ORIGINAL ARTICLE

# Free Serum Ceftriaxone Levels In Children: Correlation With Protein Energy Malnutrition (PEM)

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**Abstract**

*The study was done on two groups of children: the non-PEM or control (group I) and the PEM or experimental group (group II), both of whom were administered with 1g ceftriaxone/24 hours for some acquired infections. The objective of the present study was to estimate the serum free ceftriaxone concentrations at 15 minutes, 8 hours and 23 hours following administration and to observe whether the serum MIC/MBC was being maintained or not, whether the free serum ceftriaxone levels had any correlation with the nutritional status of children.*

## Introduction

In developing countries, about half of all childhood deaths (4.9 million) are caused by no more than five conditions: pneumonia, diarrhoeal diseases, malaria, measles and malnutrition (UN report'90). Despite various national and global consensus, pediatric health care is yet to be enriched a lot to reduce infant mortality rate. Besides, millions of children die every year in diarrhea, pneumonia, meningitis and many other curable diseases in Bangladesh. About 93.4% of children aged 6-71 months suffer from various degrees of malnutrition in Bangladesh[1].

Nutrition is a major concern for any over-crowded population particularly living at or below poverty level like Bangladesh. Many national and international strategic plans are underway to fight back the curse of malnutrition throughout the world. Bangladesh is a country facing different natural disaster round the year including epidemic of diseases. Moreover uncontrolled population growth with lack of education, housing, and financial constrains precipitate the severity of malnutrition particularly in children and making them more vulnerable to the infectious diseases. A vicious cycle is established with malnutrition giving rise to diseases and *vice versa*, a situation that is commonly found in Bangladesh. According to a WHO-UNHCR report (2000), protein energy malnutrition (PEM) is by far the most lethal form of malnutrition and PEM affects every fourth child worldwide. Geographically, more than 70% of PEM children live in Asia, 26% in Africa and 4% in Latin America and Caribbean. In 1990, the UN Subcommittee on Nutrition estimated that around 184 million children of pre-school age (34%) suffered from growth faltering due to a combination of factors such as insufficient diets and infectious diseases[2]. From a study of 53 countries, it has been reported that the effects of persistent PEM can be devastating. Even in its mild to moderate form PEM contributes 56% of all childhood deaths due to its potentiating effects on childhood infectious diseases[2]. It is one of the common causes of mortality and morbidity in children of Bangladesh[3].

Many antimicrobial agents are being used to treat the life-threatening diseases worldwide. Some of the drugs are preferred in case of children for high efficacy, broader spectrum, pharmacokinetic advantages and safety. Ceftriaxone is one of the commonest, widely used antimicrobial agents in the

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treatment of lower respiratory tract infection, infection of the skin, urinary tract, bone and joint, intra-abdominal, and central nervous system. It is also indicated for the treatment of septicemia/bacteremia[4]. Ceftriaxone is a third-generation parenteral cephalosporin with a relatively long half-life, which is stable to β-lactamases, particularly those produced by gram-negative organisms. Ceftriaxone was introduced in the United States in 1984. Since then, it has gained considerable popularity with clinicians providing pediatric health care. With its favourable spectrum of activity, long half-life, ease of administration, and relatively few adverse effects, ceftriaxone has become a frequent choice for empiric antimicrobial therapy in hospitals, emergency departments and in ambulatory care settings[5].

The choice of an antimicrobial agent generally depends on the lowest concentration of the drug that is sufficient to inhibit or kill the microorganisms, that is, the MIC or MBC. MIC is the minimum concentration of that drug that inhibits the growth of one isolate of a pathogen for 24 hours under specific *in vitro* conditions[6]. MBC is the minimum drug concentration that kills the microorganism. However, the therapeutic efficacy of an antibiotic depends not only on its *in vitro* MIC value but also upon its actual concentrations in serum and at the site of infection[7]. Considering the overall pharmacokinetic impact, socioeconomic burden of treatment and nutritional status of children, the conventional dosing of widely used drugs should be justified and evaluated repeatedly in a poor country like Bangladesh. Therefore it is of great importance to determine the free serum concentrations of a highly protein-bound drug like 'ceftriaxone' in children suffering with protein energy malnutrition (PEM) to evaluate the maintenance of MIC against common microorganisms in our country.

## Methodology

The study was carried out in the Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU) during the period of July 2002 to June 2003.

The experiment was designed to determine the free serum concentrations of ceftriaxone at different points of time (after intravenous administration) and nutritional states (PEM and non-PEM). A total number of 42 children (21 PEM and 21 non-PEM aged, 2 to 10 years) admitted in the paediatric wards of three hospitals of Dhaka city, namely- Bangabandhu Sheikh Mujib Medical University (BSMMU) hospital, Dhaka Medical College Hospital (DMCH) and Holy Family Red Crescent Medical College Hospital (HFRCMCH) were selected for the study. Informed consent from their parents or appropriate guardian was obtained.

Children were grouped in to Group-I (children without PEM but otherwise sick and receiving Inj.Ceftriaxone 1gm i.v.) considered as the control group and Group-II (children suffering from PEM in association with existing disease and received Inj. Ceftriaxone 1 gm i.v.) was considered as the experimental group.

Nutritional assessment (separating criteria of PEM and non-PEM children)

Prior to grouping of the children included in the study, the children were classified into PEM (21) and non-PEM (21) on the basis of nutritional assessment after obtaining the anthropometric measurements: Age (months), Body wt (kg), Height (cm), MAC- mid arm circumference (cm).

The data were recorded in separate sheets for each child and then compiled and analyzed for Z-score by using the 'NutStat' computer software as mentioned in the 'Child Nutrition Survey of Bangladesh 2000'. The Z-score was done for statistical determination of nutritional status and has been recommended by the W.H.O for measuring and analyzing anthropometric indices by setting the cut-off value at–3.00 sd (WHO 1995).

Blood was collected aseptically from the median cubital vein of the children each morning

between 9:30 – 10:30 AM and stored in sterile test-tube containing EDTA as an anticoagulant. The first sample was obtained fifteen (15) minutes after 1 g ceftriaxone i.v. in one hand. 2-5 ml blood was drawn from the median cubital vein of the other hand and labeled as 'a' (1ml) for estimation of ceftriaxone. The second and third samples were collected from the same patient about 8 hours and 23 hours respectively following the first sample. These were labeled as 'b' and 'c' respectively. Samples were centrifuged at 2000 rpm for 10 minutes and serum was collected in small eppendorf tubes. They were preserved at –70 °C. until estimation of the drug at HPLC.

The free serum ceftriaxone concentrations were estimated by High Performance Liquid Chromatography (HPLC) using protein precipitation method where the plasma proteins were precipitated by cold methanol stored at 4 °C [8,9].

HPLC settings: Column C18. (4.0 mm x 15 cm) that contain packing material L1–Octadecyl Silane Chemically bonded to porous silica. Wave length: 274 nm, Flow rate: 1.0 ml /min, Pressure: 2000 lb/in$^2$

The blood samples (a, b and c) were treated with methanol (kept at 4°C) for precipitating the serum protein. The method of Kohlhepp *et al.* (1998) states that the protein bound form of ceftriaxone would precipitate if the serum was centrifuged following treatment with cold methanol. For this purpose, 0.1 ml of serum was added to 0.9 ml cold methanol, stored at 4°C. The mixture was vortexed and the centrifuged at 3,500 rpm for 5-10 minutes. A white precipitate was formed at the bottom, which contained the protein bound form of ceftriaxone sodium. The supernatant was collected and kept at –70 °C until it was assayed for ceftriaxone.

The method of free serum ceftriaxone determination by HPLC was evaluated by:

Obtaining a standard curve on each day prior to the running of the samples.

The linearity of the standard solutions was also determined by calibration of external standard solutions at five different concentrations (2, 4, 8, 16, 32 µg/ml). The results were found linear (r=0.998) in replicated standard solutions. These standards were submitted to the same procedure used for the samples, including the addition of the internal standards[10].

Optimization of the method by series of dilutions of ceftriaxone were done (conc. 5-50 µg/ml). These dilutions were prepared from the ceftriaxone sodium donated by the Aventis Limited, Bangladesh. The recovery of internal standard 5µg/ml and 50µg/ml was 98.02% and 99.13% respectively and the method was found reproducible on 3 successive determinations.

Inter-day and intra-day precision of the method by determining coefficient of variation. The accuracy was determined after five replicate injections of same sample and the mean peak-areas were calculated and the standard deviation was less than 2%.

The nutritional analysis was done by computer software 'NutStat' of EpiInfo-2002 version. All data obtained for concentrations of free ceftriaxone, serum albumin and total protein were compiled and analyzed by using statistical software SPSS version 10. The comparison between control and experimental groups were done by mean, standard error and unpaired 't' test.

## Result

The mean (± se) age of children in 'Group I' (control group) was 5.6 ± 0.8 years and in 'Group II' (PEM-group) was 5.8 ± 0.1 years. The mean height was 110.2 ± 2.3 cm and 96.1 ± 2.9 cm in the group I and II respectively. The mean (± se) weight of the children in 'I' group was 18.1 ± 1.7 kg and in children of the 'II' group was 10.5 ± 1.1 kg. The mean (± se) value of MAC (mid upper arm circumference) of the children was found 15.3 ± 1.8 cm in 'Group I' and 9.4 ± 0.9 cm in 'Group II'.

Table I-Mean age, height, weight, MAC and Z score of children in group-I and group-II

| (n=21) | Age in years | Height in cm | Weight in kg | MAC in cm | Z score* | | |
|---|---|---|---|---|---|---|---|
| | | | | | HAZ | WAZ | WHZ |
| Group I (control group) | 5.6 ± 0.8 | 110.2 ± 2.3 | 18.1 ± 1.7 | 15.3 ± 1.3 | -0.4 | -0.6 | -0.2 |
| Group II (PEM group) | 5.8 ± 0.1 | 96.1 ± 2.9 | 10.5 ± 1.1 | 9.4 ± 0.9 | -3.6 | -4.1 | -3.4 |

* Z scoring was done by statistical software 'NutStat'

After intravenous infusion of 1 gram Ceftriaxone sodium (at the dose of 1g/day), the mean (± se) concentrations of serum ceftriaxone in 'Group I' and 'Group II' after 15 minutes was 125.5 ± 1.5 µg/ml and 146.7 ± 3.8 µg/ml respectively. The values of the group I were significantly higher ($P < .001$) compared to those of the group I. The free ceftriaxone levels in serum collected after 8 hours following intravenous infusion were 76.6 ± 1.5 µg/ml in 'Group I' and 90.0 ± 2.9 µg/ml in 'Group II'. These values showed significantly higher concentrations ($P< 0.01$) of free serum ceftriaxone in the group II.

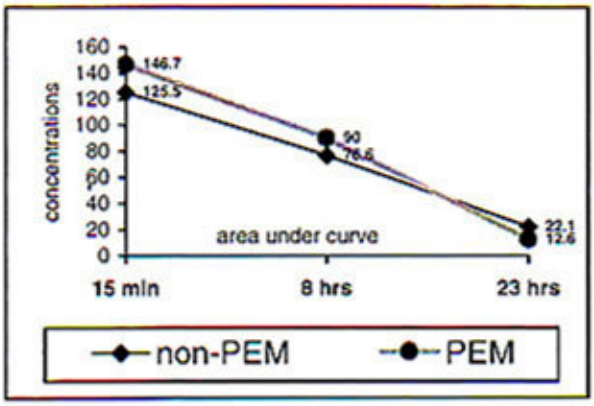
The mean (± se) concentrations of free ceftriaxone in serum samples collected after 23 hours was 22.1 ± 2.3 µg/ml in 'Group I'. The concentration was 12.6 ± 1.2 µg/ml in 'Group II' and was found significantly ($P <.001$) lower in comparison to those of the control group.

Table II-Free serum ceftriaxone concentrations in Group-I and Group-II

| | after 15 minutes (a) µg/ml | after 8 hours (b) µg/ml | after 23 hours (c) µg/ml |
|---|---|---|---|
| Group I (non-PEM children) | 125.5 ± 1.5 | 76.6 ± 1.5 | 22.1 ± 2.3 |
| Group II (PEM children | 146.7 ± 3.8 | 90.0 ± 2.9 | 12.6 ± 1.2 |

after specified time intervals * = P<0.05, ** = P<0.01, *** = P<0.001

Figure 1: Free serum concentrations and area under curve in non-PEM and PEM children at specified time interval.

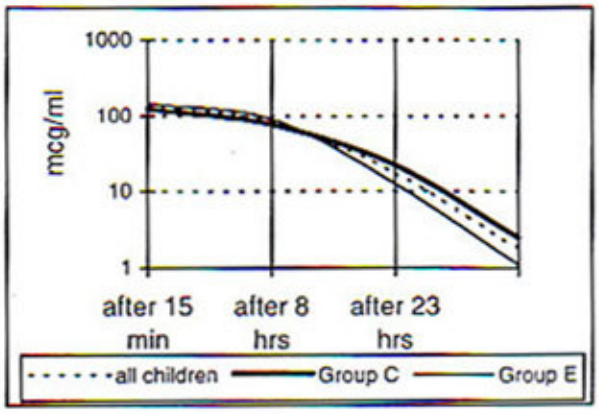



The mean concentrations of ceftriaxone after 8 hours of infusion (a-b) respectively in group I and group II were 48.9 µg/ml and 56.7 µg/ml. The mean concentrations reduced from 8 to 23 hours (b-c) were more prominent in the experimental group (Group II) than those of the control (Group I) and the values were 54.5 µg/ml and 77.4 µg/ml respectively. The reduction of free serum ceftriaxone concentrations after 23 hours (a-c) were 103.4 µg/ml in children without PEM (Group I) and 134.1 µg/ml in children with PEM (Group II). There was higher rate of reduction in the experimental group in comparison to those of the control.

Table III-Percent reduction of free serum ceftriaxone reduced in specified time intervals in children of control (I) and the experimental (II) groups

| (n=21) | Percent reduction (%) | | |
|---|---|---|---|
| | 15 min-8 hours (a-b) | 8-23 hours (b-c) | 15 min-23 hours (a-c) |
| (control group) | 38.96 % | 71.14 % | 82.39 % |
| (PEM group) | 38.65 % | 86.0 % | 91.84 % |

Figure 2-Logarithmic distribution of free serum ceftriaxone concentrations in all children, group-I and group-II




## Discussion

The present study shows the results of the antropometric measurements (age, height, weight, MAC) together with the Z score between non-PEM children (control group) and the PEM-children (experimental group). The table suggests that although the mean age of children of both groups were similar (5.6 and 5.8 years respectively), significant differences were present between the control and the experimental group in relation to height, weight and MAC. The mean height in experimental group (children with PEM) was 96.1 ± 2.9 cm that was significantly ($P<0.05$) lower than the control (children without PEM) group. This might be considered as indicator of malnutrition (stunting) in the experimental group in comparison to that of the healthy group. Similarly, the mean weight was significantly lower ($P<0.001$) in the children with PEM (10.5 ± 1.1 kg) in comparison to that in the control group (18.1 ± 1.7 kg). Measurement of mid-arm circumference (MAC) gives a rough composite gauge of stores of protein and fat in children (WHO 1997). In this study, the mean MAC of children with PEM (group II) was 9.4 ± 0.9 cm, which was significantly lower ($P< 0.01$) than those of the non-PEM (group I) children. The lower value of mean MAC could be a reflection of wasting which is an obvious feature of malnutrition.

Ceftriaxone shows high degree of protein binding which limit the amount of free drug in plasma or tissue, although the free and concentrations may remain effective (microbiologically active)[8]. The pharmacokinetics of ceftriaxone is reported to be unchanged in different age groups. Luderer *et al.* reported in a study that no significant changes were observed in plasma concentrations, elimination half-life, apparent volume of distribution, non-renal clearance of ceftriaxone between healthy children and adult[11]. But, in another study by Hayton and Stoeckel, plasma protein binding of ceftriaxone was observed about 70% in neonates and it was increased throughout childhood to the adult value of 90 to 95%[12]. This may indicate the probability of free ceftriaxone levels in plasma would be higher in children than in adult. On the other hand, if the amounts of plasma protein in children remaining less, the amount of free (unbound) drug might be more in plasma. In certain ill ness or in renal impairment when plasma albumin becomes lesser than usual (hypo-albuminemia), much caution is required to administer highly protein-bound drugs like ceftriaxone. In case of deficient children like those suffering from PEM, one may face dilemma to decide as to the amount of drug (eg. ceftriaxone) to be administered, although ceftriaxone (the cephalosporin in general) is a relatively safer drug.

In the present study, free serum ceftriaxone concentrations were found much more higher in the children with PEM after 15 minutes and 8 hours of infusion of 1g ceftriaxone i.v; but the mean free serum concentrations were higher in children without PEM at 23 hours. This might be due to the rapid metabolism or elimination of unbound (free) drugs. The higher concentrations of free ceftriaxone in hypo-albuminemic and hypo-proteinemic children of the PEM group were similar to the findings of Mimoz *et al.* (2000) where hypoalbuminemia

were induced by hydroxyethyl starch in eleven patients and free ceftriaxone levels were measured to compare with healthy volunteers[13]. In the present study, the mean serum concentrations of ceftriaxone after 15 minutes of infusion was found 125.5 ± 1.5 µg/ml in control group (children without PEM) that correspond with the findings of other studies carried out on newborn children who has recorded mean serum concentrations 116–131 µg/ml[14].

The only way to knowing what concentrations are attained after a given dose of a drug is to measure the plasma or serum levels[15]. This may give an idea over the efficacy of a drug and also whether the MIC/MBC are being maintained or not, although the therapeutic efficacy of an antibiotic depends not only on its *in vitro* MIC/MBC value but also upon its actual concentration in serum and at the site of infection[7]. To observe the MIC/MBC values and elimination rate, the serum free ceftriaxone levels were estimated in this study again after 23 hours of the single dose ceftriaxone (1g i.v.) in children. The mean concentrations of free ceftriaxone were 22.1 ± 2.3 µg/ml and 12.6 ± 1.2 µg/ml in group I and in group II respectively. The concentrations in children with PEM (group II) were significantly lower ($P<0.001$) at 23 hours than those in group I. The reduction rates were 103.4 µg/ml in control group and 134.1 µg/ml in experimental group at 23 hours. Probably the rapid elimination of the higher amount of free drug occurred in the PEM group and therefore the concentration in the PEM group were significantly *($p<0.001$)* lower at 23 hours compared to those in the control (group I). This was similar to the findings of Joos *et al.* (1984), who in a study on hospitalized patients (with severe infections) obtained free serum ceftriaxone values as 10 µg/ml at 24 hours[16]. The more rapid elimination in group II could be due to more metabolism or elimination of free serum ceftriaxone in children with low serum albumin and total protein. Because the total serum albumin and serum total protein in group II were also lower. A point to note is that, at about 15-16 hours (Fig 3.1) the concentrations of free serum ceftriaxone was similar in both groups. This indicates that, irrespective of the renal tubular secretion rate (or the elimination rate), both PEM and non-PEM children had attained almost same free serum concentrations at about 15-16 hours.

## Summary

The present study was aimed to outline the correlation or disparity if any, existed regarding the serum concentrations of free ceftriaxone when administered either to PEM or non-PEM children. It was observed that after 15 minutes of administration, there was significantly higher *($p<0.001$)* concentrations of free serum ceftriaxone in the PEM children (Group II), compared to those in non-PEM (Group I) children. A reduction of 38.9% was observed at 8 hours. Ceftriaxone remains bound principally to albumin in the body. Therefore it may be feasible that whence the PEM children had lower concentrations of serum albumin, there would be higher concentrations of free drug in serum, and this was observed in this study.

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