

## COMPARISON OF SERUM CALCIUM LEVELS AMONG EXTREMELY PRETERM AND SEVERELY PRETERM NEONATES AT A PERIPHERAL MILITARY HOSPITAL

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### ABSTRACT

**Objective:** To determine the frequency and compare serum calcium levels amongst extremely preterm and very preterm neonates.

**Study Design:** Comparative cross sectional study.

**Place and Duration of Study:** Combined Military Hospital, Multan Pakistan, from May to Oct 2018.

**Methodology:** A total of 217 preterm neonates, born at Combined Military Hospital Multan up to the gestational age of 32 weeks completed from last menstrual period, were included in the study. They were divided in to two group's i.e. very preterm and extremely preterm neonates. Those born between 28-32 weeks completed of gestation were categorized as severely preterm. Extremely preterm were those born between 24-28 weeks completed of gestation. Venous blood samples were acquired at approximately 36 hours after birth and total serum calcium levels were analyzed. Neonates having total serum calcium levels <7 mg/dL were declared as suffering from hypocalcaemia.

**Results:** Out of 217 neonates, 117 (53.9%) were male while 100 (46.1%) were female. Mean gestational age was  $30.98 \pm 2.12$  weeks while mean parity of the mothers was  $2.82 \pm 1.08$  children. Mean birth weight was  $1510.26 \pm 142.38$  grams. One hundred and seventeen (53.9%) of the preterm neonates suffered from hypocalcaemia with the mean total serum calcium level being  $6.58 \pm 0.86$  mg/dL. Hypocalcaemia was more common in extremely preterm neonates compared to very preterm neonates ( $p < 0.05$ ).

**Conclusion:** Hypocalcaemia is a frequent complication encountered in preterm neonates born between 24-32 weeks gestation. Within these neonates, the frequency of hypocalcaemia is higher in extremely preterm compared to severely preterm babies.

**Keywords:** Frequency, Hypocalcaemia, Neonates, Preterm, serum calcium level.

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### INTRODUCTION

Various trace elements occur in the human body and execute numerous functions which are vital for survival. Calcium is one such trace element that performs a multitude of essential functions like muscle contraction including cardiac muscles, nerve conduction, blood flow through vessels, blood coagulation and formation of vital enzymes. Dairy products, nuts and green vegetables are rich dietary sources of calcium. It occurs in the human body mainly as a constituent of bones and teeth. Calcium plays a crucial part for the maintenance of integrity and strength of bones in all ages including fetal and neonatal bones. During pregnancy there is movement of maternal calcium to the fetus through the placenta, resulting in the mineralization and normal development of fetal bones<sup>1</sup>. Almost 99% of calcium in the body exists in the form of bone as hydroxyapatite, while the remaining 1% exists in the extracellular fluid either in ionized form or bound to other ions and albumin<sup>2</sup>. Calcium levels in

the body are intricately regulated by the levels of phosphate, magnesium and bicarbonate in the body. Vitamin D also plays a crucial role in calcium metabolism as Yilmaz *et al*<sup>3</sup>. conducted a study regarding the vitamin D levels in neonates and its relation to hypocalcaemia. He concluded that a significant number of neonates having low levels of vitamin D, develop hypocalcaemia.

Infants, who are delivered before the completion of 37<sup>th</sup> week of gestation, are characterized as preterm neonates. They can be further divided into sub categories including extremely preterm neonates (born before gestational age of 28 completed weeks), very preterm (born between 28-32 completed weeks) while the remaining are labeled as moderate preterm and late preterm neonates. Neonates belonging to the extremely preterm and severely preterm categories experience exceedingly high mortality rates while survivors have high risk of long-term sequelae like learning difficulties and developmental delays. Many of them who survive experience chronic health disorders, the most frequent being cerebral palsy, impaired vision and hearing abnormalities<sup>4</sup>. Bajwa *et al*<sup>5</sup>, concluded that hyper-

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bilirubinemia and other metabolic anomalies like hypocalcaemia and hypoglycemia were very frequent in preterm infants, thus requiring timely management to prevent any loss of life. Preterm birth rates continue to rise all over the world, partially owing to increased rates of multiple pregnancies, as the rate of preterm birth is almost 40-60% in multiple pregnancies whereas in single pregnancies it is only 5-10%<sup>6</sup>. This has led to an overall increase in preterm neonates predisposed to metabolic abnormalities, infections and other life-threatening conditions, requiring prolonged hospitalizations in neonatal intensive care units.

Hypocalcaemia is a condition described as reduced levels of serum calcium. At birth, it is described in term infants or preterm infants with weight >1.5 kg as serum calcium level <8 mg/dL or ionized calcium levels <4.4 mg/dL whereas in preterm infants with weight <1.5 kg, it is delineated by total serum level <7 mg/dL or ionized calcium <4 mg/dL<sup>7</sup>. Some common risk factors predisposing to development of neonatal hypocalcaemia include newborns who are born premature, are small for gestational age, being formula fed, parathyroid hormone deficiency, maternal diabetes and reduced serum levels of vitamin D<sup>8</sup>. Similarly, perinatal asphyxia in newborns also leads to development of hypocalcaemia and other metabolic disorders like hypoglycemia or hypomagnesemia<sup>9</sup>. Hypocalcaemia in newborns may remain asymptomatic or becomes life-threatening and lead to a wide array of potentially fatal complications like seizures, tetany, laryngospasm, cardiomyopathy, cardiac arrest and respiratory failure<sup>10</sup>. Therefore, early detection followed by timely management of hypocalcaemia is imperative for the survival of the preterm neonates to prevent these potentially lethal complications. Maternal nutrition greatly alters the serum calcium levels in the newborn therefore pregnant females are recommended to take 400-600 IU/day vitamin D during their entire duration of pregnancy.

This study was done to evaluate the preterm infants born before completing 32 weeks gestation for hypocalcaemia as they are more vulnerable to be afflicted by this condition and compare serum calcium levels between extremely preterm and severely preterm neonates. This study had been planned especially to cater for the population of Southern Punjab. This would provide some data to determine the degree and intensity of hypocalcaemia effecting preterm babies born in our region and devise remedial measures to overcome this health dilemma leading to reduction in neo-

natal morbidity. It would also prove advantageous to health institutions to devise strategies for reduction of neonatal deaths resulting from hypocalcaemia.

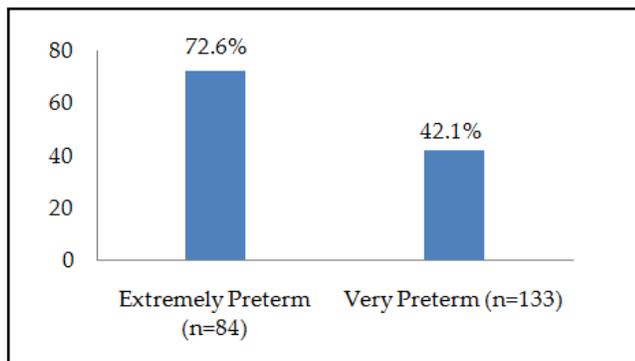
## METHODOLOGY

This comparative cross-sectional study was conducted in Pediatrics department of Combined Military Hospital Multan, from May to October 2018. Sample size of 217 was calculated by the use of Epi-Info software taking frequency of hypocalcaemia in preterm infants born before completing 32 weeks gestation as 83%<sup>11</sup>, and margin of error as 5%. Non-probability, purposive sampling was performed. All the serum samples were analysed for total calcium using Cobas 501 automated chemistry analyser by Roche diagnostics. The study was approved by the Ethical Committee of Combined Military Hospital Multan.

Preterm infants of either gender, born between 24-32 weeks of gestational age completed according to date of last menstrual period, were included in the study. Babies having suspected chromosomal anomalies or those whose mothers were taking calcium therapy or vitamin D supplements were excluded from the study. The scope of study was explained to parent of each neonate included in the study and informed written consent was obtained to participate in the study. The preterm neonates were further segregated into two groups: extreme preterm neonates (those born at 24-28 weeks of gestational age completed) and very severe preterm neonates (born at 29-32 weeks gestational age completed), 3ml of venous blood was obtained from the right sided cephalic vein at the level of elbow, at about 36 hours after birth, using a 5ml disposable syringe. A new syringe was used for every patient. The collected blood sample was placed in appropriate sample bottles and dispatched to the Pathology department. Serum total calcium levels were determined in each case by the pathologist. Those preterm babies having levels of total serum calcium <7 mg/dL, were labeled as having hypocalcaemia while patients having serum calcium levels  $\geq 7$  mg/dL were declared normal. A prewritten performa was employed to record all the essential information and findings of the patient and the parents such as gender, gestational age, birth weight, socioeconomic status, parity of the mother and educational qualification of the mother. The collected data was analyzed using SPSS-22 and the frequency of hypocalcaemia was calculated in both extremely preterm and severely preterm group using chi-square test. The results were considered significant at the  $p$ -value  $\leq 0.05$ .

**RESULTS**

During the study, 217 preterm neonates were evaluated for hypocalcaemia, out of which 117 (53.9%) were male and 100 were female (46.1%). The mean gestational age of the preterm neonates was 30.98 ± 2.12 weeks with 133 patients (61.3%) having gestational age more than 28 weeks completed. Mean parity of the mothers in our study was 2.82 ± 1.08 children with 116 mothers (53.5%) having history of previous preterm births as well (figure). Mean birth weight in our



**Figure: Comparison of hypocalcaemia between extremely preterm and severely preterm neonates.**

study came out to be 1510.26 ± 142.38 grams with 110 neonates (50.7%) having birth weight >1.5 kg. 134 (61.8%) of the cases were resident of urban areas while 83 (38.2%) cases were living in rural areas. Most of the mothers 175 (80.6%) were illiterate, 42 (19.4%) had educational level up to secondary level while none of them had any higher qualification.

One hundred and seventeen of the preterm neonates (53.9%) suffered from hypocalcaemia having serum calcium levels <7 md/dL. The mean total serum calcium level came out to be 6.58 ± 0.86 mg/dL with the range being between 5.2-8.3 mg/dL. Extreme preterm neonates (n=84) showed hypocalcaemia in 61 cases (72.6%) while 56 (42.1%) of the very preterm neonates (n=133) were found to have hypocalcaemia. The result was significant at p<0.005 (table).

**Table : Serum calcium and gestational age wise distribution of preterm neonates (n=217).**

Gestational Age (Weeks)	Patients With Hypocalcaemia	Patients with Normal Serum Calcium	p-value
24-28	61 (72.6%)	23 (27.4%)	<0.001
29-32	56 (42.1%)	77 (57.9%)	

**DISCUSSION**

Hypocalcaemia defined as reduced serum calcium levels in the body, is a frequently encountered

biochemical anomaly, notably in preterm neonates, infants of diabetic mothers and neonates with perinatal asphyxia<sup>12-15</sup>. Therefore, preterm neonates, in addition to other serious complications, are predisposed to developing hypocalcaemia, and its occurrence enhances with earlier gestational age at the time of birth. Same was evident in our study, in which 53.9% of the preterm neonates suffered from hypocalcaemia (serum calcium levels <7 md/dL taken as hypocalcaemia). The mean serum calcium level came out to be 6.58 ± 0.86 mg/dL. Extremely preterm neonates, born at gestational ages between 24-29 weeks, were more susceptible and showed hypocalcaemia in 72.6% cases. Very preterm neonates born between 29-32 weeks of gestational age, were relatively less predisposed, and showed hypocalcaemia in 42.6% cases.

Hypocalcaemia in preterm neonates is a neglected issue and studies on this topic are extremely scarce, especially studies carried within the last 5 years. Extensive research only yielded few previous related studies, most of them conducted almost decades ago. Vuralli *et al*<sup>7</sup> studied the clinical approach to hypocalcaemia in newborn period and infancy. He concluded that most neonates suffering from with hypocalcaemia are generally asymptomatic so serum calcium levels is compulsory in preterm infants (born at gestational age <32 weeks), infants of diabetic mothers, small for gestational age infants and infants having severe prenatal asphyxia with a 1 min APGAR score of <4. Management of hypocalcaemia must be started immediately in neonates with hypocalcaemia.

Mimouni *et al*<sup>16</sup>, carried a case-control study of hypocalcaemia in predisposed infants and showed that hypocalcaemia was significantly related with low gestational age at time of birth (p<0.01), reduced APGAR score (p<0.01) and white race (p<0.01) (R2=0.457). These results agree with the findings of our study, which showed that hypocalcaemia was significantly higher in extremely preterm neonates as compared to those belonging to very preterm group.

Venkataraman *et al*<sup>14</sup>, conducted a study on 19 preterm neonates regarding early neonatal hypocalcaemia in extremely preterm infants. He showed that the incidence of hypocalcaemia in preterm neonates was 37% by 12 hours, 83% by 24 hours, and 89% by 36 hours. In extremely preterm infants, the frequency of hypocalcaemia is higher with earlier onset. In our study, 53.9% of the preterm newborns were diagnosed with hypocalcaemia at 36 hours after birth. The difference in frequency may be due to difference in sample

size or gestational age of the sample population. The results of all the above mentioned studies were in accordance with the results of our study and conclude an increased incidence of hypocalcaemia in preterm newborns. No national study was found on this topic and more studies would be required to validate and reinforce our study.

There are few limitations in our study. First of all, it does not correlate the level of serum calcium with other minerals like magnesium or phosphate. The plasma glucose levels were not accounted for. There are some additional factors that need to be addressed in relation to the development of hypocalcaemia in newborns. Certain procedures in the neonates like phototherapy have been known to influence the calcium levels in the newborn. Khan *et al*<sup>17</sup>, performed a study regarding hypocalcaemia in jaundiced newborns undergoing phototherapy. He concluded that considerable number of newborns having jaundice, undergoing phototherapy, developed hypocalcaemia. Therefore, he advocated that serum calcium levels should be cautiously scrutinized in such patients considering the hazards associated with hypocalcaemia. On the other hand, a contrasting study by Asl *et al*<sup>18</sup>, concluded that phototherapy may lead to higher urinary excretion of calcium, but it does not predispose to hypocalcaemia. Therefore, the effect of phototherapy on serum calcium level is still under evaluation and further research is mandatory to validate it. However, this was beyond the scope of our study.

## CONCLUSION

Hypocalcaemia is a glaring health issue affecting preterm neonates, with a high proportion suffering from this dilemma. Extremely preterm neonates are at an increased risk of developing hypocalcaemia compared to the relatively more mature group of at risk neonates that are categorized as severely preterm. Therefore, serum calcium levels must be prescribed as a mandatory investigation in all preterm neonates.

## CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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