Original Article

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Estimation of Serum Calcium Level among Hospitalised Infants with Acute Bronchiolitis: A Cross-sectional Study

Paediatrics Section

SUMAN GUPTA[1], NIRMAL KUMAR[2], AMARDIP KAUR[3], DEEPAK KUMAR GUPTA[4], SHIFALI GUPTA[5]

ABSTRACT

Introduction: Acute bronchiolitis is a common viral respiratory illness with clinical features ranging from mild to severe forms, requiring intensive care management. Calcium, a micronutrient, plays a significant role in numerous intracellular and extracellular events. Vitamin D deficiency is known to be associated with respiratory infections, and vitamin D metabolism regulates serum calcium and Alkaline Phosphatase (ALP) levels. Thus, calcium levels may be the actual causative factor at the molecular level to determine the severity of respiratory infections.

Aim: To estimate and compare serum calcium levels in infants with acute bronchiolitis and controls. Additionally, it aimed to investigate the effect of sunlight exposure on calcium levels in infants presenting at a tertiary care centre.

Materials and Methods: A cross-sectional study was conducted from October 2004 to April 2006, over two consecutive years, among 446 infants in the paediatric unit of a tertiary care centre in Delhi, North India. A total of 223 infants (<1 year of age) hospitalised with a diagnosis of acute bronchiolitis, presenting with the first episode of wheeze based on American Academy of Paediatrics (AAP) criteria (including coryza, fever, tachypnoea, tachycardia, paroxysmal wheezy cough, and irritability), were considered as cases. An equal number of age-matched controls (n=223), admitted for non respiratory illnesses, were included. Blood samples were collected for total serum calcium, phosphorus, and ALP, along with other relevant investigations related to their diagnosis. Statistical analysis was performed using Student's t-test, Kruskal-Wallis's test, and Chi-square test.

Results: The study included 223 patients in both the case and control groups. In the control group, there were 76.58% males and 23.42% females, while in the cases, there were 81.53% males and 18.47% females. The mean age of cases was 5.78±3.45 months, and controls were 4.77±2.83 months, with a mean difference of 1.01 (95% CI, 0.42-1.59). The mean calcium levels were 9.00±1.43 mg/dL and 8.71±1.51 mg/dL in controls and cases, respectively, with a mean difference of 0.29 (95% CI-0.02 to 0.56), which was statistically significant (p-value=0.037). There was no significant difference in phosphorus and ALP levels between the two groups.

Conclusion: This study found an association between calcium levels and acute bronchiolitis. The findings suggest that low serum calcium levels might serve as a risk factor for acute bronchiolitis.

Keywords: Immunity, Lower respiratory tract infection, Rickets, Sunlight exposure, Viral infection

INTRODUCTION

Bronchiolitis is a viral lower respiratory tract illness that commonly affects infants, leading to a significant health burden worldwide. Respiratory Syncytial Virus (RSV) is the most validated cause of bronchiolitis, resulting in a large number of hospitalisations among children under five annually [1]. In developed countries like the United States of America (USA), bronchiolitis is the leading cause of hospital admissions in infants during their first year of life. However, data from developing countries is limited, likely due to compromised hygiene, poor infection control, overcrowding, and poverty [2-4].

RSV bronchiolitis typically occurs from late October with peak activity during winters and early spring. Various risk factors for frequent bronchiolitis in infancy have been postulated, including prematurity, exposure to smoking, living in crowded houses without proper sunlight exposure, and urban living conditions [5]. Mechanisms such as staying indoors, crowding, humid atmosphere, and inhalation of cold mist leading to impaired ciliary function have been suggested [6].

Vitamin D deficiency has been closely linked to severe bronchiolitis and the need for intensive care unit admission in infants [7]. Assessing vitamin D levels before the bronchiolitis season and providing appropriate supplementation has been proposed as a protective measure against severe bronchiolitis. Vitamin D levels have also been checked in nasopharyngeal secretions and found to be associated with a higher risk of positive pressure ventilation in children [8].

Calcium and phosphate homeostasis is complex, and three important hormones modulate most of the extracellular control of these minerals. Parathyroid hormone plays a role in maintaining or restoring serum calcium levels [9]. Calcium is crucial for cellular processes, metabolic and signaling pathways, survival, and immune functions. Low serum calcium has been associated with higher mortality and complications, making it a prognostic factor for the severity of viral diseases [10].

To the best of our knowledge, no previous similar study has been conducted on infants regarding the association of calcium with acute bronchiolitis. Therefore, the findings from this small prospective study warrant further investigation on a larger scale and in different at-risk populations. The hypothesis is that serum calcium levels in patients with acute viral bronchiolitis may correlate with this common paediatric problem. The aim was to study and compare the serum calcium levels in infants with acute bronchiolitis and without bronchiolitis (controls), and also to find an association between sunlight exposure and calcium levels in both cases and controls.

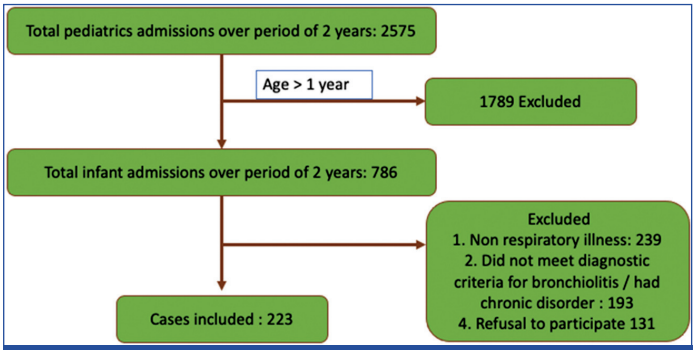
MATERIALS AND METHODS

This cross-sectional study was conducted from October 2004 to April 2006 for two consecutive years in the paediatric unit of St. Stephen Hospital in Delhi, North India. The study was approved by the Institutional Ethical Committee (IEC no: Thesis/NBE/74/2492), and informed written consent was obtained from parents or legal caregivers for inclusion in the study.

Inclusion criteria: Infants younger than one year old who were referred to the paediatric emergency department for acute bronchiolitis were included as cases. The severity of bronchiolitis was assessed using the AAP guidelines for the Diagnosis and Management of Bronchiolitis [11]. Patients who met the diagnostic criteria for acute bronchiolitis were included in the study. Controls were defined as patients in the same age group with acute febrile illness (fever >38.5°C, duration >24 hours) but without respiratory symptoms.

Exclusion criteria: Children with chronic disorders that could influence the severity and course of bronchiolitis, such as chronic lung disease, congenital heart disease, suspected inborn errors of metabolism, global developmental delay, hypotonia, suspected central nervous system disorders, or syndromic features were excluded from the study.

Sample size: Out of a total of 2,575 patients admitted during this period, 786 were infants. Of these, 223 were included as cases and 223 as controls, selected through purposive sampling based on the inclusion criteria [Table/Fig-1].



[Table/Fig-1]: Study flow diagram.

Data collection: A proforma was filled for all patients upon referral to the emergency department, including demographic data and information on sunlight exposure (less than or more than 30 minutes, swaddled or not). Data regarding past medical history, risk factors for asthma, and family history were also collected to follow the inclusion/exclusion criteria.

Blood samples: 5 mL of blood samples were obtained from all study subjects under aseptic precautions, and serum was separated after spinning 1 mL of the sample for one minute at 3000 rpm. Levels of total calcium, phosphorus, and ALP were estimated using a fully automated Hitachi analyser. Age-appropriate reference ranges for calcium (8.8-10.8 mg/dL), phosphorus (3.8-6.5 mg/dL), and ALP (145-420 U/L) were used for comparison [12].

STATISTICAL ANALYSIS

The collected data were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) software version 21.0. Continuous variables such as age, weight, length, total calcium, phosphorus, and albumin were compared using the Student's t-test. The continuous non parametric variable, ALP, was compared using the Kruskal-Wallis test due to the wide variation in values. The nominal variable, gender, was compared using the Chi-square test.

RESULTS

A total of 446 infants were recruited, with 223 cases and 223 controls. Cases and controls had similar baseline demographic characteristics. Males outnumbered females in both cases and controls, but the gender difference was not statistically significant (p-value=0.2). Variables such as length and weight were analysed, and no significant difference was found among the groups [Table/Fig-2].

In the present study, the mean calcium level in the control group was 9.00±1.43 mg/dL, while in the cases, it was 8.71±1.51 mg/dL. The mean difference was 0.29 (95% CI 0.016 to 0.56), which was statistically significant (p-value=0.037). Therefore, there was a significant difference in calcium levels between cases and controls, but no significant difference was observed in phosphorus and alkaline phosphatase levels [Table/Fig-3]. After regressing age difference, the calcium level remained borderline significant (p-value of 0.05), and the phosphorus level was significant (p-value of 0.009).

Characteristics		Controls (n=223) Mean±SD	Cases (n=223) Mean±SD	Mean difference (95% CI)	p-value
Age (months)		5.78±3.45	4.77±2.83	1.01 (0.42-1.59)	0.008
Gender (%)	M	170 (76.58%)	181 (81.53%)	-	0.2
	F	53 (23.42%)	42 (18.47%)		
Weight (in Kgs)		5.98±1.85	5.86±1.62	0.12 (-0.21-0.46)	0.47
Length (in cm)		60.39±6.55	59.47±5.33	0.92 (-0.22-2.07)	0.11

[Table/Fig-2]: Demographic profile of cases and controls.

Parameters	Controls	Cases	Mean difference (95% CI)	p-value
Calcium (mg/dL)	n=223*	n=223*	0.29 (0.02-0.56)	0.037
	9.00±1.43	8.71±1.51		
Phosphorus (mg/dL)	n=194*	n=213*	0.19 (-0.04-0.43)	0.10
	5.46±1.19	5.27±1.23		
Alkaline phosphatase (U/L)	n=201*	n=215*	-27.67 (-76.33-20.99)	0.26
	343.93±248.66	371.60±256.17		

[Table/Fig-3]: Comparison of biochemical parameters between cases and controls. *In cases where the sample was inadequate, calcium levels were preferably done, and the rest were deferred

The association of sun exposure with these biochemical parameters within the control group showed that calcium levels were low and phosphorus levels were high in the non exposed group (sunlight exposure <30 minutes with significant swaddling) compared to the exposed group (sunlight exposure >30 minutes without cover), which was statistically significant. However, there was no statistically significant difference in ALP levels among the groups [Table/Fig-4a].

Parameters	Sun-exposure		Mean difference (95% CI)	p-value
	Not present	Present		
Calcium (mg/dL)	n=153	n=70	-0.55 (-0.95 - -0.15)	0.007
	8.83±1.41	9.38±1.40		
Phosphorus (mg/dL)	n=127	n=67	0.41 (0.06-0.76)	0.021
	5.60±1.21	5.19±1.09		
Alkaline phosphatase (U/L)	n=131	n=70	60.18 (-12.11-132.47)	0.10
	364.89±260.53	304.71±221.24		

[Table/Fig-4a]: Effect of sun-exposure on biochemical parameters in controls.

The association of sunlight exposure with calcium levels within the cases showed no difference in total serum calcium, phosphorus, and alkaline phosphatase levels between the exposed and non-exposed groups [Table/Fig-4b]. Clinical and/or radiological features of rickets were seen in 9.42% of cases compared to 5.38% of controls [Table/Fig-5,6].

Parameters	Sun-exposure		Mean difference (95% CI)	p-value
	Not present	Present		
Calcium (mg/dL)	n=165	n=58	0.04 (-0.41-0.49)	0.86
	8.72±1.52	8.68±1.50		
Phosphorus (mg/dL)	n=158	n=55	0.24 (-0.14-0.62)	0.21
	5.32±1.21	5.08±1.28		
Alkaline phosphatase (U/L)	n=157	n=58	10.69 (-67.06-88.46)	0.79
	374.49±256.55	363.79±257.21		

[Table/Fig-4b]: Effect of sun exposure on biochemical parameters in cases.

Rickets (X-ray proven) (Image 1)	Cases (n=223) (n, %)	Controls (n=223) (n, %)	OR (95% CI)	p-value
Present (n=33)	21 (9.42)	12 (5.38)	1.82 (0.87-3.81)	0.109

[Table/Fig-5]: Prevalence of rickets among cases and controls.

[Table/Fig-6]: Radiograph showing features of rickets in 10-month-old child.

DISCUSSION

This study suggests that low serum calcium levels are associated with acute bronchiolitis compared to controls of a similar age group. Calcium is a micronutrient that plays a role in vital reactions throughout the body. It interacts with various proteins in different cellular compartments and is involved in major body systems such as muscle contraction, enzyme activation, cell differentiation, immune response, programmed cell death, and neuronal activity [13-15]. Hypocalcaemia is known to be associated with sepsis and the release of endotoxins and cytokines [16]. Deficiency or lower levels of calcium could be associated with respiratory infections due to its direct role in immune system regulation and action [17]. Treatment of hypocalcaemia has been emphasised in various studies on sepsis management [18]. However, studies on the association of low serum calcium levels with viral infections are scarce, and the results of this study may represent pioneering work in this direction.

Another important factor is ciliary motility and mucosal surface defense mechanisms, which require calcium in various pathways at the molecular level [19]. In the presence of low levels of calcium, these mechanisms may be compromised, leading to a compromised primary defense against respiratory pathogens. Another important mechanism is increased chest wall compliance in vitamin D deficiency, leading to poor respiratory secretions and an increased predisposition to respiratory tract infections. Calcium also plays a role in gene regulation and the production of proteins involved in immunomodulation [20].

Taken together, these mechanisms support present study hypothesis that low serum calcium levels, as found in this cohort, may be an important predictor for acute viral lower respiratory infection or acute bronchiolitis.

Another objective of the study was to compare serum calcium levels in the cases and control groups. It was found that serum calcium levels were significantly lower in cases compared to age-matched controls with illnesses other than respiratory infections. Hypocalcaemia has been shown to be associated with wheeze in infants in a previous study [21]. Furthermore, hypocalcaemia has been well-documented as a complication of acute viral bronchiolitis [22]. Hence, patients with low serum calcium levels at onset may be more prone to and have increased severity of electrolyte imbalances, leading to morbidity and mortality. A study by Saboktakin L has shown that hypocalcaemia can be considered a prominently poor prognostic factor for clinical outcomes in Paediatric Intensive Care Unit (PICU) patients with pneumonia, but hypomagnesemia and hypophosphatemia do not predict clinical outcomes [23].

The present study also showed that rickets was present in 9.42% of cases compared to 5.38% in controls. In a previous study, the incidence of rickets in infants with wheezy bronchitis was 24%. Other studies have reported a high prevalence of vitamin D deficiency and rickets in cases of bronchiolitis [24]. Similarly, severe vitamin D deficiency, non exclusive breastfeeding, and inadequate sunlight exposure were found to be significant risk factors for respiratory infections by Narang GS et al., [25].

Limitation(s)

There were several limitations to this study. Firstly, the study was conducted only on admitted infants, which automatically excluded many mild cases. This may have introduced bias and limited the generalisability of the findings to the broader population.

Secondly, the correlation between sunlight exposure and bronchiolitis was attempted, but the measurement of adequate versus inadequate sunlight exposure could not be objectively quantified. This may have introduced uncertainty in the analysis and interpretation of the results.

Additionally, the sample size of the study was limited, which may have affected the statistical power and the ability to detect significant associations. A larger sample size would have provided more robust results and increased the generalisability of the findings.

Furthermore, the study was unable to validate the appropriate dose of calcium and vitamin D supplements that can prevent or reduce the incidence of bronchiolitis. This is an important aspect to consider for future research, as it could provide valuable insights into preventive measures for bronchiolitis.

CONCLUSION(S)

This study found an association between calcium levels and acute bronchiolitis. The findings suggest that low serum calcium levels might serve as a risk factor for acute bronchiolitis. The study also found that rickets is more common in these patients, highlighting the importance of proper sunlight exposure and adequate supplements. Another major finding was that serum calcium levels at the onset of illness may serve as a prognostic marker for severity, as the cohort included patients who were admitted for severe disease. Therefore, a widely available and low-cost serum marker can help anticipate sickness and improve outcomes for this severe yet common ailment.

To the best of our knowledge, no previous similar study has been conducted on infants with acute bronchiolitis, so the findings from this small prospective study warrant further investigations on a larger scale and future randomised controlled trials in different at-risk populations.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Paediatrics, Adesh Institute of Medical Science and Research, Bathinda, Punjab, India.
2. Head, Department of Paediatrics, St. Stephen's Hospital, Delhi, India.
3. Associate Professor, Department of Paediatrics, Adesh Institute of Medical Science and Research, Bathinda, Punjab, India.
4. Associate Professor, Department of Emergency Medicine, Adesh Institute of Medical Science and Research, Bathinda, Punjab, India.
5. Senior Resident, Department of Genetic Metabolic Unit, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Deepak Kumar Gupta,
House No. 45, Phase 2, Model Town, Bathinda-151001, Punjab, India.
E-mail: deepakguptak@yahoo.com

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