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Effect of Enteral Administration of Granulocyte-Colony Stimulating Factor (G-CSF) on Feeding Tolerance in Very Low Birth Weight and Extremely Low Birth Weight Neonates; a Historical-Controlled Clinical Trial

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ABSTRACT

Background: The current study aimed to investigate the effect of enteral Granulocyte-Colony Stimulating G-CSF (Factor) on feeding tolerance in very low birth weight (VLBW) and extremely low birth weight (ELBW) neonates.

Methods: This historical-controlled clinical trial was conducted on VLBW and ELBW neonates admitted to Mahdieh Hospital, affiliated to Shahid Beheshti University of Medical Sciences, Tehran, Iran, between July 2016 and March 2017. In the intervention group, 81 neonates with birth weights of 710-1480 were given enteral 5 µg/kg/day of G-CSF (which has been approved by the US FDA) for 7 consecutive days. On the other hand, the control group included 191 neonates who did not receive G-CSF with birth weights of 600-1490 admitted during 24 months prior to the study. The two groups were compared in terms of adverse effects of treatment, primary and secondary outcomes.

Results: The mean of gestational age and birth weight in the G-CSF group were reported as 29.96±2.47 weeks and 1204.81±201.68 grams, and these values in the control group were measured at 29.77±2.13 weeks and 1189.47±207.89 grams, respectively. Neonates who received G-CSF demonstrated better feeding tolerance, as reflected by the earlier achievement of 50, 75, 100, full enteral feeding of 150, and maximal enteral feeding of 180 mL/kg/day ($p < 0.05$), with earlier weight gain and a shorter hospital stay. The rate of necrotizing enterocolitis (NEC) in the G-CSF group was measured at 3.7% that was significantly lower, as compared to the control group (P=0.005). Approximately 8.9% of the neonates in the control group expired which was higher than the G-CSF group (P=0.06). All neonates tolerated the treatment and there was no statistically significant difference between the two groups.

Conclusion: As evidenced by the obtained results, the enteral administration of G-CSF to VLBW and ELBW neonates improved feeding tolerance and it was well tolerated without any associated side effects.

Keywords: Granulocyte colony-stimulating factor, Feeding tolerance, Neonate, Very low birth weight

Introduction

Feeding intolerance is one of the most common problems in premature neonates admitted to the neonatal intensive care unit (NICU) manifested as abdominal distention, vomiting, diarrhea, and bloody stool (1-3) and affects the length of hospital stay (4). Withholding enteral feeding defects the development of the gut and causes mucosal atrophy and additional feeding intolerance after restarting the milk (4). Some studies were focused on the trophic effects of growth factors, such as insulin-like growth factor-1(IGF-1), interleukin-8(IL-8), granulocyte colony-stimulating

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factor (G-CSF), and erythropoietin (EPO), on the growth and development of the gastrointestinal system (5-8). Recent studies have focused on Granulocyte-colony stimulating factor (G-CSF) and erythropoietin (EPO) as recombinant human factors (9-11).

G-CSF and EPO which are present in amniotic fluid, colostrum, and human milk promote the growth and development of gut villi by attaching to their intestinal receptors (12). Efficacy and safety of enteral GCS-F, good tolerance without absorption, and any systemic effects have been demonstrated in some studies (13, 14). G-CSF has been associated with rare complications, such as bone pain, fever, allergic responses, drowsiness, and increased enzymes of lactate dehydrogenase (LDH) and alkaline phosphatase (11-13).

The effect of enteral G-CSF on the improvement of feeding tolerance (15), prevention and treatment of feeding intolerance (16), prevention and treatment of necrotizing enterocolitis (NEC) has been investigated in preterm neonates (17). In a study conducted by El-Ganzoury, it was revealed that neonates with G-CSF demonstrated better feeding tolerance and reached full feeding (150mL/kg/day) earlier than the control group. In these groups, hospital stay and necrotizing enterocolitis (NEC) risk were significantly lower, as compared to the control groups (18).

A few studies have been performed on the relationship between G-CSF and feeding tolerance with a small sample size and different dosages (18-20). No previous data is available on the effect of enteral G-CSF on feeding tolerance in very low birth weight (VLBW) and extremely low birth weight (ELBW) neonates. Therefore, the present study aimed at investigating the effect of G-CSF on feeding tolerance in VLBW and ELBW neonates.

Methods

Study design

This historical-controlled clinical trial was conducted in Mahdieh Hospital affiliated to Shahid Beheshti Medical University (SBMU), Tehran, Iran, between July 2016 and March 2017. The participants were provided with detailed information regarding the intervention. Written informed consent was obtained from parents. The study was performed according to Good Clinical Practice and Declaration of Helsinki principles. Approval was obtained from the Ethics Committee of SBMU in 2016 (IR.SUMS.SM.REC.1395.12). The trial has been registered at Iranian Registry of Clinical Trials (IRCT2016051427886N1, https://www.irct.ir/trial/22754).

Study population

The study population included 321 eligible premature neonates with a lower than 1500 gr birth weight who were admitted to the NICU of Mahdieh Hospital. Thereafter, 89 neonates who were born in a nine-month period were assigned to the G-CSF group and 232 neonates who were born during two years before the study (July 2014-July 2016) were regarded as the control group. Neonates with congenital or acquired anomalies of the gastrointestinal tract (e.g., omphalocele, gastroschisis, tracheoesophageal, gastrointestinal perforation, intestinal obstruction, necrotizing enterocolitis), other congenital major anomalies (e.g., congenital cyanotic heart disease, neural tube defect, diaphragmatic hernia, trisomies), history of asphyxia, Apgar Score≤ 6 at fifth minute, and death during first two weeks (death before the start of oral feeding) were excluded from the study.

Procedures

All the neonates who participated in our study had a documented perinatal history, had undergone a clinical examination and had received standard neonatal care. Laboratory investigations included a complete blood count (CBC), blood culture, serum electrolytes c-reactive protein and the number of white blood cells (WBC) and neutrophils were measured at baseline and on days 10-14.

Based on ward policy, all neonates were nothing per os (NPO) during the first day and received the serum, parenteral nutrition, antibiotics, and some type of respiratory support as indicated. Upon admission, demographic information of all neonates was recorded. This information included gender, gestational age, birth weight, delivery mode, prenatal complications (gestational diabetes (GDM), premature rupture of membrane (PROM), intrauterine growth retardation (IUGR) and preeclampsia/hypertension), as well as Apgar score.

Intervention

In the intervention group, the neonates received enteral G-CSF, a single daily dose of recombinant human granulocyte colony-stimulating factor (rhG-CSF), 5μg/kg/day (Filgrastim 300 μg /0.5mL, PooyeshDarou Biopharmaceuticals Company, Tehran, Iran). The daily dose for each neonate was diluted with 0.5 mL sterile distilled water in accordance with the manufacturer's instructions and kept in a separate opaque aliquot at 2-8º C. Thereafter, it was allowed to warm to

room temperature before administration by nasogastric/orogastric tube concurrent with feeding since the starting day and continued for 7 days. Simultaneously, minimal enteral nutrition (breast milk, formula or mixture of them, 20mL/kg/day) was initiated and increased gradually per day, as tolerated. On the other hand, in the historical control group, enteral feeding began according to the ward's feeding policy without any intervention.

Feeding policy

Ward feeding follows a policy that initiates oral feeding for infants without respiratory distress after 24 hours and for neonates with respiratory distress after the signs of stabilization are observed at least three days after birth. The unit's feeding policy was to initiate early trophic feeding beginning with 10-20 mL/kg/day, ideally breast milk if available, and advancing by 10-20 mL/kg/day for as long as tolerated (4) (as judged by the neonatologist). This approach was applied during the study period.

Feeding intolerance is defined as the presence of some factors which interfere with the written enteral feeding plan. They include emesis, increased abdominal girth, abdominal distension, gastric residual of ≥25% of the previous feed volume, or the presence of macroscopic blood in stools. Based on the reported documents, no specific value was required for an increase in abdominal girth or volume of emesis (21).

Withholding feeding is indicated in cases of feeding intolerance with a gastric residual ≥25% of the previous feed volume, suspected or proven NEC, heavily bile-stained or large gastric residuals or vomiting, clear abdominal pathology, significant abdominal distension or discoloration, blood in stool, or an unstable condition causing clinical concern, including significant cardiorespiratory instability.

Outcomes and measurements

In the present study, 150 and 180 mL/kg of milk were regarded as full and maximal enteral feedings, respectively. Therefore, the primary outcome was defined as the mean time required to reach full and maximal feeding with milk. Feeding tolerance data were recorded, including times to reach 50mL/kg/day, 75mL/kg/day, 100mL/kg/day, full enteral feedings (150 mL/kg/day), and maximal enteral feedings (180mL/kg/day).

Secondary outcomes consisted of days of withheld feeding, age of feeding initiation, feeding intolerance rate, drug administration for feeding intolerance (ranitidine and/or domperidone), day of birth weight regain, mechanical ventilation, duration of parenteral nutrition, hospital stay duration, rate of NEC, rate of late-onset sepsis (LOS), age of beginning LOS, days of antibiotic therapy, number of antibiotics, and death after the second week.

The adverse effects of treatment were recorded, including emesis, increased gastric residual volume, increased abdominal girth, diarrhea, gastrointestinal bleeding, skin rash, leukocytosis, leukopenia, neutropenia, and c-reactive protein (CRP) value. All data derived from the G-CSF group and control group were recorded and compared.

Statistical analysis

The sample size was calculated considering a 95% confidence level, estimating error (d) of 2 days, an alpha of 0.05, a beta of 0.2, and a standard deviation of 5.3 days. Accordingly, the sample size was calculated at a minimum of 73 neonates in the intervention group and 220 newborns in the control group. The data were analyzed in SPSS software (version 16.0). Descriptive statistics (mean ±standard deviation) were used to present the data. Student's t-test was used to compare quantitative parametric variables between the two groups and Chi-square test was used for data analysis of qualitative variables. To investigate the effect of eternal G-CSF on feeding tolerance, survival analysis was performed by Cox regression analysis. A p-value less than 0.05 was considered statistically significant.

Results

A total number of 321 neonates were assessed for eligibility (89 and 232 neonates in G-CSF and control group, respectively). Thereafter, 8 and 41 neonates were excluded from the G-CSF group and control groups, respectively. On a final note, 81 neonates were included in the G-CSF group and 191 neonates were assigned to the control group (Figure 1).

Out of 81 neonates in the intervention group, 42(51.9%) were male and 39 (48.1%) were female. On the other hand, out of 191 neonates in the control group, 92 (48.2%) were male and 99 (51.8%) were female which was not statistically significant. There were no significant difference between G-CSF and control neonates in terms of gender, gestational age, birth weight, delivery mode, Apgar score, WBC baseline, WBC in the second week, neutrophil baseline, neutrophil in the second week, the rate of maternal

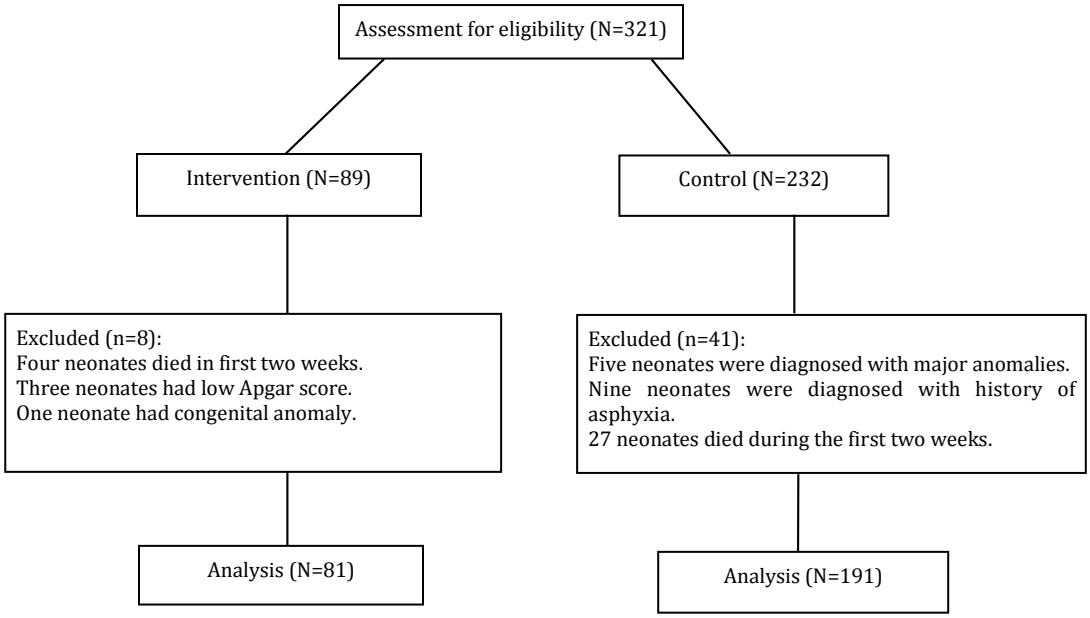

Figure 1. Flowchart of study

complications (diabetes, premature rupture of membrane, preeclampsia/hypertension, IUGR), transferred neonates, and multiple birth (Table 1). The mean gestational age in the GCSF and control groups were measured at 29.77 and 29.96 weeks, respectively. The general parameters of the two G-CSF and control groups are presented in Table 1.

The median days to reach 50, 75, 100, 150, and 180 mL/kg/day were 6.4±4.2, 9.2±5.6, 12.28±7.1, 16.9±8.9, and 21.4±10.5 days, respectively. Neonates who received G-CSF demonstrated better feeding tolerance, as reflected by the earlier achievement of 50, 75, 100, 150, and 180 mL/kg/day (P< 0.05). The likelihood of reaching the dose of 150 and 180 was 44% higher in neonates who received G-CSF, as compared to the control group. The result of the comparison of days to reach different volumes of milk feeding between the two G-CSF and control groups are presented in Table 2.

The median days to reach 150 mL/kg/day of milk in neonates under 1000 (500-999) grams in the G-CSF group was reported as 16.2±4.8 days, whereas this value was measured at 19.7±5.6 days in the control group, and this difference was statistically significant (P<0.001). In addition, the median days to reach 150 mL/kg/day of milk in 1000-1249 grams neonates in the G-CSF group was 11.6±2.9 days and in the control group was 14±4.3 days, and this difference was also statistically significant (P<0.004). Nonetheless, the median days to reach 150 mL/kg/day of milk in 1250-1500 grams neonates in the G-CSF group was measured at 10.4±2.8 days and in the control group was 11.8±3.1 days, and this difference was not statistically significant(P<0.07). The comparison of secondary outcomes between the two G-CSF and control groups is demonstrated in Table 3.

As illustrated in Table 3, although the mean days of withheld feeding due to feeding intolerance in the intervention group was shorter, as compared to the control group (1.88±2.1 vs. 3.6±1.3 days), the difference was not statistically significant (P=0.05). Moreover, there was no significant difference in the age of feeding commencement between the two groups. Feeding intolerance rate was lower in the G-CSF group and this difference was statistically significant (P=0.002). Compared to the G-CSF group, more neonates in the control group received treatment of intestinal dysmotility due to feeding intolerance which was statistically significant (P=0.002; Table 3).

As illustrated by the result of the study, age of regaining birth weight, mechanical ventilation, duration of parenteral nutrition, length of hospital stay, rate of LOS (clinical, probable and definite), age at the beginning LOS, days and numbers of antibiotic therapy between the two groups was statistically significant. The rate of NEC in the G-CSF group was lower, in comparison to the control group and this difference was statistically significant (P=0.005). About 8.9% of

Table 1. Comparison of general parameters between the two granulocyte-colony stimulating factor group and control group

Variable		Granulocyte-colony stimulating factor Group (n=81)	Control Group (n=191)	P-value
Gender, n (%)	Male Female	42(51.9) 39(48.1)	92 (48.2) 99(51.8)	NS*
Gestational age, w	Min-Max mean±SD	25-36 29.77±2.13	25-36 29.96±2.47	NS**
Birth weight, gr	Min-Max mean±SD	710-1480 1202±201	600-1490 1204±216	NS**
Delivery mode, n (%)	NVD C/S	11(13.6) 70(86.4)	32(16.7) 159(83.3)	NS*
Apgar score	1 5 minute	7.3 8.5	7.6 8.8	NS**
WBC baseline	mean±SD	8713±987	9380±1080	NS**
WBC in the second week	mean±SD	11146±1695	10453±1365	NS**
Neutrophil baseline	mean±SD	4427±670	5024±1010	NS**
Neutrophil in the second week	mean±SD	7206±1458	6404±1211	NS**
The rate of maternal complication (diabetes, premature rupture of membrane, preeclampsia / hypertension, IUGR), n (%)		36(44.4%)	89(46.6%)	NS*
Transferred neonates, n (%)	Yes No	19(23.5) 62(76.5)	43(22.5) 148(77.5)	NS*
Multiple births, n (%)	1 2 3 4	50(61.7) 19(23.8) 8(10) 4(5)	108(56.6) 51(26.7) 22(11.53) 10(5.24)	NS*

Abbreviations: n; number, w; week, gr; gram, SD; standard deviation, NVD; normal vaginal delivery, C/S; cesarean section
*Chi-squared test
**Student's t test

Table 2. Comparison of days to reach different volumes of milk feeding between the two granulocyte-colony stimulating factor group and control groups using cox regression.

Variable	Granulocyte-colony stimulating factor group (n=81)	Control group (n=191)	Hazard Ratio	95%CI	P-value
	mean ± SD				
Days to reach 50 mL/kg	5.38±3.46	6.92±4.80	1.39	1.06-1.83	0.02
Days of reach to 75 mL/kg	7.84±4.64	9.87±5.97	1.42	1. 09-1.87	0.01
Days to reach 100 mL/kg	10.35±5.58	13.09±7.73	1.39	1.09-1.88	0.009
Days to reach 150 mL/kg	14.33±7.48	18.17±9.50	1.44	1.10-1.90	0.008
Days to reach 180 mL/kg	18.24±9.41	22.87±10.83	1.44	1.09-1.89	0.009

Table 3. Comparison of secondary outcome between the two granulocyte-colony stimulating factor group and control group

Variables		Granulocyte-colony stimulating factor (n=81)	Control group (n=191)	P-value
Withholding feeding, d,	mean ± SD	1.88±2.1	3.6±1.3	0.05*
Age of start feeding, d	mean ± SD	3.54±1.69	3.76±1.92	0.1*
Feeding intolerance rate	n (%)	10(12.3)	57(30)	0.002**
Start drug for feeding intolerance (ranitidine and/or domperidone)	n (%)	10(12.3)	57(30)	0.002**
Regain birth weight, d	mean±SD	11.39±4.30	12.35±4.84	0.13*
Mechanical ventilation, d	mean±SD	1.77±3.12	3.93±7.87	0.28*
Duration of parenteral nutrition, d	mean±SD	16.74±8.11	20.70±9.97	0.21*
Hospital course, d	mean±SD	31.80±13.73	34.41±16.05	0.34*
Rate of NEC	n (%)	3(3.7)	40(21)	0.005**
Rate of LOS	n (%)	46(57)	115(60)	0.36**
Age at the beginning LOS, d	mean±SD	16.58±4.2	15.4±3.9	0.44*
Days of antibiotic therapy	mean±SD	14.96±8.6	18.87±14.9	0.22*
Numbers of antibiotics	mean±SD	3.35±1.41	3.95±1.97	0.05*

Abbreviation: d; day, n; number
*Student's t test
**Chi-squared test

Table 4. Comparison of adverse effects between the two granulocyte-colony stimulating factor group and control group

Variables		Granulocyte-colony stimulating factor (n=81)	Control group (n=191)	P-value
Emesis	N (%)	0(0)	12(6.3)	NS*
Increased gastric residual volume	N (%)	3(3.7)	8(4.2)	NS*
Increased abdominal girth	N (%)	4(4.9)	9(4.7)	NS*
Diarrhea	N (%)	4(4.9)	9(4.7)	NS*
Gastrointestinal bleeding	N (%)	1(1.2)	2(1.1)	NS*
Skin rash	N (%)	3(3.7)	8(4.1)	NS*
Leukocytosis >25000	N (%)	0(0)	5 (6)	0.02*
Leukopenia	N (%)	6(7.4)	15(7.8)	NS*
Neutropenia <1500	N (%)	0(0)	5(6)	0.02*
CRP value	mean ± SD	20.1±14.2	32.2±16.4	0.01**

*Student's t-test
**Chi-squared test

the neonates in the control group died and it was higher than the death rate in the G-CSF group (P=0.006; Table 3).

As displayed in Table 4, all neonates tolerated the treatment. In the control group, 6% of neonates had leukocytosis and severe neutropenia, while no leukocytosis and severe neutropenia were reported in the G-CSF group (P=0.02). Leukopenia was reported in both groups; however, there was no statistically significant difference. The mean of CRP level in the G-CSF group was lower than in the control group, and this difference was statistically significant (P=0.01; Table 4). The result of adverse effects is displayed in Table 4.

Discussion

Feeding intolerance is one of the most common problems in premature neonates admitted to NICU (4). Accordingly, the present study was designed to assess the effects of enteral G-CSF on the improvement of feeding tolerance in VLBW and ELBW neonates. It also aimed at investigating other benefits, such as decreasing withheld feeding days, age of feeding commencement, drug administration for feeding intolerance, mechanical ventilation, length of hospital stay, rate of NEC, rate of LOS, number of antibiotics, and rate of neonatal death. The presented data indicated that the enteral G-CSF improved feeding tolerance in VLBW and ELBW neonates and is well tolerated without any associated side effects.

Based on the results, neonates who received G-CSF had better feeding tolerance, as reflected by the earlier achievement of 50, 75, 100, 150, and 180 mL/kg/day. The mean days to reach 150 mL/kg/day of enteral feeding in the current study was 14.33±7.48 days in the G-CSF group and 18.17±9.50 days in the control group. The likelihood of reaching the dose of 50, 75, 100, 150, and 180 mL/kg/day was approximately 40% higher in the G-CSF group, in comparison to the control group. In accordance with these findings, in a study conducted by El-Ganzoury, enteral G-CSF was well tolerated for up to 7 days of administration. In comparison with the control group, the treatment group demonstrated a significantly (P<0.05) shorter time to achieve one-half (75 mL/kg/day), two-thirds (100 mL/kg/day), and full enteral feeding (150 mL/kg/day). The mean days to reach 150 mL/kg/day was calculated at 12.6±5.4 days in the G-CSF group, whereas it was measured at 16.3±5.3 days in the control group (18). In a study performed by Gad, the G-CSF group demonstrated better feeding tolerance as reflected by earlier achievement of 50, 100, 120 and full enteral feeding growth factors, including epidermal growth factor, G-CSF, and erythropoietin (EPO) (22), which have the practical advantage of being available as sterile human recombinant factors (23). with higher enteral caloric intake 7 days after the administration (P<0.05) (22). G-CSF presented in liquids swallowed by the fetus and neonate, specifically amniotic fluid, colostrum, and human milk (12, 24) is essential for normal small bowel development by attaching to their intestinal receptors (22). The ingested G-CSF is highly protected from digestion in the neonatal intestine and remains biologically active. It binds to specific receptors expressed on the surface of fetal villous enterocytes (18). However, the result of a study conducted by Calhounet et al. demonstrated that orally administered rhG-CSF is not absorbed in significant quantities. They speculate that the G-CSF swallowed by the fetus and neonate has local but not systemic effects (24).

The effect of enteral G-CSF was more obvious in neonates weighing 1250-1500 grams. Contrary to this finding, in the study carried out by El-Ganzoury, there were significant negative linear correlations between birth weight and the time

needed to achieve one-half (r = −0.414; P=0.001), two-thirds (r = −0.386; P=0.002), and full enteral feeding (r= −0.487; P< 0.001) (18).Since the results are conflicting, additional well-designed trials are needed to confirm these early results (25). The rate of feeding intolerance in neonates who received rhG-CSF was significantly less, in comparison to the control group (12.3% vs. 30%). In similar studies, it has been demonstrated that GCSF treatment could significantly reduce feeding intolerance (18, 26). Furthermore, in agreement with the findings reported in other studies, neonates in the treatment group had a shorter duration of feeding withholding, fewer medicines administered for feeding intolerance, fewer antibiotics (18, 20, 24, 27). Nonetheless, unlike the other studies (18, 20), the neonates in the G-CSF group did not have better weight gain, faster hospital discharge, or shorter duration of parenteral nutrition. These discrepancies can be ascribed to differences in the weight of the neonates, gestational age, sample size, and the dosage of the administered drug.

Another significant finding was the reduced incidence of NEC in the neonates who received enteral growth factors, as compared to the neonates in the control group (3.7 vs 21%). This is an exceptionally low figure not previously reported in the literature; in other words, we need to provide seven neonates with rhG-CSF to prevent one additional case of NEC. In line with our findings, in the study conducted by El-Ganzoury, the risk of NEC reduced from 10% to 0% in the rhG-CSF treatment group (P<0.05). In the study performed by Canpolat, enteral rhG-CSF treatment could prevent the progression of mild (stage I) NEC to further stages and decrease the time required for the resolution of clinical and radiological signs of the disease (20). In the pathophysiology of NEC, intestinal ischemia and bacterial colonization have been demonstrated to be the major factors leading to a common inflammatory process and tissue damage mediated by several cytokines. EPO-like growth factors and cytokines could play major roles in the growth, development, and protection of the GI tract on the immature intestine in experimental NEC studies (20, 28). One of the important cytokines is G-CSF and the presence of specific G-CSF receptors has been demonstrated in the fetal and neonatal GI tract (27). Studies have suggested that G-CSF levels increase in amniotic fluid by gestational age and simultaneously its receptors increase in the bowel villi. This factor has stimulating effects on intestinal growth and development, apart from its hematologic effects (18, 20, 29). Canpolat demonstrated the protective effect of enteral administration G-CSF on intestinal damage in an experimental rat model of NEC. Histopathologically, the lesions in the untreated rats were similar to those observed in neonatal NEC, with the destruction of villi and crypts and extension to the muscularis in some cases (20).

In the present study, the number of deaths was significantly fewer in the group receiving G-CSF, as compared to the control group (1.23% vs. 8.9%). In a similar study conducted by Gathwala et al. in India, GCSF treatment could significantly reduce all-cause mortality rate (30). In another research conducted in London, Russel et al. studied 28 neonates with birth weights of 500-1500 grams and the number of deaths was significantly fewer in the GCSF group (31).

All neonates tolerated oral diluted G-CSF and it caused no adverse effects since the safety of oral administration of G-CSF had been approved in previous studies (24, 32). The present study indicated that oral G-CSF did not affect the average number of WBC and neutrophils; however, the levels of neutropenia and leukopenia and CRP decreased during sepsis. These results are indicative of the probable hypothesis that orally administered G-CSF improves the immune system, while these results may cast doubt on previous studies performed on the inactivation of systemic oral G-CSF (18, 24).

Limitations of the study

Every study has limitations that should be addressed in the paper. Not to be an exception, the present study had some limitations. To begin with, the lack of random assignment into intervention and control groups may lead to non-equivalent test groups which can limit the generalizability of the results to a larger population. Apart from the lack of randomization and reduced internal validity, the conclusions about causality are less definitive in this study. In addition, very limited evidence exists concerning the safety of enteral G-CSF in VLBW and ELBW neonates. Another limitation of this study was the unknown prevalence of breastfeeding and formula feeding either alone or mixed with breast milk. Accordingly, it is recommended to investigate the effect of the growth factors on large randomized trials on feeding intolerance, NEC, and sepsis with and without absolute breastfeeding.

Conclusion

As evidenced by the results of the present

study, enteral G-CSF in VLBW and ELBW neonates improves feeding tolerance and decreases the age of reaching full (150mL/kg) and maximal (180 mL/kg) enteral feeding. Enteral administration of G-CSF was well tolerated without any associated side effects. To confirm these results, it is suggested that further studies be carried out with different doses, larger sample sizes, and longer duration of enteral administration of G-CSF.

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Conflicts of interests

The authors declare that they have no conflict of interest regarding the publication of the current paper.

Authors' contributions

MS is the main researcher, SAA, ARSh, and NKH are scientific advisor, LA is research assistant, and NKH is the approver of the manuscript.

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