primary cause of PPROM pathogens (9). Zinc acts as a co-factor in collagen synthesis (28), and is an essential part of steroid hormone receptors (e.g. estrogen

Table 1. The Comparison of Socio-Demographic Characteristics of the Research Subjects in Zinc and Placebo Recipient Groups[a]

	Zinc, n = 54	Placebo, n = 54	Statistical Indices
Maternal age, y			
Under 20	1 (1.9)	4 (7.4)	
21 - 30	26 (48.1)	17 (31.5)	T-test =-0.489
Above 31	27 (0.50)	33 (1.61)	df = 106
Mean (SD)	30.5 (5.3)	30.4 (5.4)	P value= 0.626
Age of marriage, y			
Under 20	30 (55.6)	33 (61.1)	
21 - 30	22 (40.7)	18 (33.3)	T-test = 0.327
Above 31	2 (7.3)	3 (6.5)	df = 106
Mean (SD)	20.4 (4.3)	20.3 (4.7)	P value = 0.745
Maternal education			
Elementary school	10 (5.18)	6 (1.11)	
Junior high school	8 (8.14)	12 (2.22)	χ^2 for trend = 0.006
High school	4 (4.7)	8 (8.14)	df = 1
High school diploma	25 (3.46)	22 (7.40)	P value = 0.941
University	7 (0.13)	6 (1.11)	
Spouse education			
Elementary school	8 (8.14)	10 (5.18)	
Middle school	9 (7.16)	19 (2.35)	X^2 for trend = 2.26
High school	5 (3.9)	3 (6.5)	df = 1
High school diploma	27 (0.50)	14 (9.25)	P value = 0.132
University	5 (3.9)	8 (8.14)	
Spouse job			
Unemployed	1 (9.1)	0	
Employee	4 (4.7)	6 (1.11)	Exact χ^2 = 3.27
Laborer	25 (3.46)	28 (9.51)	df = 4
Shopkeeper	10 (5.18)	5 (3.9)	P value = 0.514
Others	14 (9.25)	15 (8.27)	
Income level			
Sufficient	23 (6.42)	34 (0.63)	Exact χ^2 for trend = 3.42
Relatively sufficient	29 (7.53)	18 (3.33)	df = 1
Insufficient	2 (7.3)	2 (7.3)	P value =0.091
Willingness for pregnancy			χ^2 = 0.393
Positive	39 (2.72)	36 (7.66)	df = 1
Negative	15 (8.27)	18 (3.33)	P value = 0.531
Body mass index (BMI)			
Between 18.5 and 24.9	24 (4.44)	19 (2.35)	χ^2 = 0.966
Between 25 and 29.9	12 (2.22)	14 (9.25)	df = 2
30 and above	18 (3.33)	21 (9.38)	P value = 0.617

[a] All data in the table, except for those expressed as mean scores (standard deviations), represent frequency (%)

and progesterone) that contain zinc-finger protein (29, 30). These hormones start decreasing at the beginning of labor with reduction in the zinc level, resulting in disruption in fetal membranes and amniotic fluid (31). Fetal membranes and decidua respond to different stimuli, such as reproductive tract infection. This response affects the activity of matrix enzymes by producing mediators, such as prostaglandins and cytokines (8). In addition, free radicals induced oxidative stress (12) during pregnancy cause damage to fatty acids existing in fetal membranes (13, 32, 33). PPROM often represents a final path associated with infection, inflammation, uterine stretch, oxidative stress and decidual bleeding. In fact, membrane weakening process and loss of extracellular matrix and collagen may be accel-

Table 2. The Comparison of Obstetrics Characteristics of the Subjects in Zinc and Placebo Recipient Groups[a,b]

	Zinc, n = 54	Placebo, n = 54	Statistical Indices
Current delivery type			
Vaginal birth	24 (4.44)	27 (0.50)	$\chi^2 = 0.33$
C-section	30 (6.55)	27 (0.50)	df = 1; P value = 0.563
Previous delivery type			
Vaginal birth	29 (7.53)	32 (3.59)	$\chi^2 = 0.33$
C-section	25 (3.46)	22 (7.40)	df = 1; P value = 0.560
Number of pregnancies			
2	22 (7.40)	20 (0.37)	$\chi^2 = 1.15$
3	13 (1.24)	18 (3.33)	df = 2
4 or more	19 (2.35)	16 (6.29)	P value = 0.560
Reason for C-section			
Planned repeat caesarean	26 (7.86)	22 (5.81)	Exact $\chi^2 = 3.18$
Lack of progress in labor	0	1 (7.3)	df = 3
Maternal problems	1 (3.3)	3 (1.11)	P value = 0.321
Fetal problems	3 (0.10)	1 (7.3)	

[a] All data in the table represent frequency (%).
[b] Analyses were conducted among those with C-section delivery, with 30 and 27 subjects from the zinc and placebo recipient groups, respectively.

Table 3. The Comparison of Mean Gestational Age at Birth in Zinc and placebo recipient groups[a]

	Zinc, n = 41	Placebo, n = 51	MD (95% CI)	Percentage of Change	Statistical Indicators
Gestational age at birth					
Between 22 to 37 weeks	9 (0.22)	17 (3.33)			T-test = 1.84
38 weeks and above	32 (0.78)	34 (7.66)	949.0		df = 65
Mean (SD)	38.2 (2.1)	37.2 (4.3)	(07.0 - 97.1)	6.2	P value = 0.07

[a] Mean difference (confidence interval).

Table 4. The Comparison of the Frequency of Preterm Premature Rupture of Membrane (PPROM) and Premature Rupture of Membrane (PROM) in Zinc and Placebo Recipient Groups[a]

	Zinc, n = 41	Placebo, n = 51	Statistical Indices	OR (95%CI)[b]
PPROM				0.385 (0.073 to 2.016)
Positive	2 (9.4)	6 (8.11)	Exact $\chi^2 = 1.35$	
Negative	39 (1.95)	45 (2.88)	df = 1	
Total	41 (100)	51 (100)	P value = 0.291	
PROM				0.800 (0.259 to 2.467)
Positive	6 (6.14)	9 (6.17)	$\chi^2 = 0.151$	
Negative	35 (4.85)	42 (4.82)	df = 1	
Total	41 (100)	51 (100)	P value = 0.697	

[a] Data in the above table represent frequency (%).
[b] Odds ratio (95% confidence interval).

erated by the aforementioned factors (3, 34).

As a major limitation of this study, we could not measure the serum zinc level of the subjects prior to inclusion in the study for financial reasons. The lack of control over serious asymptomatic infections in PPROM, such as ureoplasma, was another limitation of the study. Regarding

Table 5. The Comparison of the Mean Birth Weight and Mean Head Circumference in Zinc and Placebo Recipient Groups[a]

	Zinc, n = 41	Placebo, n = 51	MD (95% CI)	Percent of Change	Statistical Indicators
Birth weight					
Below 2500 g	2 (9.4)	6 (8.11)			T-test = 0.690
Above 2500 g	39 (1.95)	45 (2.88)	6.111		df = 90
Mean (SD)	17.3192 (22.614)	52.3080 (52.876)	(-9.432 to 6.20)	6.3	P value = 0.492
Head circumference					
Less than 35 cm	30 (2.73)	33 (7.64)			T-test = -0.568
More than 35 cm	11 (8.26)	18 (3.35)	-0.175		df = 86
Mean (SD)	63.34 (47.1)	81.34 (41.1)	(-78.0 to 43.0)	-0.51	P value = 0.572

[a] All data in the table, except for those expressed as mean (standard deviations), represent frequency (%).

that the research samples had a history of PPROM, random allocation of them removed this limitation to some extent. In addition, samples were investigated at visits and during follow-up for symptoms and abnormal discharge.

Since this study used specific types of women, results are not generalizable to whole population. However, we tried to overcome this weakness through completely randomized sampling, using randomized block design, and complete assimilation of the experimental and control groups. In addition, sampling was done based on the rate of recurrence among women with a history of pregnancy. The sampling can be done based on other strong risk factors, such as multiple pregnancy, polyhydramnius, amniocentesis, cerclage, etc. In this way, the incidence of this problem can be decreased even in the first pregnancy.

Among the strengths of this study is that it is the first trial into the effect of zinc supplement on the prevention of PPROM in pregnant women with previous history of pregnancy; therefore, this study is a foundation for further studies into the effect of zinc supplementation, after the removal of aforementioned limitations. In addition, it can be a foundation for similar studies using other micronutrients and minerals influencing on the mechanism of PPROM, and maternal and neonatal risk prevention. The use of double -blind methodology and random allocation are other strengths of this study.

5.1. Conclusion

The result of this comparative study into the zinc sulfate supplement and placebo showed that adding 40 mg zinc sulfate supplement to routine care of pregnant women with a history of PPROM in the second trimester and early third trimester had no preventive impact on the PPROM and PROM. In addition, we did not observe a significant clinical effect on the improvement of mean gestational age at birth and neonatal anthropometric measurements, such as birth weight and head circumference. Despite these findings, due to the importance of prenatal mortality, maternal and neonatal complications associated with preterm labor, specifically because of PPROM and recurrent of this problem, conduction of studies into preventive measures is essential.

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