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# Zinc supplementation in neonatal bronchopulmonary dysplasia: Is it beneficial?

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## Abstract

**Background: Neonatal Broncho Pulmonary Dysplasia (BPD) is a chronic chest disease that is caused by prolonged ventilation and oxygenation, leading to sever neonatal disability.**
**Aim of the study: Determination the role of zinc supplementation in neonatal bronchopulmonary dysplasia.**
**Patients and methods: Prospective Randomized Clinical Trial (RCT) study which was done at Tanta University Hospital (TUH) from July 2016 to March 2018 on 100 preterm neonates who had Respiratory Distress (RD) and were put on Mechanical Ventilation (MV). The studied neonates were divided into 2 groups: Group 1, which received Zn supplementation, and group 2, which received placebo. Urinary β2-Microglobulin (B2M) and plasma Krebs van den Lungen-6 (KL-6) levels were estimated on the 3rd day and the10th day of presence in the hospital.**
**Results: Significant decline in urinary B2M and plasma KL-6 levels occurred in neonates of group 1 who were given Zn compared with those in group 2 neonates, who received placebo (P<0.05). There was a significant decline in the time of incubator stay in group 1 neonates, compared with that of group 2 neonates (P<0.05). Group 1 neonates showed a significant decline in the development of established cases of BPD if compared with group 2 (P<0.05).**
**Conclusion: Zn supplementation is beneficial in the prevention of neonatal BPD.**

**Keywords:** Neonate, Zinc, Bronchopulmonary dysplasia.

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## Introduction

BPD is a respiratory disease which occurs mainly in premature neonates who are exposed to MV and high O2 therapy. BPD is a chronic chest disease where the normal structure of the lung is replaced by fibrous tissue causing neonatal RD [1,2]. BPD lead to a chronic O2 requirement due to prolonged O2 exposure to more than 28 days with chest X-rays revealing constant opacification or increased density [3].

BPD is classified as mild, moderate and severe according to the severity of the disease and the O2 requirement of neonates [4]. Mild BPD is aneonatal treatment with >21% O2 for at least twenty-eight days in addition to the ability to keep in room air without RD at discharge. Moderate BPD is aneonatal treatment with >21% O2 for at least twenty-eight days in addition to the ability to respire <30% O2 without RD at discharge [3]. Finally, severe BPD is a neonatal treatment with >21% O2 for at least twenty-eight days in addition to the need for ≥ 30% O2 for normal respiration without RD at discharge [4].

KL-6 is a glycoprotein that is formed by type II pneumocytes, its levels is increased in chronic lung diseases and is considered as an excellent predictor marker for the occurrence of neonatal BPD [5]. B2M is a protein that is present on the cell surface, especially the lung cells and is secreted by B lymphocytes into the serum. B2M is found in various body fluids, mainly the urine. Urinary B2M level is a non-invasive marker for neonatal BPD [6].

Zinc (Zn) is a trace element that is important for a various body function especially in neonates. Zn is required for the synthesis of many enzymes involved in many essential metabolic patterns (*i.e.*, protein formation, integrity of immune functions). Zn is essential to activate T lymphocytes (T cells) in the body [7,8]. Preterm neonates have lower Zn reserves than those in term neonates as most of the Zn which transferred from the mother to the foetus occur in the 3rd trimester of pregnancy, [9,10] in addition to the defective Zn absorption (due to immature digestion and absorption) with increasing the Zn requirements in preterm neonates to catch up growth. Thus, premature neonates need high zinc requirements [11,12]. The aim of this study was to show the role of zinc as adjuvant therapy in the prevention and management of subsequent BPD in those neonates.

## Patients and Methods

This research was a prospective RCT that was performed at TUH from July 2016 to March 2018 on 100 neonates suffering from RD needing MV and exposed to FIO2>21%, Ethical Committee of the college of Medicine, TUH approved it, and informed consent was taken from the fathers of the studied neonates. The examined neonates who are 100 cases were

divided into 2 groups, in which group 1 consisted of 50 preterm neonates who received Zn supplementation, and group 2 consisted of 50 preterm neonates who received placebo and lottery method were done for simple random sample.

Plasma KL-6 and urinary B2-microglobulin were measured at the 3rd and 10th days of admissions and used as markers for the presence of neonatal BPD.Established cases of BPD were diagnosed as a condition with a chronic O2 requirement due to prolonged O2 exposure with chest x-rays revealing constant opacification or increased density [3].

### *Inclusion criteria*

Preterm neonates suffering from severe RD needing MV and exposed to FIO2>21%.

### *Exclusion criteria*

Full term neonates, neonatal sepsis, congenital anomalies, death (2 in group 1 and 4 in group 2) and transfer to another hospital (3 in groups 1 and 4 in group 2). In cases of death and transfer to another hospital, the cases were replaced by other neonates according to inclusion criteria to maintain 50 neonates in each group.

Group 1 (n=50): had received Zn as 0.6 cm of zinc origin/kg/day orally through orogastric tube divided into 2 doses (12 hours) which is equal 1.2 mg elemental zinc/kg/day orally for 7 days (zinc origin 10 mg/5 ml syrup is produced by Egyptian Group for pharmaceutical Industries “EGPI” for: Origin international pharma, every 100 ml syrup contain 0.8793 g Zn sulfate hepta-hydrate which contain 0.2 g elemental Zn). Group 2 (n=50): Had received placebo (distilled water). The neonatal enteral intake recommendations of elemental Zn is from 0.8 in term to 3 mg/Kg/day in preterm neonates [13].

### *Determination of plasma KL-6*

The plasma KL-6 levels were detected using ELISA kits (Kamiya Biomedical Co. ®, USA) by the manufacturer's instructions using an Awareness Technology® (USA) ELISA Reader. The KL-6 concentration was expressed in U/ml.

### *Estimation of urinary B2M*

The urinary B2M level was estimated by ELISA using commercial kits (ORGENTEC Diagnostika®, Germany) in accordance with the manufacturer's instructions using Awareness Technology® (USA) ELISA Reader. The B2M concentration was expressed in mg/L.

**Statistical analysis:** The computer program which had been done for every statistical calculation was SPSS version 21, IBM, Armonk, NY, USA. Using the mean, standard deviation and chi-square test P<0.05 was considered as a significant.

## Results

The studied neonates were 100 who were divided into group 1 consisted of 50 preterm neonates who received Zn, and group 2 consisted of 50 preterm neonates who received placebo (Tables 1-4).

| | | Group 1 (n=50) | Group 2 (n=50) | Test | P value | | |
|---|---|---|---|---|---|---|---|
| Weight (g) | Mean ± S.D. | 1879.8 ± 96 | | 1883.9 ± 91 | T: 0.217 | 0.827 | |
| Gestational age (weeks) | Mean ± S.D. | 32.8 ± 1.2 | | 32.9 ± 1.25 | T: 0.408 | 0.684 | |
| Down score | Mean ± S.D. | 8.2 ± 0.16 | | 8.19 ± 0.12 | T: 0.352 | 0.724 | |
| Duration of mechanical ventilation (days) | Mean ± S.D. | 9.8 ± 0.2 | | 9.82 ± 0.14 | T: 0.581 | 0.564 | |
| Duration of exposure to FI02>21% (days) | Mean ± S.D. | 8.4 ± 0.2 | | 8.33 ± 0.18 | T: 1.835 | 0.069 | |
| | | N | % | N | % | X2 | P value |
| Mode of delivery | NVD | 14 | 28 | 15 | 30 | 0.052 | 0.826 |
| | CS | 36 | 72 | 35 | 70 | | |
| Sex | Male | 33 | 66 | 33 | 66 | 0 | 1 |
| | Female | 17 | 34 | 17 | 34 | | |

***Table 1.*** Comparative characteristics between studied groups. P: Significant if <0.05. NVD: Normal Vaginal Delivery, CS: Caesarean Section.

Table 1 showed group 1 which included 50 preterm neonates (32.8 ± 1.2 weeks) who weighed 1879.8 ± 96 grams, were admitted with severe RD with a Down score of 8.2 ± 0.16, were incubated on MV for a duration of 9.8 ± 0.2 days, exposed to FIO2>21% for 8.4 ± 0.2 days, and received Zn for 5 days from the 4th day to the 9th day of presence in the hospital. Table 1 showed also group 2 which included 50 preterm neonates (32.9 ± 1.25 weeks), who weighed 1883.9 ± 91 grams, were admitted with severe RD and a Down score of

8.19 ± 0.12, were incubated on MV for a duration of 9.82 ± 0.14 days, were exposed to FIO2>21% for 8.33 ± 0.13 days, and did not receive Zn. There were no significant contrasts in weight, gestational age, Down score, duration of MV, duration of exposure to FIO2>21%, mode of delivery and sex between group 1 and group 2, and the P- value was>0.05.

| | | Group 1 (n=50) | Group 2 (n=50) | t test | P value |
|---|---|---|---|---|---|
| Plasma KL-6 at the 3rd day (U/ml) | Mean ± S.D. | 101.8 ± 42 | 101.6 ± 40 | 0.023 | 0.981 |
| Plasma KL-6 at the 10th day (U/ml) | Mean ± S.D. | 79.1 ± 30 | 102.8 ± 41.8 | 3.263 | 0.002* |
| P value | | 0.002* | 0.884 | | |
| Urinary B2-microglobulin at the 3rd day (mg/L) | Mean ± S.D. | 3.3 ± 1.8 | 3.1 ± 1.6 | 0.586 | 0.558 |
| Urinary B2-microglobulin at the 10th day (mg/L) | Mean ± S.D. | 2 ± 1.3 | 3.3 ± 1.7 | 4.302 | 0.001* |
| P value | | 0.001* | 0.546 | | |

***Table 2.*** Plasma KL-6 and urinary B2-microglobulin at the 3rd and 10th days of admission.*P: Significant if <0.05.

Table 2 showed that in group 1, plasma KL-6 on the 3rd of presence in the hospital was 101.8 ± 42 U/ml, while plasma KL-6 at the 10th day of presence in the hospital was 79.1 ± 30 U/ml, with significant difference( P-value<0.05), while in group 2, plasma KL-6 on the 3rd day of presence in the hospital was 101.6 ± 40 U/ml, and the plasma KL-6 level at the 10th day of presence in the hospital was 102.8 ± 41.8 U/ml with no significant contrasts (P-value>0.05). Moreover, there were no significant contrasts in plasma KL-6 between the groups (P-value>0.05) on the 3rd day, but there was a significant difference in plasma KL-6 between group 1 and 2 (P-value<0.05) on the 10th day in the hospital.

Table 2 illustrated that in group 1, urinary B2M at the 3rd day of admission was 3.3 ± 1.8 mg/L, while urinary B2M at the 10th day of presence in the hospital was 2 ± 1.3 mg/L, with a significant decline at the 10th day in comparison to the 3rd day in the hospital (P-value<0.05). In group 2, urinary B2M at the 3rd day of presence in the hospital was 3.1 ± 1.6 mg/L, and urinary B2M at the 10th day of presence in the hospital was 3.3 ± 1.7 mg/L, with no significant contrast (P-value>0.05). Moreover, there were no significant contrasts in urinary B2M between groups 1 and 2 (P-value>0.05) on the 3rd day, but there was a significant difference in the urinary B2M between the two groups (P-value<0.05) at the 10th day in the hospital.

| | | Group 1 (n=50) | Group 2 (n=50) | t test | P value |
|---|---|---|---|---|---|
| Duration of stay of neonates in the incubator (days) | Mean ± S.D. | 22.5 ± 8.5 | 30.5 ± 9.5 | 4.439 | 0.001* |

***Table 3.*** Duration of the stay of neonates in the incubator.*P: Significant if <0.05.

Table 3 showed that the duration of incubator stays of neonates in group 1 was 22.5 ± 8.5 days, while the duration of incubator stays of neonates in group 2 was 30.5 ± 9.5 days, with a statistically significant difference between the two groups (P-value<0.05).

| | Group 1 (n=50) | Group 2 (n=50) | t test | P value |
|---|---|---|---|---|
| Number of neonates with established BPD | 3 (6%) | 12 (24%) | 6.35 | 0.012* |

***Table 4.*** The number of cases that developed BPD in both groups.*P: Significant if <0.05.

Table 4 showed that there were 3 cases of BPD in group 1, where there were 12 cases of BPD in group 2, with significant difference between group 1 and 2 (P-value<0.05).

## Discussion

BPD is a chronic neonatal lung disease that occurs mainly in preterm due to MV and prolonged high oxygenation [1].

KL-6is considered an excellent predictor marker for the occurrence of BPD [5]. Urinary B2M is are liable predictor marker in the prediction of the occurrence of neonatal BPD [14,15]. High oxygen exposure of the premature neonates will cause release of harmful oxidant products like oxygen-free radicals, these products will cause a chronic inflammatory

destructive process in the neonatal lung leading to occurrence of neonatal BPD, and this harmful process could be counteracted by safe antioxidants like Zn [16,17]. This study revealed that the levels of urinary B2M in neonates of group 1 who received Zn were declined after giving Zn indicating that Zn administration lead to decline in urinary B2M which is a good marker for the development of neonatal BPD.

This study revealed that the level of plasma KL-6 in group 1 neonates who received Zn was 101.8 ± 42 U/ml at the 3rd day of presence in the hospital. Moreover, there was a decline in the level of plasma KL-6 after giving Zn to 79.1 ± 30 U/ml at the 10th day of presence in the hospital, indicating that Zn administration had led to decline in plasma KL-6, which is a marker for the development of neonatal BPD; this finding was in comparison with the results of neonates in group 2, where there was an elevation of plasma KL-6 at the 10th day compared with the 3rd day of presence in the hospital. Zn maybe helpful in counteracting chronic inflammation of the lung tissue which occur in neonatal BPD as a result of prolonged exposure of neonatal lung tissues especially the premature to high levels of oxygen [18,19]. In agreement with our study, certain researches revealed that neonates with BPD and treated with Zn had lower levels of pro-inflammatory cytokines which are markers of chronic inflammation which occurred in the diseased lung tissues [20].

Some studies concluded that giving Zn was associated with decline in the inflammatory cytokines and improvement in the clinical outcome of BPD and these studies had attributed these beneficial results due to the anti-inflammatory effect of the Zn [21,22]. In agreement with the present study, a study stated that Zn could act as an antioxidant which protects the lung tissue from the damage by these harmful oxidants [23]. Urinary B2M levels are used as a good marker of chronic inflammatory chest disease or BPD, so B2M was used in this study as a reliable predictor for the neonatal BPD [24]. Many studies had proven that elevated urinary B2M and serum KL-6couldbe used as an excellent predictor for the early neonatal BPD diagnosis [25,26]. Serum levels of KL-6 are correlated with the occurrence and severity of neonatal BPD [27,28]. In another study, the cord blood and plasma levels of KL-6 proved to be a good marker for BPD [29,30]. Zn which is considered as antioxidant and anti-inflammatory trace element that could protect lung tissue against the harmful effect of harmful oxidants that released from lung exposure to high levels of O2 which will lead to occurrence of neonatal BPD [31-34].

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