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Original paper

Selenium Supplementation for Prevention of Late-Onset Sepsis in Very Low Birth Weight Preterm Neonates

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ABSTRACT

Background: Neonatal mortality continues to be a significant problem in the Indian setting, especially in very low birth weight (VLBW) neonates. Selenium (Se) has been shown to possess antioxidant properties, and some recent studies have shown a reduction in the sepsis-attributable neonatal mortality with its use. India is a Se-deficient country. Blood Se concentrations in newborns are lower than those of their mothers and lower still in preterm infants.

Objective: To evaluate the efficacy of Se in preventing the first episode of late-onset sepsis in VLBW preterm neonates.

Methods: Ninety neonates weighing <1500 g and period of gestation <32 weeks, asymptomatic at birth and admitted to the neonatal intensive-care unit (NICU) in the first 12 h of birth with no maternal risk factors for sepsis were analyzed in the study. Se or placebo was supplemented orally once daily from 1st to 28th day of life to the test ($n = 45$) or control ($n = 45$) groups, respectively, followed by daily clinical assessment for signs or symptoms of sepsis in the hospital and weekly after discharge.

Results: Preterm VLBW neonates (mean birth weight 1464.22 ± 50.14 g and mean gestational age 221.75 ± 4 days) are Se deficient at birth, with mean (SD) Se levels 31.1 ± 14.8 μg/l. Se supplementation at 10 μg/day increased serum Se levels significantly (63.9 ± 13.9 μg/l on Day 28 in Se vs. 40.9 ± 17.3 on Day 28 in placebo; $p < 0.01$). The incidence of the first episode of culture-proven late-onset sepsis was significantly lower in the Se than in the placebo group. [0/45 (0%) in Se vs. 6/45 (13.3%) in placebo; $p = 0.033$]. The incidence of probable sepsis was found to be significantly lower in the Se group [7/45 (15.55%)] than in the placebo [16/45 (35.55%)]; $p = 0.02$. The total incidence of any late-onset sepsis (i.e. culture-proven plus probable sepsis) was also significantly reduced by Se supplementation. [7/45 (15.55%) in Se vs. 22/45 (48.88%) in placebo; $p = 0.001$].

Conclusion: Preterm VLBW neonates are Se deficient at birth. Se supplementation at 10 μg/day resulted in getting the Se levels into the acceptable normal level and reduced the incidence of the first episode of late-onset sepsis in these neonates.

KEYWORDS: VLBW preterm neonates, late-onset sepsis, selenium

WHAT IS KNOWN ABOUT THIS TOPIC

Preterm infants have inadequate Selenium stores at birth.

For reasons of compromised antioxidant defense and the fact that much of neonatal morbidity is oxygen free radical-mediated, Selenium supplementation in preterm infants is likely to have beneficial effects on their outcomes.

WHAT THIS STUDY ADDS

10 μg/day is possibly a more effective dose for Selenium supplementation in the very low birth weight (VLBW) neonates.

The present study documented that the Se levels in preterm VLBW neonates were 31.1 ± 14.8 μg/l, which are considerably lower than the acceptable normal levels of 50–150 μg/l.

INTRODUCTION

Selenium (Se) is a trace element of tremendous importance in human health. It is a constituent of the antioxidant enzyme Glutathione peroxidase and therefore is vital to antioxidant defense. Several diseases of the neonate have been shown to be caused, at least in part, by oxygen free radicals. These include bronchopulmonary dysplasia, retinopathy of prematurity (ROP), necrotizing enterocolitis, periventricular leukomalacia and neuronal injury of hypoxic ischemic encephalopathy [1, 2]. Antioxidant capacities are inadequate in preterm newborns, both because of placental-fetal transfer interruption of antioxidant molecules and insufficient endogenous production [3, 4].

Se is also known to have a role in immunocompetence. Neutrophils and macrophages from Se-deficient animals have low GPx activity, which may affect their antimicrobial properties, and animal studies suggest that Se supplements may enhance immunoglobulin M and immunoglobulin G antibody response [5]. Limited data from human subjects suggest that Se supplementation enhances cytotoxic and natural killer cell development, and Se deficiency after prolonged parenteral nutrition is associated with abnormal leukocyte function [6, 7].

The plasma Se concentrations of the majority of healthy neonates at birth and up to 3 months of age fall within the range of 50–150 μg/l [8]. Se levels in the full-term Indian neonates were reported to be 54.17 ± 3.4 μg/l [9, 10]. Preterm infants have levels that are even lower.

Two major causes of newborn deaths in India are prematurity (35%) and neonatal infections (33%) [11]. Se supplementation can impact both these outcomes positively and contribute to improved neonatal survival. We planned the present RCT with the research question that in preterm very low birth weight (VLBW) infants, supplementation with Se compared with placebo would decrease the incidence of late-onset sepsis (LOS).

MATERIALS AND METHODS

This randomized, double-blind, placebo-controlled trial was conducted in the Neonatal Services Division, Department of Pediatrics, Pt. B.D.S. PGIMS, Rohtak. Ours is a tertiary care medical college teaching hospital. The study period was from July 2013 to August 2014. Ethical clearance was obtained from the Institution P.G. Board of studies in Pediatrics. An informed consent was obtained from the parents of enrolled infants.

Intramural, VLBW infants with period of gestation <32 weeks and asymptomatic at birth were enrolled. Neonates with gross congenital malformations, severe birth asphyxia (Apgar score <3–5 min or cord pH <7), history of chorioamnionitis in mother or suspected congenital infections were excluded. Neonates with early-onset sepsis (EOS; positive sepsis screen or blood culture) and those with death within 72 h of life were also excluded as also those where parental consent was not given.

Primary outcome was incidence of culture-proven bacterial and *Candida* sepsis at any day, after 72 h and up to 28 days of life, during the hospital stay or during follow-up. Secondary outcomes were incidences of ROP needing treatment according to ETROP [12] and all-cause mortality after 72 h of life, during the hospital stay or during follow-up till Day 28 of life.

The sample size was calculated for a superiority trial with a null hypothesis that Se supplementation to VLBW infants will not decrease incidence of sepsis as compared with control/placebo therapy. It is estimated that incidence of sepsis in VLBW infants is around 20% [13]. To reach a predictive power of

90% with an alpha error of 5% and an attrition rate of 10%, 30 patients are required in each treatment group to show a clinically meaningful difference of at least 40%. One hundred thirty-six infants were assessed for eligibility, of whom 99 were enrolled, and 90 completed the trial for primary analysis (Fig. 1).

Randomization was done on the day of admission using computer-generated random number sequences. The allocated group was mentioned in sealed opaque envelope. The envelopes were picked up serially at the time of enrolment, and the allocated group was communicated telephonically to the staff nurse involved in the care of those newborns. Infants were randomized to receive either Sachet A or Sachet B (Sachet A being Glucon-D powder plus Se powder, and sachet B, Glucon-D powder alone). All sachets were similar looking and prepared by the hospital pharmacy.

The infant was given sachets containing the same code, daily till Day 28 of life. The test sachets contained 10 µg of Se plus 100 mg Glucon D. The sachets being used as placebo contained 100 mg Glucon-D alone. Hospitalized neonates were assessed everyday for any clinical signs or symptoms of sepsis. The neonates discharged from the hospital were asked to follow-up weekly in the neonatal follow-up clinic. In addition, the child could be brought to the neonatal services division in the department for consultation if the need be felt. At each visit, the child was assessed for any signs and symptoms of sepsis. All neonates were screened for ROP as per protocol.

All neonates with a clinical suspicion of sepsis were admitted for sepsis work-up and treated as per the unit protocol. The sepsis work-up included blood culture and a sepsis screen. CRP was done by the qualitative method of Latex agglutination, and an agglutination in 1 : 1 dilution (12 mg/l) was considered as positive. A total leukocyte count (TLC) of <5000 was taken as positive. An immature to total neutrophil ratio of $>20\%$ was considered positive. The absolute neutrophil count (ANC) was considered positive if the value fell outside the limits of normalcy as per Mouzinho's charts [14]. The micro-erythrocyte sedimentation rate (micro-ESR) was considered positive if it was above 'age in days + 3 mm in first hour' in the first week of life or >10 mm in the first hour thereafter. The sepsis screen was considered positive if any two of these parameters were positive.

Serum Se levels were estimated on Day 1 and Day 28 of life using Inductively Coupled Plasma Optical Emission Spectrometer [JobinYvon make, model Ultima2] [15].

The gestational age was assessed on the basis of the first day of the last menstrual period (DLMP) or first trimester ultrasound. In addition, the gestational age was assessed by the Expanded New Ballard Score (ENBS) [16]. If details of DLMP or first trimester ultrasound were not available or if the difference in gestational age as assessed by them and ENBS was >2 weeks, the gestational age as assessed by ENBS was considered final for deciding the eligibility for inclusion in the study. The classification of appropriate-for-gestational-age was done according to the charts prepared by Battaglia and Lubchenco [17].

All data were entered in Microsoft Excel spread sheet by the principal investigator. Data were analyzed by using SPSS version 20.0 statistical software. All quantitative variables were described as Mean (SD) or Median (interquartile range), and all qualitative variables were depicted as number (proportion). For determining the statistical significance between the two groups, the Chi-square/Fischer exact test was applied for categorical variables, whereas the unpaired student *t*-test/nonparametric Mann–Whitney test was used for comparing continuous variables. The level of significance was set at <0.05.

RESULTS

Fig. 1 gives the flow of patients in the study. A total of 136 intramural neonates were assessed for eligibility, of which 114 were randomized into Se and placebo groups. Of these, six in the Se group and nine in the control group were excluded because they developed EOS or died within 72 h. Forty-nine in the Se group and 50 in the control group were finally enrolled. Of those enrolled, four in the Se group and five in the control group were lost to follow-up. Baseline characteristics of neonates and mothers were comparable between the groups, except for the incidence of ante-partum hemorrhage, which occurred significantly more often in the group that had received Se as compared with the placebo [5/45 (11.1%) in Se group vs. 0/45 in control group; $p = 0.020$] (Table 1).

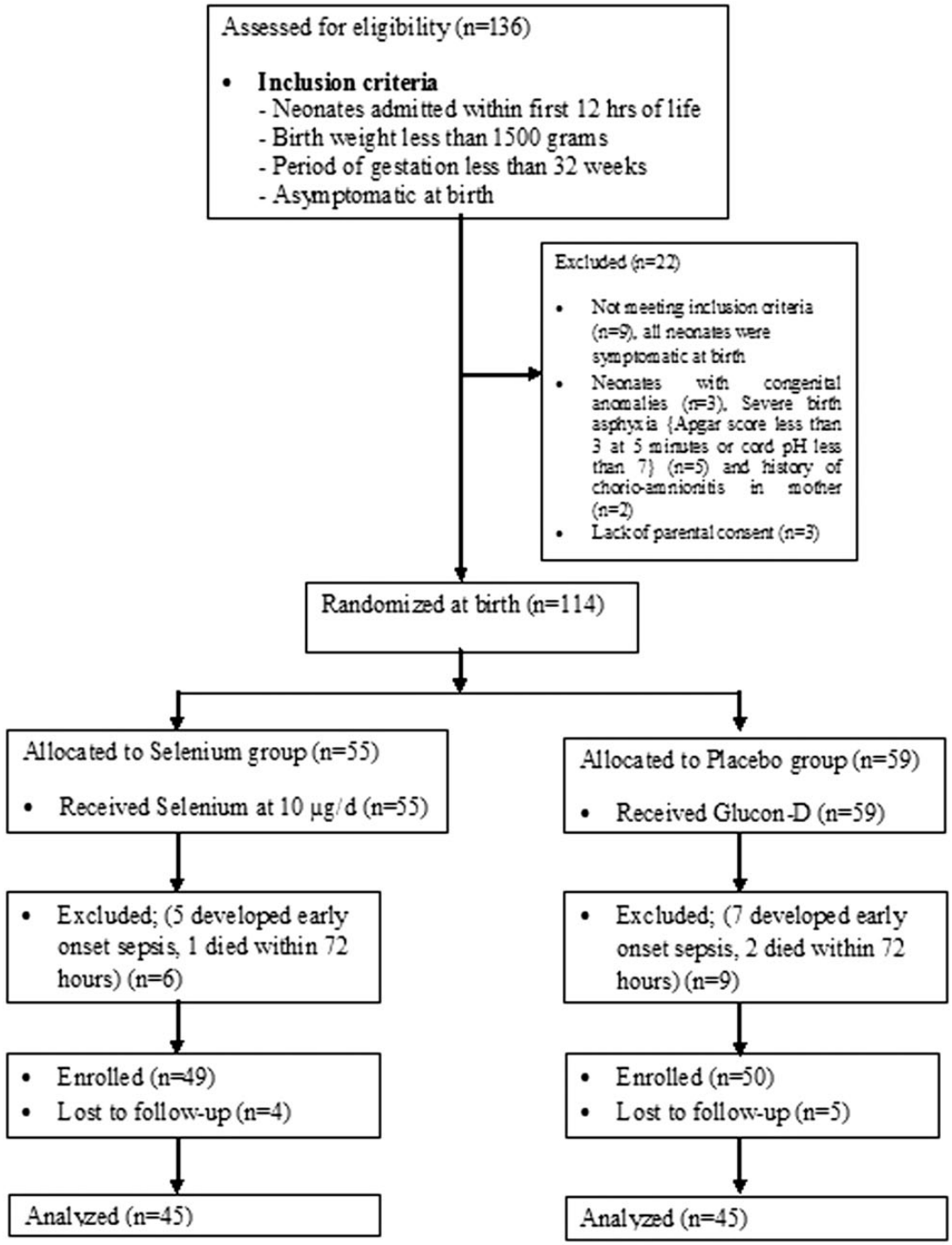

Fig. 1. Study flow.

Table 1. Baseline characteristics of mothers and neonates

Demographic/clinical parameters	Se ($n = 45$)	Placebo ($n = 45$)	p
Age of mother (years) [mean (SD)]	24.13 (±2.70)	23.75 (±2.66)	0.505
Booked [number (proportion)]	43 (95.55%)	42 (93.33%)	0.645
Duration of maternal leaking (hours) [mean (SD)]	9.06 ± 24.17	23.26 ± 57.51	0.130
Vaginal delivery [number (proportion)]	40 (88.88%)	41 (91.11%)	0.559
Antenatal steroids received by mother			
One dose received [number (proportion)]	26 (57.77%)	26 (57.77%)	0.805
Two doses received [number (proportion)]	10 (22.22%)	12 (26.66%)	
Not received [number (proportion)]	9 (20%)	7 (15.55%)	
PIH in mother [number (proportion)]	3 (6.66%)	2 (4.44%)	0.645
Gestational age (days) [mean (SD)]	222.17 ± 3.92	221.33 ± 4.08	0.320
Birth weight (grams) [mean (SD)]	1473.11 ± 45.86	1455.33 ± 54.42	0.090
Male gender [number (proportion)]	24 (53.33%)	22 (48.88%)	0.673
One min Apgar Score [mean (SD)]	6.20 ± 0.404	6.24 ± 0.434	0.616
Five min Apgar score [mean (SD)]	8.08 ± 0.358	8.2 ± 0.504	0.231

Note. PIH = pregnancy-induced hypertension; SD = standard deviation.

On Day 1, Se levels in the entire cohort ($n = 90$) were 31.1 ± 14.8 μg/l.Serum Se levels were comparable between the two groups on Day 1, but were significantly higher on Day 28 in the group that received 10 μg/day of Se supplementation. Se supplementation increased serum Se levels significantly (63.9 ± 13.9 μg/l on Day 28 in Se vs. 40.9 ± 17.3 on Day 28 in placebo; $p < 0.01$) (Table 2).

Incidence of the first episode of culture-proven late-onset bacterial sepsis (after 72 h of life) was significantly lower in the Se group than in the placebo group [0/45 (0) in Se vs. 6/45 (13.33%) in placebo; $p = 0.03$] The absolute risk reduction (ARR) in the incidence of late-onset culture-proven sepsis was 13.3% in the neonates who received Se as compared with the neonates who did not receive it. The number needed to treat (NNT) was 7.5, implying that 7.5 neonates would have to be supplemented with Se to avoid one case of culture-proven LOS (Table 2).

Pseudomonas aeruginosa was isolated among the two culture-positive patients in the placebo group. *Klebsiella*, *Citrobacter*, Coagulase-negative *Staphylococcus aureus* and *Enterococcus* grew in one culture each. *Candida* was not isolated from any patient in the study.

To account for culture-negative LOS, our study also analyzed the incidence of probable sepsis. The diagnosis of probable sepsis was made when the sepsis work-up of a clinically symptomatic patient suggested abnormalities of two or more of the laboratory parameters (i.e. CRP, ANC, TLC, micro-ESR or IT ratio) along with no growth on blood culture. The incidence of probable sepsis was significantly lower in the Se group [7/45 (15.55%)] than in the placebo [16/45 (35.55%)]; $p = 0.020$. The ARR in the incidence of probable sepsis was 20.0%, and the number NNT was 5 (Table 2).

On analyzing the total incidence of any LOS (i.e. culture-proven plus probable sepsis), a significant reduction was found in the group that had been supplemented with Se when compared with the unsupplemented group. [7/45 (15.55%) in Se vs. 22/45 (48.88%) in placebo; $p = 0.001$] The ARR in the incidence of any sepsis was 33.33%, and the NNT was 3 (Table 2).

The all-cause mortality after 72 h of life, during the hospital stay or during follow-up for 4 weeks, was comparable in the two groups [2/45 (4.44%) in Se vs. 3/45 (6.66%); $p = 0.645$]. Three of the five deaths (both of Se group and one of placebo group) occurred outside our hospital. Babies were declared brought dead by the private clinics they were taken to, in two of the deaths. In the third case, the baby expired after getting treatment for 2 days.

Table 2. Serum Se levels and outcome variables

Outcome measure	Se ($n = 45$)	Placebo ($n = 45$)	p value	Relative risk (95% CI)	ARR (95% CI)	NNT (95% CI)
Se levels (μg/l)						
Day 1	29.9 ± 8.7	32.5 ± 20.1	0.388			
Day 28	63.9 ± 13.9	40.9 ± 17.3	**<0.01**			
Culture-proven sepsis	0	6 (13.33%)	**0.030**	–	13.33 (2.7–26.1)	7.5 (4–36)
Probable sepsis	7 (15.55%)	16 (35.56%)	**0.020**	0.437 (0.019–0.960)	20 (1.91–36.57)	5 (3–52)
Any sepsis	7 (15.55%)	22 (48.88%)	**0.001**	0.318 (0.141–0.669)	33.3 (14.12–49.46)	3 (2–7)
All-cause mortality	2 (4.44%)	3 (6.66%)	0.645	0.666 (−0.0904–3.80)	–	–
ROP					–	–
Stage I	2 (4.44%)	2 (4.44%)	1	1 (−0.108–6.79)		
Stage II	3 (6.66%)	6 (13.33%)	0.291	0.5 (−0.0657–1.87)		

Note. CI = confidence intervals.

In the two deaths that occurred in our hospital, both of the placebo group, sepsis screen was positive in both the cases, while blood cultures were sterile (Table 2).

Incidence of ROP was again comparable in the two groups, and none of the babies needed treatment. In our study, 13 patients developed ROP, which involved Zone 2 in all the cases. In 4 of 13 patients, a maximum of Stage I was reached [2/45 (4.44%) in Se vs. 2/45 (4.44%) in placebo group; $p = 1.000$]. In the remaining nine patients, a maximum of Stage II was reached [3/45 (6.66%) in Se vs. 6/45 (13.33%) in placebo group; $p = 0.291$] (Table 2).

The incidence of meningitis was found to be comparable in the two groups [0/45 in Se vs. 3/45 (6.66%) in placebo group; $p = 0.240$]. Of these three neonates, two were affected by *Enterococcus* and *Citrobacter*. The third neonate had a negative blood culture.

The mean duration of NICU stay was significantly lesser in the group that had received Se as compared with the placebo [94.93 ± 57.19 h in the Se group vs. 137.6 ± 104.66 h in the placebo group; $p = 0.010$]. Overall, Se was a well-tolerated drug with no apparent side effects noted. In fact, the neonates who received oral Se suffered much less from the episodes of feed intolerance [11/45 (24.44%) in Se group vs. 23/45 (51.11%) in placebo; $p = 0.010$].

DISCUSSION

Our study found a significant reduction in the incidence of LOS by using Se supplementation among VLBW neonates. However, the all-cause mortality and incidence of ROP was comparable between the two groups. The present study also documented these VLBW neonates are Se deficient at birth. Their Se levels at 31.1 ± 14.8 μg/l were well below the acceptable normal levels of 50–150 μg/l [8].

The Food and Nutrition Board recommends a RDA of Se for full-term infants as 15 μg/day [18]. There is no recommendation for preterm neonates. An earlier study that supplemented VLBW preterm neonates with Se at 5 μg/day did not result in achieving Se levels in the normal range [19]. As infants enrolled in the present study were VLBW preterm neonates, we took a considered decision to supplement at 10 μg/day to be on the safer side.

In a previous study, 36 preterm [<1500 g; mean (SD) 975 (122) g and gestational age 27 (1) weeks] infants were randomly assigned to the Se supplementation and control groups. In the supplemented group ($n = 18$), infants received 4.8 mg of Se-enriched yeast containing 5 μg Se daily. In the supplemented group, the serum Se concentration increased from 36.1 (± 12.8) μg/l to 43.5 (7.9) μg/l, and in the non-supplemented group, it decreased from 34.4 (20.4) μg/l to 26.1 (16.6) μg/l from birth in 2 weeks [19]. In our study, Se supplementation at 10 μg/day increased serum Se levels significantly (63.9 ± 13.9 μg/l on Day

28 in Se vs. 40.9 ± 17.3 on Day 28 in placebo; $p < 0.01$), and resulted in getting the Se levels into the acceptable normal level (50–150 μg/l). The more effective rise in Se achieved in the present study was possibly because of higher supplementation at 10 μg/day vs. 5 μg/day used in the earlier study. Although that study achieved a rise of Se levels to 43.5 μg/l by using 5 μg/day, it did not manage to bring it to the acceptable normal level (50–150 μg/l). Therefore, 10 μg/day is possibly a more effective dose for supplementation in the VLBW neonates.

The reported Se levels in the Indian full-term neonate are 54.17 ± 3.4 μg/l [9, 10]. The present study documented that the Se levels in preterm VLBW neonates were 31.1 ± 14.8 μg/l, which are considerably lower than the acceptable normal levels of 50–150 μg/l. Preterm VLBW neonates, we know, are more vulnerable to oxygen free radical injury, and Se supplementation for them makes great sense. There is no data on Se levels in the preterm VLBW Indian neonate, the present report being the first.

In a study of 38 preterm infants receiving parenteral nutrition, 19 were randomized to Se supplementation and 19 to placebo. Mean birth weight was 1129 (±42) g in the Se group and 1211 (±65) g in the placebo group. Mean gestational age was 29.0 (±0.5) weeks in the Se group and 28.0 (±0.5) weeks in the placebo group. Those receiving 3 μg/kg/d of Se had a lower incidence of sepsis than nonsupplemented infants (42%; $n = 8$ vs. 79%, $n = 15$; $p < 0.05$) [20]. In another study, a total of 529 infants were studied, 268 being randomized to Se supplements and 261 to placebo. Mean birth weight was 1047 (±255) g in the Se group and 1039 (±270) g in the placebo group. Mean gestational age was 28.0 (±2.3) weeks in the Se group and 27.8 (±2.5) weeks in the placebo group [21]. Infants randomized to treatment received 7 μg/kg/d of sodium selenate added to the amino-acid solution when fed parenterally and 5 μg/kg/d of sodium selenite (0.5 ml/kg of a colorless 10 μg/ml solution) when fed enterally. Fewer infants in the Se group had an episode of nosocomial sepsis after the first week of life (25.1 vs. 33.3%; $p = 0.038$). The result obtained in our study, showing significant reduction in the culture-proven sepsis, is consistent with that of the previous studies. The mechanism of the observed beneficial effects of Se in LOS remains unclear. Decreased serum levels of soluble L-selectin are found among patients with severe sepsis and has been linked to increased mortality. Soluble L-selectin is biologically active and may prevent leukocyte adhesion to endothelial

cells. Therefore, its decreased levels may lead to an overwhelming recruitment of leukocytes within the microcirculation. Once the local release of cytokines and production of reactive oxygen species have reached a certain level, there may be severe damage to local tissues, potentially leading to end-organ failure. Se supplementation leads to increased shedding of L-selectin from monocytes, thereby resulting in increased levels of soluble L-selectin, which may represent the underlying mechanism of therapeutic effect of Se on the inflammatory response in patients with severe sepsis [22].

In this study, Se supplementation in VLBW infants did not improve neonatal ROP outcome. There are a number of possible explanations why Se supplementation did not give much benefit. First, higher doses of Se supplementation might have been required. Alternatively, tissues such as the retina may maintain sufficient concentrations of glutathione peroxidases despite plasma Se concentrations being low. Supplementation increases plasma GPx (which is made in the kidney) only slightly, whereas total Se concentrations get almost doubled [21]. Animal studies have shown that key Se-dependent enzymes are preferentially conserved, and different organs show differing responses to Se deficiency [23]. Also, antioxidant protection involves both enzymatic and nonenzymatic components, and it may be that combinations of antioxidant therapies are required to prevent free radical injury [24]. The animal diseases associated with Se deficiency frequently respond better to a combination of Se and vitamin E [25].

MERITS OF THE STUDY

The strength of our study is its robust design, which ensured adequate double-blinding and elimination of the investigator/observer bias. Our study setting was a tertiary care hospital catering to subjects from all strata of the society. There was less than the expected loss to follow-up. Moreover, this attrition was almost similar in the two groups.

LIMITATIONS OF THE STUDY

Our study has a few limitations. Firstly, our study results can be extrapolated only to neonates with a similar clinical and demographic profile in a similar setting of a developing nation like ours. Secondly, our study subjects included children with birth weight ranging between 1000 grams and 1499 grams. Neonates <1000 grams who could benefit from this drug were not studied. So, the role of Se in this group of neonates cannot be commented on. Thirdly, our study has been done in a geographical area recognized as having low soil and population concentrations of Se. There are good theoretical reasons to expect that, if Se supplementation does result in decreased morbidity, this would be more readily apparent in studies from populations with low Se concentrations. Nevertheless, the fact that no large trials have been carried out in populations with higher concentrations does mean that the results may not be generalizable. Lastly, our follow-up period is limited to 1 month only. A longer follow-up period would have been desirable to effectively monitor for any long-term adverse effects associated with the use of Se in the neonates.

CONCLUSION

Preterm VLBW infants had Se levels considerably below the normal at birth. Se supplementation in these infants at 10 μg/day resulted in bringing the Se levels in the normal range. Preterm VLBW infants supplemented with Se at 10 μg/day had a significantly lesser incidence of culture-proven LOS compared with the placebo.

Good Se nutrition may therefore be especially important to this group of infants and must be instituted alongside other nutritional supplements.

GUIDELINES WERE FOLLOWED DURING THE COURSE OF THE RESEARCH

Consort guidelines.

LICENSE STATEMENT

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