HIGHER VERSUS LOWER PROTEIN PARENTERAL NUTRITION EFFECT ON SERUM CYSTATIN C IN PRETERM NEONATES; TWO CENTRES STUDY

By

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Running Title: Protein Parenteral Nutrition and Cystatin

ABSTRACT

Background: *Nutritional interventions are crucial to optimal outcomes in preterm neonates. The effect of early high protein administration for preterm neonates on glomerular function remains controversial.*

Objectives: *The primary objective is to study the effect of protein parenteral nutrition on serum cystatin C as a marker for renal function in preterm neonates. Secondary objectives are to compare the sensitivity of serum cystatin to serum creatinine in acute kidney injury in preterm neonates and to study the effect of protein parenteral nutrition on weight gain.*

Subjects and Methods: *A case-control study was conducted at the neonatal intensive care units of Ain Shams University Hospital and Manshet El Bakery Hospital. Eighty four preterm neonates 36 weeks gestation or less were enrolled during the period from March 2016 to March 2017; 28 neonates who received enteral feeding and did not receive PPN comprised the control group, 28 neonates received low PPN at Manshet EL Bakery Hospital and 28 neonates received high PPN at Ain Shams University Hospital. Complete blood count, serum creatinine and blood urea nitrogen, serum Na, serum K, pH were measured on day 3 and 7 of life. Serum cystatin C was measured by ELISA on day 7.*

Results: *On day 7 there were no significant differences regarding serum cystatin C between the high and low PPN groups (P=0.289,). There were no significant differences between the high and low PPN regarding weight gain till day 7.*

Conclusion: *Neither high nor low protein parenteral nutrition has significant effect on s. Cystatin C consequently high protein parenteral nutrition can be used to minimize weight loss without increasing the risk of metabolic acidosis or renal impairment in preterm neonates.*

Key words: *amino acids, acidosis, AKI.*

INTRODUCTION

Nutritional management of preterm infants varies widely, controversies exist regarding mode of feeding, when to initiate nutrition, energy requirements, and composition of enteral and parenteral feeds **(Moltu et al., 2021)**.

Early administration of adequate protein with optimal mixture of essential and nonessential amino acids (AA) is required to achieve a positive nitrogen balance leading to growth **(Burattini et al. 2013)**. This can decrease weight loss, improves neurodevelopmental outcome, and reduce the risk of mortality and later adverse outcomes, such as bronchopulmonary dysplasia and necrotizing enterocolitis **(Christmann et al., 2013** & **Moyses et al., 2013)**.

There has been a concern about high protein parenteral nutrition for fear of potential AA toxicity, uremia and metabolic acidosis. These complications were noticed during the earlier days of parenteral nutrition when the used solutions were unbalanced with a relatively high non-essential, potentially, toxic AA **(Thureen, 2003)**.

For glomerular function assessment, serum creatinine is only a rough estimate because it reflects changes with low sensitivity and specificity. Its value depends on muscular mass and thus it depends on age and sex and tubular secretion **(Delanaye et al., 2017)**.

The use of renal biomarkers could improve the early diagnosis of AKI (acute kidney injury) in preterm infants and guide response to therapy and potential impact of nephrotoxic medications **(Branagan et al., 2022)**.

Cystatin C (Cys C), a protein of the cysteine protease inhibitor family, is produced by all nucleated cells and is measurable in body fluids **(Donadio et al., 2001)**. It is a low molecular weight protein (13-kDa) that is

almost completely filtered by the glomerulus and largely catabolized by proximal tubular cells. Its concentration in adults is closely related to the glomerular filtration rate (GFR) **(Stickle et al., 1998)**.

Studies of the premature neonates have tried to establish normal reference ranges for Cys C **(Armangil et al., 2008)**. **Demirel et al., 2013** suggested that Cys C can overcome the reflection of maternal serum creatinine on neonatal creatinine.

AIMS OF THE WORK

The primary objective is to study the effect of protein parenteral nutrition on serum cystatin C as a marker for renal function in preterm neonates.

Secondary objectives are to compare the sensitivity of serum cystatin to serum creatinine in acute kidney injury in preterm neonates and to study the effect of protein parenteral nutrition on weight gain.

PATIENTS AND METHODS

Patients:

This case control study was conducted at the neonatal intensive care units (NICU) of Ain Shams University Hospital and Manshet El Bakery Hospital.

Ethical Consideration:

- An informed consent was obtained from parents of neonates before enrolment in the study.
- The study was ethically approved by the Council of the Pediatric Department, Faculty of Medicine, Ain Shams University.
- The data of the study are confidential, and the care giver has the right to keep it.
- The care giver has the right to refuse and withdrew from the study.
- The researcher explained to the caregiver the aim of the study.
- There is no conflict of interest regarding the study or the publication.
- The authors report no financial fund or support regarding the study or the publication.

Sample size:

Sample size was calculated and showed that examining 75 babies would have a power of 100% in detecting significant difference as regard s.Cystatin C level using a two-sided F-test with a confidence level of 99% (type 1 error, 0.01).

Inclusion criteria:

Preterm neonates 36 weeks gestation or less consecutively

admitted to NICU during the period from March 2016 to March 2017 were enrolled to the study.

Exclusion criteria:

Neonates with congenital renal anomalies, other significant congenital anomalies, perinatal asphyxia and those receiving nephrotoxic medications were excluded from the study.

Study Design:

The study included 84 neonates; 56 neonates who received early protein parenteral nutrition (PPN) during their admission to NICU comprised the PPN group and 28 neonates who received enteral feeding and did not receive PPN comprised the control group.

They were classified into:

Study Groups:

1. **Group 1:** Enteral feeding group; 28 neonates who started enteral feeding in the form of breast milk and did not need any PPN at the NICU of Ain Shams University Hospital.

PPN group was further subdivided to either:

2. **Group 2:** Low PPN group; 28 neonates who started PPN at 0.5-1 g/kg/day and gradually increased targeting 2 g/kg/day at the NICU of Manshet EL Bakery Hospital.
3. **Group 3:** High PPN group; 28 neonates who started PPN ≥ 2g/kg/day and gradually increased targeting 4 g/kg/day at the NICU of Ain Shams University Hospital.

Parenteral nutrition (PN) was started in the 2nd day of life, with 1.0 g/kg/day lipids an advanced by 0.5 – 1.0 g/kg/day targeting 3g/kg/day for both groups. Trophic breast milk feds of 10-20 cc/kg/day were given when tolerated and was gradually increased for both groups.

Initial assessment:

All the included neonates were subjected to comprehensive history taking, thorough clinical examination, and routine neonatal care. The gestational age was determined by maternal last menstrual period and further confirmed by using the Ballard.

Score **(Ballard et al., 1991)** the birth weight and the weight on day 7 was documented.

Laboratory investigations:

On day 3 and 7 of life, complete blood counts were done using Max M Coulter Beckman Coulter, Inc., 22 Raio Juste - Olivier, 1260 Nyon - Switzerlan, serum creatinine (s.Cr), blood urea nitrogen (BUN), serum Na (s.Na), serum K (s.K) were measured for all included neonates using

Synchron CX – 9 Delta autoanalyzer (Beckman Instrument Inc; Scientific Instrument Division, Fullerton, CA 92634, 3100, USA), pH were measured for all included neonates using ABL 800 blood gas analyzer. Glomerular filtration rate (GFR) was calculated according to Schwartz formula **(Schwartz et al., 2007)**. Serum cystatin C (s.Cys C) was measured on day 7 of life for all included neonates by ELISA technique using BioVendorVR Human Cystatin C ELISA kit (BioVendor, Czech Republic).

Statistical analysis:

Analysis of data was done using Statistical Program for Social Science version 21 (SPSS Inc., Chicago, IL). Qualitative data were presented as number and percentages while quantitative data were presented as ranges, mean and standard deviations or median and interquartile range. In order to compare quantitative parametric variables between three groups. Analysis of Variance (ANOVA) test with post hoc test was used for comparing the two groups, Student t-test was applied. Comparison of nonparametric variables was carried out using Mann-Whitney tests. Qualitative variables were compared using Chi-square (v2) test or Fischer's exact test when frequencies were below five. Receiver operating characteristic curve (ROC) was used to assess the best cut off point with its area under curve (AUC), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The confidence interval was set to 95% and the margin of error accepted was set to 5%. P value<.05 was considered significant in all analyses.

RESULTS

The results of our study will be demonstrated in the following tables and figures:

Table (1): Demographic data of all studied groups

	Group 1 Control N=28	Group 2 Low PPN N=28	Group 3 High PPN N=28	X^2/f	Overall P-Value	Low PPN vs. control	High PPN vs. control	Low vs. high PPN
Male gender N (%)	16(57)	13(46)	14(50)	2.111	0.670	0.550	0.550	0.801
GA (Weeks)	31.2±1.26 29-34	32.32±0.99 29-34	30.64±1.73 29-34	8.777	0.425	0.120	0.444	0.314
Birth weight (Kg)	1.78±0.36 1.42-2.14	1.88±0.32 1.65-2.20	1.75±0.39 1.34-2.14	5.690	0.785	0.882	0.993	0.539
PROM N (%)	16(64)	14(56)	15(60)	1.276	0.400	0.283	0.399	0.863
CS Delivery N (%)	16(57)	19(68)	21(75)	0.621	0.714	0.536	0.446	0.985

GA (Gestational age), PROM (Premature rupture of membranes), CS (Cesarian section).
P > 0.05: Non-significant; P < 0.05: Significant; P < 0.01: Highly Significant.

This table shows insignificant difference regarding demographic data between the three groups.

Table (2): Comparison between Low and High PPN Groups Regarding the Administrated Total Parenteral Nutrition on Day 3

Day 3	Group 2 Low PPN N =28	Group 3 High PPN N =28	t	P-Value
Protein (g/kg/d) Mean±SD	1.45±0.54	2.63±0.73	-5.540	0.004
GIR (m/kg/min) Mean±SD	4.39±0.49	4.76±0.86	-1.685	0.497
Fluids (mL/kg/d) Mean±SD	88.63±9.00	91.36±13.07	-3.074	0.734

GIR (Glucose infusion rate)

Table (3): Comparison between Low and High PPN Groups Regarding the Administrated Total Parenteral Nutrition on Day 7

Day 7	Group 2 Low PPN No. =28	Group 3 High PPN No. =28	t	P-Value
Protein (g/kg/d) Mean±SD	2.08±0.36	4.32±0.21	-10.093	0.034
GIR (m/kg/min) Mean±SD	7.24±0.81	7.13±0.74	0.468	0.537
Fluids (ml/kg/d) Mean±SD	157.45 ±9.77	168.36±26.17	-9.630	0.843

GIR (Glucose infusion rate)

Table 2 and **Table 3** show insignificant difference between low and high PPN groups regarding the administrated total parenteral nutrition on day 3 and on day 7; low PPN group started PPN at 0.5-1 g/kg/day and gradually increased targeting 2 g/kg/day while high PPN group started PPN ≥ 2 g/kg/day and gradually increased targeting 4 g/kg/day. Lipids started on a dose of 1.0 g/kg/day and advanced by 0.5 – 1.0 g/kg/day targeting 3g/kg/ day for both groups.

Table (4): Weight changes among the studied groups

		Group 1 Control N=28	Group 2 Low PPN N=28	Group 3 High PPN N=28	X^2	Overall P-Value	Low PPN vs. Control	High PPN vs. Control	Low vs. High PPN
Birth weight (kg)	Mean±SD Range	1.78±0.36 1.42-2.14	1.88±0.32 1.56-2.20	1.75±0.39 1.34-2.14	5.690	0.785	0.882	0.993	0.539
Weight on day 7 (kg)	Mean±SD Range	1.64±0.31 1.42-2.14	1.77±0.31 1.56-2.20	1.68±0.37 1.31-2.05	5.080	0.991	0.998	0.999	0.995
Increment of the weight (kg)	Mean±SD Range Percent of change	- 0.18±0.05 -0.24-0.20 -9%	- 0.11±0.01 -0.10-0.12 -7%	- 0.07±0.02 -0.05-0.09 -4%	5.187	0.000	0.000	0.000	0.000

Table 4 shows comparison between the study groups regarding weight changes and revealed that there was weight loss with highly significant difference in the three study groups, but it was more in the control group followed by the low PPN group with the least weight loss noticed in the high PPN group.

Table (51): Laboratory Results among the Studied Groups on Day 3

	Control N=28	Low PPN N=28	High PPN N=28	X^2/f	Overall P-Value	Low PPN vs. control	High PPN vs. control	Low vs. high PPN
Hb (g/Dl)	14.32±3.35	12.37±2.12	13.43±2.58	1.737	0.307	0.221	0.352	0.211
PLT ($\times10^3$/µL)	210.48±34.53	224.16±78.30	245.68±73.35	0.914	0.405	0.415	0.288	0.334
WBC ($\times10^3$/µL)	8.06±3.37	7.86±5.14	9.29±2.43	1.030	0.362	0.871	0.145	0.214
pH	7.38±0.11	7.38±0.15	7.35±0.08	1.503	0.764	0.272	0.966	0.704
s.Na (mEq/L)	135.08±4.35	138.20±3.95	139.33±5.42	4.308	0.861	0. 858	0.828	0.81
s.K (mEq/L)	3.95±0.39	4.52±0.47	4.02±0.35	2.923	0.847	0.868	0.939	0.781
s.Creatinine (mg/dL)	0.77±0.11	0.82±0.11	0.76±0.08	6.800	0.148	0.199	0.147	0.108
BUN (mg/dl)	13.64±3.82	12.24±3.00	11.84±3.14	5.240	0.618	0.860	0.544	0.919
GFR (mL /min/1.73 m^2)	18.49±2.99	16.73±2.52	16.97±2.29	5.080	0.992	0.991	0.991	0.937

Hb (haemoglobin), PLT (platelets), WBC (white blood cells count), BUN (blood urea nitrogen) and GFR (glomerular filtration rate).

This table shows insignificant difference between the three study groups regarding Hb, PLT, WBC, PH, s.Na, s.K, s. Creatinine, BUN and GFR on day 3.

Table (62): Laboratory Results among the Studied Groups on Day 7

	Group 1 Control N=28	Group 2 Low PPN N=28	Group 3 High PPN N=28	X^2/f	Overall P-Value	Low PPN vs. Control	High PPN vs. Control	Low vs. High PPN
Hb (g/dL)	12.32±1.35	11.86±1.24	11.40±1.16	1.946	0.012	0.017	0.007	0.005
PLT ($\times10^3/\mu L$)	257. 6±67.53	248.36±62.46	244.63±61.04	2.783	0.319	0.246	0.119	0.315
WBC ($\times10^3/\mu L$)	11.39±3.46	10.1±2.09	10.63±2.01	4.664	0.987	0.998	0.982	0.486
pH	7.38±0.11	7.30±0.13	7.29±0.15	2.133	0. 875	0.831	0.663	0.815
s.Na (mEq/L)	140.36±3.90 136-144	138.84±2.89 136-142	137.16±3.07 135-141	5.280	0.388	0.386	0.931	0.990
s.K (mEq/L)	4.45±0.73	4.09±0.09	3.97±0.37	4.240	0.998	0.937	0.801	0.621
s.Creatinine (mg/dL)	0.67±0.12	0.75±0.25	0.75±0.24	2.481	0.148	0.103	0.118	0.143
BUN (mg/dL)	12.44±2.26	11.92±2.41	11.63±2.25	4.520	0.201	0.336	0.249	0.532
GFR (ml/ min/1.73m^2)	21.66±4.43	20.17±4.82	22.13±3.27	4.640	0.301	0.324	0.147	0.156

Hb (haemoglobin), PLT (platelets), WBC (white blood cells count), BUN (blood urea nitrogen) and GFR (glomerular filtration rate).

This table shows insignificant difference between the three study groups regarding Hb, PLT, WBC, PH, s.Na, s.K, s. Creatinine, BUN and GFR on day 7.

Table (73): S. Cystatin C among the Studied Groups on Day 7

	Group 1 Control N=28	Group 2 Low PPN N=28	Group 3 High PPN N=28	X^2/f	Overall P-Value	Low PPN vs. Control	High PPN vs. Control	Low vs. High PPN
s. Cystatin C (mg/L)	1.60±0.35	1.33±0.32	1.27±0.23	6.920	0.289	0.193	0.419	0.709

This table shows insignificant difference between the three study groups regarding s. Cystatin C on day 7.

ROC curve was constructed and showed that s.Cystatin C can predict acute kidney injury, the area under the curve (AUC) was 0.762, at cutoff point of > 1.4 mg/l, with sensitivity of 82.14% and specificity of 64.29%. On contrast, s. creatinine has poor sensitivity and specificity (71.43% and 50.00% respectively).

DISCUSSION

Potential benefits of higher parenteral AA intake for improved nitrogen balance, growth, and infant health may be outweighed by the infant's ability to utilise high intake of parenteral AA, especially in the days after birth **(Osborn et al., 2018)**.

Amino acids should be provided soon after birth in order to prevent protein breakdown and promote growth. The weight gain during the postnatal growth in the preterm infants is often not achieved because extrauterine life require higher energy expenditure due to intensive care environment, illness, inadequate nutrition, and other adverse conditions **(Velaphi, 2011)**.

Controversially, endogenous protein breakdown is fairly affected by supplemental exogenous protein, it is not clear if a positive protein balance is beneficial. Fasting enhances recycling of cellular components into amino acids for cellular fuel and according to theory of adaptive hibernation it promotes the down regulation of mitochondrial activity to maintain cell life **(Patel et al., 2018** & **Moonen and Van Zanten, 2020)**.

Preterm infants tend to lose weight (about 15%) in the first 7 days of life **(Schanler, 2005)**. In the current study there were no significant differences between the high and low PPN regarding weight gain till day 7. Upon studying the decrement of weight, we found that the percentage of weight loss were 9%,7%, and 4% in the control group, low PPN group and high PPN group respectively with higher weight loss in the control group.

Ho et al., 2012 found that aggressive early simultaneous AA administration plus enteral feeding during the first few days of life for preterm infants was associated with improved weight gain and earlier full enteral feeding.

In the current study on day 7 there were no statistical differences between the control, low and high PPN groups regarding acidosis, serum creatinine, BUN or s. Cystatin C. **Porcelli and Sisk, 2002**, found that preterm neonate with very low birth weight tolerated 4 g/kg/day but had higher BUN. Failure to fully metabolize amino acid substrate into protein synthesis can result in elevated blood urea levels. In the fetus, amino acids are significant source of energy beyond the needs for protein accretion. In ELBW an increased urea concentration may reflect an acceptable metabolic byproduct and not protein

intolerance **(Thureen et al., 2003)**. Increased creatinine in preterm neonates may be related to immature vessel structure **(Sonntag et al., 1996)**.

In the current study, serum cystatin c was more sensitive marker for detection of acute kidney injury compared to Creatinine. This goes with the study of **EL-Gammacy et al., 2018** who found that serum cystatin C is a useful detection marker of AKI and may detect AKI one to two days earlier than creatinine.

On the other hand, **Clark et al. (2007)** found that the use of higher initial dose, faster administration and higher maximal dose of AA in parenteral nutrition did not promote improved growth (weight gain or change in length and head circumference), compared with lower dose of amino acids supplementation.

LIMITATIONS

The limitation of this study is that we do not have reference value for s.Cystatin c in neonates. We did not follow up clinical and laboratory data for longer period.

CONCLUSION

Neither high nor low protein parenteral nutrition has significant effect on s. Cystatin C hence not leading to AKI.

Consequently, high protein parenteral nutrition can be used to minimize weight loss without increasing the risk of metabolic acidosis or renal impairment in preterm neonates.

RECOMMENDATIONS

To start high protein parenteral nutrition early in preterm infants to improve weight gain with no concern about the hazardous effect on renal function. Further studies are needed for longer duration to confirm the current results.

Author Contributions:

All authors contributed to data interpretation and manuscript writing and have read and approved the final submission. Rania IH Ismail and Tarek M El-Gammasy. conceptualized and designed the study. Rania IH Ismail and Fatema EE Mohamed supervised data collection. Rania IH Ismail and Tayseer MM Gad reviewed manuscript, and approved the final manuscript as submitted. Rania IH Ismail and Fatema EE Mohamed contributed to data collection and performed data analysis. Manal MK El Din. contributed to the laboratory ELISA analysis.

REFERENCES

1. **Armangil D, Yurdakök M, Canpolat FE, Korkmaz A, Yigit S, and Tekinalp G., (2008):** Determination of reference values for plasma cystatin C and comparison with creatinine in premature infants. Pediatr Nephrol. 23:2081–3. doi: 10.1007/s00467-008-0867-1.
2. **Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, and Lipp R., (1991):** New Ballard Score, expanded to include extremely premature infants. J Pediatr. Sep;119(3):417-23. doi: 10.1016/s0022-3476(05)82056-6. PMID: 1880657.
3. **Branagan A, Costigan CS, Stack M, Slagle C, and Molloy EJ., (2022):** Management of Acute Kidney Injury in Extremely Low Birth Weight Infants. Front Pediatr. Mar 30; 10:867715. doi: 10.3389/fped.2022.867715. PMID: 35433560; PMCID: PMC9005741.
4. **Burattini I, Bellagamba MP, Spagnoli C, D'Ascenzo R, Mazzoni N, Peretti A, Cogo PE, and Carnielli VP; Marche Neonatal Network., (2013):** Targeting 2.5 versus 4 g/kg/day of amino acids for extremely low birth weight infants: a randomized clinical trial. J Pediatr. Nov;163(5):1278-82.e1. doi: 10.1016/j.jpeds.2013.06.075. Epub 2013 Aug 12. PMID: 23941670.
5. **Christmann V, Visser R, Engelkes M, de Grauw AM, van Goudoever JB, and van Heijst AF., (2013):** The enigma to achieve normal postnatal growth in preterm infants--using parenteral or enteral nutrition? Acta Paediatr. May;102(5):471-9. doi: 10.1111/apa.12188. Epub 2013 Feb 25. PMID: 23398476.
6. **Delanaye P, Cavalier E, and Pottel H., (2017):** Serum Creatinine: Not So Simple! Nephron. 136(4):302-308. doi: 10.1159/000469669. Epub 2017 Apr 26. PMID: 28441651.
7. **Demirel G, Celik IH, Canpolat FE, Erdeve O, Biyikli Z, and Dilmen U., (2013):** Reference values of serum cystatin C in very low-birthweight premature infants. Acta Paediatr.102:e4–7. 10.1111/apa.12041.
8. **Donadio C, Lucchesi A, Ardini M and Giordani R., (2001):** Cystatin C, beta 2-

microglobulin, and retinal-binding protein as indicators of glomerular filtration rate: comparison with plasma creatinine. J Pharm Biomed Anal; 24: 835-842.

9. **El-Gammacy TM, Shinkar DM, Mohamed NR, and Al-Halag AR., (2018):** Serum cystatin C as an early predictor of acute kidney injury in preterm neonates with respiratory distress syndrome. Scand J Clin Lab Invest. Sep;78(5):352-357. doi: 10.1080/00365513.2018.1472803. Epub 2018 May 22. PMID: 29786454.

10. **Ho M, Yen Y, Chen H, Chien S, Hsieh M and Yang Y., (2012):** "Effect of Aggressive Early High-Dose Intravenous Amino Acid Infusion and Early Trophic Enteral Nutrition on Very Low Birth Weight Infants," Food and Nutrition Sciences, Vol. 3 No. 11, 2012, pp. 1604-1608. doi: 10.4236/fns.2012.311209.

11. **Moltu SJ, Bronsky J, Embleton N, Gerasimidis K, Indrio F, Köglmeier J, de Koning B, Lapillonne A, Norsa L, Verduci E, and Domellöf M; ESPGHAN Committee on Nutrition., (2021):** Nutritional Management of the Critically Ill Neonate: A Position Paper of the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr. Aug 1;73(2):274-289. doi: 10.1097/MPG.0000000000003076. PMID: 33605663.

12. **Moonen HPFX, and Van Zanten ARH., (2020):** Mitochondrial dysfunction in critical illness during acute metabolic stress and convalescence: consequences for nutrition therapy. Curr Opin Crit Care. 2020 Aug;26(4):346-354. doi: 10.1097/MCC.0000000000000741. PMID: 32487844.

13. **Moyses HE, Johnson MJ, Leaf AA and Cornelius VR., (2013):** Early parenteral nutrition and growth outcomes in preterm infants: a systematic review and metaanalysis. Am J Clin Nutr; 97:816.

14. **Osborn DA, Schindler T, Jones LJ, Sinn JK, and Bolisetty S., (2018):** Higher versus lower amino acid intake in parenteral nutrition for newborn infants. Cochrane Database Syst Rev. Mar 5;3(3):CD005949. doi: 10.1002/14651858.CD005949.pub2. PMID: 29505664; PMCID: PMC6494253.

15. **Patel JJ, Martindale RG, and McClave SA., (2018):**

Controversies Surrounding Critical Care Nutrition: An Appraisal of Permissive Underfeeding, Protein, and Outcomes. JPEN J Parenter Enteral Nutr ;42:508-15.

16. **Porcelli PJ and Sisk PM., (2002):** Increased parenteral amino acid administration to extremely low birth weight infants during early postnatal life. J Pediatr Gastroenterol Nutr; 34:174.
17. **Schanler RJ., (2005):** Enteral Nutrition for the High-Risk Neonate. In Taeusch HW, Ballard RA, Gleason CA eds. Avery's Diseases of the Newborn, 8th ed. Philadelphia, Saunders; 1043-60.
18. **Chwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, and Furth SL., (2009):** New equations to estimate GFR in children with CKD. J Am Soc Nephrol. Mar;20(3):629-37. doi: 10.1681/ASN.2008030287. Epub 2009 Jan 21. PMID: 19158356; PMCID: PMC2653687.
19. **Sonntag J, Prankel B, and Waltz S., (1996):** Serum creatinine concentration, urinary creatinine excretion and creatinine clearance during the first 9 weeks in preterm infants with a birth weight below 1500 g. Eur J Pediatr 155(9): 815-819.
20. **Stickle D, Cole B, Hock K, Hruska KA and Scott MG., (1999):** Correlation of plasma concentrations of cystatin C and creatinine to inulin clearance in a pediatric population. Clin Chem; 44: 1334-1338.
21. **Thureen PJ, Melara D, Fennessey PV and Hay WW., (2003):** Effect of low versus high intravenous amino acid intake on very low birth weight infants in the early neonatal period. Pediatr Res; 53:24.
22. **Velaphi S., (2011):** Nutritional requirements and parenteral nutrition in preterm infants, South African Journal of Clinical Nutrition, 24:sup3, 27-31, DOI: 10.1080/16070658.2011.11734377.