**Original Research Article**

# Prevalence of Vitamin D deficiency in Duchenne muscular dystrophy in Salem District

**P. Kanchana[1], Sathyasuresh[2*]**

[1,2]Assistant Professor, Department of Pediatric Medicine, Government Mohan Kumuramangalam Medical College & Hospital, Salem, Tamil Nadu, India
[*]Corresponding author email: **sureshkruthik@gmail.com**

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## Abstract

**Background:** Duchenne muscular dystrophy (DMD) is the most common congenital muscular disorder in children. It is an X-chromosome-linked recessive disorder, occurring in 1 in every 3500 male births. Although congenital, the onset of clinical symptoms and disease progression is variable.
**Aim and objective:** To study the prevalence of vitamin D deficiency among children affected with DMD by assessing 25(OH) vitamin D levels.
**Materials and methods:** This prospective descriptive study was done in Mohan Kumaramanglam Medical College in the year 2019. Height and weight were plotted on growth charts growth charts of normal Indian children as recommended by Indian Association of Pediatrics (CDC) Blood sample was collected for following investigations - Complete Hemogram (CBC), Renal Function Test (RFT), Liver Function Test (LFT), serum calcium ($Ca^{2+}$), serum phosphorous$Po_4^-$, Serum Alkaline Phosphatase (ALP), serum vitamin D (25 OH) levels using Automated chemiluminescent immunoassay, Polymerase chain reaction (PCR) for mutation analysis.
**Results:** Calf muscle hypertrophy was present in 84% and valley sign had prevailed in 74% hypertrophy of Brachioradialis and Deltoid occurred in 58%. 74% of the children (23 out of 31) showed positive valley sign Among our Duchenne muscular dystrophy group 23% had cardiac and 3% had CNS involvement. The most common deletion was the deletion of 49 (11x) followed by 50 (10x) and 46, 47 and 48 (9x).4 out of 7 DMD children with cardiac involvement had a deletion of 49 and 50.
**Conclusion:** 87% suffers from vitamin-D deficiency. Skeletal changes, cardiac involvement and CNS involvement is common in Vitamin-D deficiency but not statistically significant. Vitamin-D deficiency correlated with < one-hour outdoor activity, urban population and sluggish tendon reflex.

**Key words**

Vitamin D deficiency, Duchenne muscular dystrophy, Prevalence.

## Introduction

Duchenne muscular dystrophy (DMD) is the most common congenital muscular disorder in children. It is an X-chromosome-linked recessive disorder, occurring in 1 in every 3500 male births. Although congenital, the onset of clinical symptoms and disease progression is variable [1]. In most affected boys, clinical manifestations appear at 3–5 years of age, with frequent falls, abnormal running and inability to jump and hop. By 8–10 years, standing and walking require braces or other aids; and by age 12–15, most patients are confined to a wheelchair [2]. Contractures and scoliosis develop frequently [3]. Proximal skeletal muscles are most commonly affected, however progressive cardiomyopathy is common and can be severe. Death most often occurs in the early twenties due to respiratory or cardiac failure [4]. Improvements in intensive care facilities in the past few years have led to a significant prolongation of life [5]. In addition to muscle pathology, varying degrees of intellectual impairment is present in about 30 %. Females are carriers; however, 10% show some disease manifestation which is milder than in boys [6]. No specific therapy is available for DMD. Glucocorticosteroids (GCs) reduce the focal inflammatory processes, reduce breakdown and necrosis of muscle fibers and have now become the standard treatment for DMD [7]. Clinically, they improve muscle strength and function, prolong autonomous walking and slow down the progression of the disease. Osteoporosis is common in DMD patients [8]. The chronic use of steroids also results in steroid-induced osteopenia and an increased risk of fractures. Vitamin D deficiency is known to result in both deficient growth and osteoporosis. There is very little data on Vitamin D levels in children with DMD and its impact on them. We wanted to study the prevalence of Vitamin D deficiency, its impact on patients with DMD [9, 10].

## Materials and methods

This prospective descriptive study was done in Mohan Kumaramanglam Medical College in the year 2019. Height and weight were plotted on growth charts growth charts of normal Indian children as recommended by Indian Association of Pediatrics (CDC) Blood sample was collected for following investigations - Complete Hemogram (CBC), Renal Function Test (RFT), Liver Function Test (LFT), serum calcium ($Ca^{2+}$), serum phosphorous$Po_4^-$, Serum Alkaline Phosphatase (ALP), serum vitamin D (25 OH) levels using Automated chemiluminescent immunoassay, Polymerase chain reaction (PCR) for mutation analysis.

**Inclusion criteria:** Boys with genetically proven Duchene muscular dystrophy, Before starting steroids, Those who had consented.

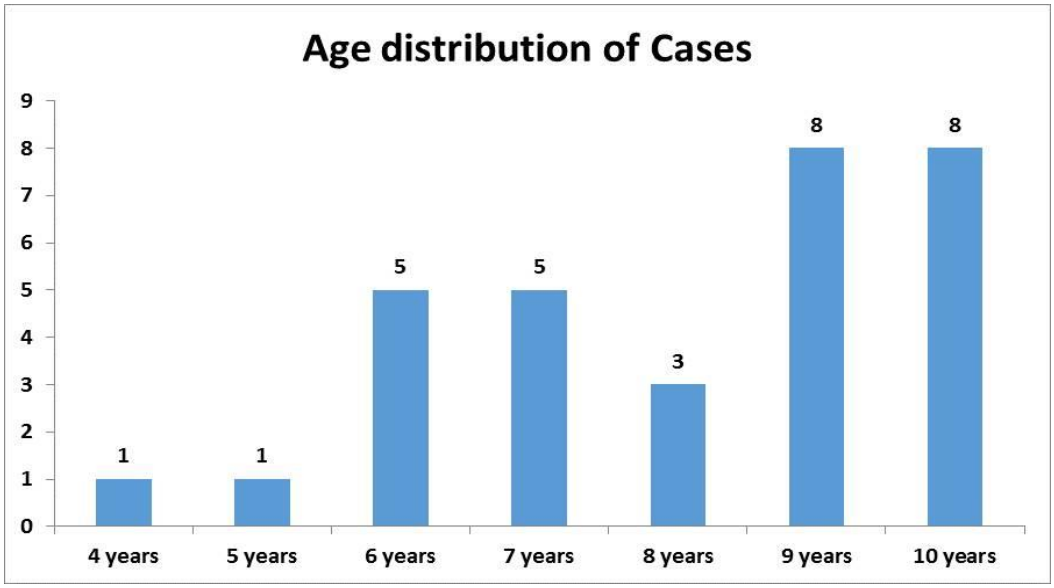
**Exclusion criteria:** Genetically proven Duchene muscular dystrophy children who were having history of drug intake like antiepileptics, steroids, Boys > 10 years not part of the study due to inability to stand and suffer from severe kyphoscoliosis were common in these patients. Most of them were immobile and has impaired respiratory function. Children who were having a chronic liver disease or renal disease or history of chronic diarrhea, Non-ambulant children, Children not given consent

**Statistical analysis:** The data were entered in a spread sheet and analyzed by SPSS software version 16. The Categorical variable was analyzed using the chi-square test. Any of the cells contain values less than 5 fishers exact test was used. For continuous variable t-test used to find significance between two means. P-value of 0.05 was considered as significant.

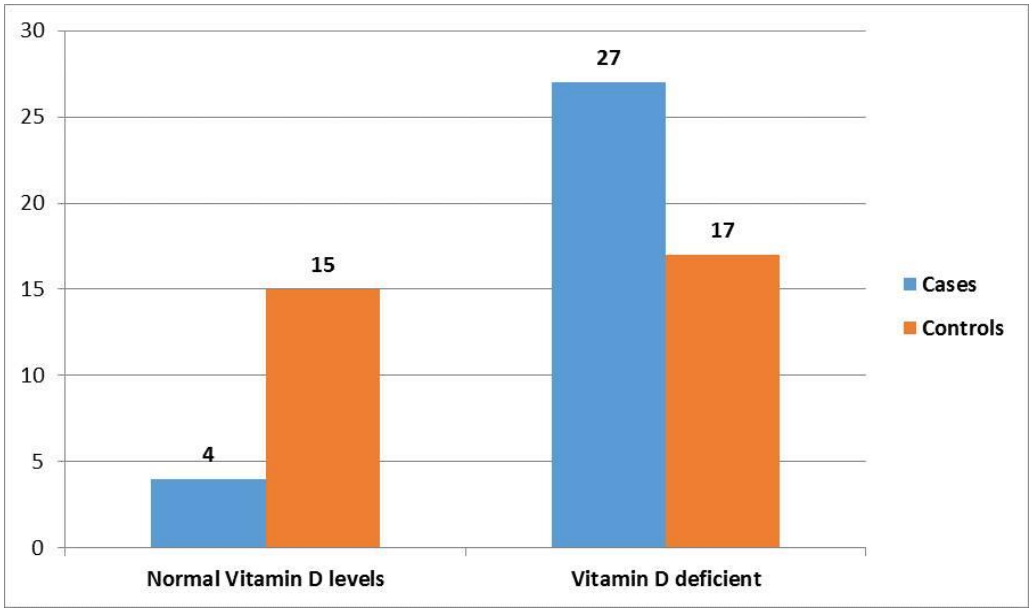
## Results

Total no. of patients was 31 with a minimum of 4 years and 10 years. 9 and 10 years being the most common age group each had 8 people (**Graph – 1**).

**Graph – 1:** Age distribution.




**Graph – 2:** Vitamin D deficiency status among cases and controls.




All the patients had disease manifested by 6 years of age. 50% in the study population disease started in 3 and 5 years earliest age of onset being 1.5 years (**Table – 1**).

**Table – 1:** Distribution of age at onset of DMD.

| Age of onset | Frequency | % |
|---|---|---|
| 1.5 | 1 | 3.2 |
| 2 | 1 | 3.2 |
| 3 | 9 | 29 |
| 4 | 5 | 16.1 |
| 5 | 9 | 29 |
| 6 | 6 | 19.4 |

The mean height of the DMD group was 117.53 cm with a standard deviation of 6.7 cm. 90.3% (28 out of 31 children) of the children measured less than 50$^{th}$ percentile for age. Only 3 children measured greater than 50$^{th}$ percentile in height for age. Only 2 children (6%) were exposed to outdoor activity for at least 1 hour or more than 1 hour. 11 children (36%) had only 30 minutes to 1 hour of outdoor exposure. 58% of the children (18 out of 31) had less than 30 minutes of daily outdoor exposure. Family history was positive in 19% (6 out of 31 children) of the children. Remaining 25 (81%) did not have any positive

family history. The distribution of vitamin D levels did not show any significant difference between different socioeconomic subgroups ($p > 0.05$). Residential pattern (rural or urban) was not found to have any significant association with vitamin D levels ($p > 0.05$). Duration of outdoor exposure was found to be an important factor in vitamin D deficiency according to the literature review. Our study also showed a significant association between duration of outdoor exposure and vitamin D deficiency ($p < 0.05$). 100% of those who were exposed to less than 30 minutes had vitamin D deficiency and 100% of those with more than 1 hour of outdoor exposure had normal vitamin D levels (**Table – 2**).

**Table – 2:** Variable analysis of study.

| Variables | Mean | Standard deviation |
|---|---|---|
| **Age (years)** | 8.06 | 1.731 |
| **Height (centimetres)** | 117.53 | 6.687 |
| **Weight (kilogram)** | 22.42 | 4.926 |
| **Age at onset (years)** | 4.245 | 1.29 |
| **Age at Walking (years)** | 1.92 | 0.35 |
| **CPK (U/L)** | 12962.06 | 8061.553 |
| **Serum Calcium (mg/dl)** | 8.774 | 0.58 |
| **Serum Phosphorus (mg/dl)** | 4.029 | 0.34 |
| **Serum Alkaline phosphatase (IU/dl)** | 437.23 | 98.9 |
| **Serum Vitamin D (ng/ml)** | 10.86 | 4.97 |

**Table – 3:** Comparison of biochemical parameters among cases and controls.

| Variables | Cases (Mean ± Standard deviation) | Controls (Mean ± Standard deviation) | P-value |
|---|---|---|---|
| **Serum Calcium levels** | 8.774 ± 0.58 | 12.43 ±0.76 | **< 0.05 (Significant)** |
| **Serum Phosphorus levels** | 4.029 ± 0.34 | 4.62 ± 0.23 | **< 0.05 (Significant)** |
| **Serum ALP levels** | 437.23 ± 98.9 | 212.04 ± 76.34 | **< 0.05 (Significant)** |
| **Vitamin D levels** | 10.86 ± 4.98 | 16.27 ± 4.98 | **< 0.05 (Significant)** |

The frequency of vitamin D deficiency was higher among cases compared to controls and the difference was statistically significant ($p < 0.05$). The mean vitamin D levels were lower among cases than controls and the difference was statistically significant when compared with t-test ($p < 0.05$). The mean serum calcium, phosphorus and alkaline phosphatase levels were compared between cases and controls. The difference in serum levels between cases and controls was statistically significant ($p < 0.05$) as per **Graph - 2**.

The mean serum calcium and phosphorus levels of the DMD group were close to the lower limits of the normal range. The mean serum alkaline phosphatase levels were high. The mean vitamin D level was 10.86 ng/ml with a standard deviation of 4.97 ng/ml. 87% (27 out of 31 children) of the DMD group had vitamin D deficiency. In this study, the mean vitamin D levels were 5.4±1 ng/ml in the corticosteroid treated group and 14± 6 in the non-corticosteroid treated group. The mean serum CPK levels were very high (Mean 12962.06 with a standard deviation of 8061.5). The mean serum calcium and phosphorus levels of the DMD group were close to the lower limits of the normal range. The mean serum alkaline phosphatase levels were high (**Table – 3**).

The most common deletion was a deletion of 49 (11x) followed by 50 (10x) and 46, 47 and 48 (9x). 4 out of 7 DMD children with cardiac involvement had a deletion of 49 and 50 (**Table – 4**).

**Table – 4:** PCR profile.

| Deletions | Frequency | | Deletions | Frequency |
|---|---|---|---|---|
| none | 8 | | 20-43 | 1 |
| 2 | 1 | | 25 | 2 |
| 3 | 1 | | 44 | 1 |
| 4 | 1 | | 45 | 5 |
| 5 | 1 | | 46 | 9 |
| 6 | 1 | | 47 | 9 |
| 7 | 1 | | 48 | 9 |
| 8 | 3 | | 49 | 11 |
| 13 | 1 | | 50 | 10 |
| 14 | 1 | | 51 | 5 |
| 15 | 1 | | 52 | 5 |
| 16 | 1 | | 53 | 4 |
| 17 | 1 | | 54 | 3 |
| 18 | 1 | | 55 | 1 |
| 19 | 1 | | | |

## Discussion

This study was done to assess the prevalence of Vitamin D deficiency among children with DMD. 31 children diagnosed with DMD, 32 age-matched children. This prospective descriptive study was done in Mohan Kumaramanglam Medical College in the year 2019 during the same period with other minor complaints that were chosen as controls. The controls were subjected to physical examination and biochemical investigations similar to the cases. 31 children were included in the study. DMD occurs as a result of mutations in the dystrophin gene (*DMD*; locus Xp21.2). This leads to an absent or defective dystrophin protein leading to progressive muscle degeneration [11]. Variable phenotypic expression is seen according to the type of mutation and its effect on dystrophin production. Milder allelic forms like Intermediate muscular dystrophy (IMD) and Becker muscular dystrophy (BMD) with exist, while the severe form DMD leads to progressive muscle degeneration and death [12]. Some dystrophin mutations also present as isolated cardiac phenotype. In most cases, clinical manifestations appear at 3–5 years of age, with frequent falls, abnormal running and inability to jump and hop. By 8–10 years, standing and walking require braces or other aids; and by age 12–15, most patients are confined to a wheelchair [13]. Contractures and scoliosis develop frequently. Proximal skeletal muscles are most commonly affected, however progressive cardiomyopathy is common and can be severe. Death most often occurs in the early twenties due to respiratory or cardiac failure [14]. Improvements in intensive care facilities in the past few years have led to a significant prolongation of life. In addition to muscle pathology, varying degrees of intellectual impairment is present in about 30% [15]. The mean age of children was 8.06 years with a standard deviation of 1.73. There were 8 children each in age group 9 and 10 constituting 51.6% of the population. The mean age at onset was 4.2 years with a standard deviation of 1.3 years. 29% of children had their onset of disease at 3 years of age and another 29% had their onset by 5 years [16]. Only 2 children (6.5%) had their onset within 2 years. The mean age at onset is slightly higher than that reported by Kristensen HL, et al. which was 3.4±1.7.16 children (52%) were from an urban area and 15 children (48%) were from the rural area. Only 2 children (6%) were exposed to outdoor activity for at least 1 hour or more than 1 hour. 11 children (36%) had only 30 minutes to 1 hour of outdoor exposure. 58% of the children (18 out of 31) had less than 30 minutes of daily outdoor exposure. The mean age at onset of walking in the group with DMD was 1.92 years. The minimum age at the onset of walking was 1.5 years. 81% had achieved this

milestone by 2 years. Family history was positive in 19% (6 out of 31 children) of the children. Remaining 25 (81%) did not have any positive family history. All 31 children with DMD showed positive Gower's sign. 74% of the children (23 out of 31) showed positive valley sign. 84% (26 out of 31 children) had calf muscle hypertrophy.58% (18 out of 31 children) presented with hypertrophy of deltoid and brachioradialis. Ankle jerk was found positive in all 31 children with DMD.68% (21 out of 31 children) had Lordosis. 7 children out of 31 children with DMD (31%) presented with cardiac manifestations. Only one child (3%) had manifestations of central nervous system involvement. All 31 children (100%) had normal renal function tests. Only 6 children (19%) fared normally in pulmonary function tests. Remaining 81% (25 out of 31) had a restrictive lung pattern on pulmonary function tests. The mean vitamin D level was 10.86 ng/ml with a standard deviation of 4.97 ng/ml. 87% (27 out of 31 children) of the DMD group had vitamin D deficiency [17]. Bikle DD, et al. compared the serum 25 (OH) D levels of 15 boys with DMD with 7 healthy siblings in Children's nutrition research center, Brisbane. The prevalence of vitamin D deficiency was 80%.The mean serum CPK levels were very high (Mean 12962.06 with a standard deviation of 8061.5). A significant finding was that all samples with DMD mutations had serum creatine kinase levels > 2000 U/L. Similar high levels of CPK were also reported by Our study also reports high CPK values in all children with a minimum value of 1340 U/L and a maximum of 30000 U/L [18]. The mean serum calcium and phosphorus levels of the DMD group were close to the lower limits of the normal range. The mean serum alkaline phosphatase levels were high. This biochemical profile is similar to that reported by Holick MF, et al. at different stages of vitamin D deficiency [19]. The distribution of vitamin D levels did not show any significant difference between different socioeconomic subgroups ($p > 0.05$). Residential pattern (rural or urban) was not found to have any significant association with vitamin D levels ($p > 0.05$). Duration of outdoor exposure was found to be an important factor in vitamin D deficiency according to the literature review [20]. Our study also showed a significant association between duration of outdoor exposure and vitamin D deficiency ($p < 0.05$). 100% of those who were exposed to less than 30 minutes had vitamin D deficiency and 100% of those with more than 1 hour of outdoor exposure had normal vitamin D levels. Valley sign, calf muscle hypertrophy, deltoid and brachioradialis hypertrophy were found to a higher occurrence among those with vitamin D deficiency but the difference was not statistically significant [21]. The association between grading of deep tendon reflexes and vitamin D levels was statistically significant ($p < 0.05$) [22]. The occurrence of lordosis was higher among those with vitamin D deficiency, but the difference was not statistically significant ($p > 0.05$) [23]. All 7 children with cardiac involvement and the single child with CNS involvement had vitamin D deficiency but the difference was not statistically significant [24, 25].

## Conclusion

On comparing biochemical profile with vitamin D levels, the frequency of low serum calcium, phosphorus, and alkaline phosphatase levels was higher among those with vitamin D deficiency, but the difference was not statistically significant. The frequency of vitamin D deficiency was higher among cases compared to controls and the difference was statistically significant. The mean vitamin D levels were lower among cases than controls and the difference was statistically significant. Skeletal changes, cardiac involvement and CNS involvement is common in Vitamin-D deficiency but not statistically significant. Vitamin-D deficiency correlated with < one-hour outdoor activity, urban population and sluggish tendon reflex. No correlation between any specific genetic lesions with Vitamin-D deficiency.

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