# The multi-directional reach test in children with Down syndrome 

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#### Abstract

Objective: This study investigated the limits of stability (LOS) and the movement patterns during reaching by applying the Multi-Directional Reach Test (MDRT) in children with Down syndrome (DS) aged 7-12 years old. Methods: Thirty children with DS and 30 age and gender typical development (TD) matched children, aged $7-12$ years old were recruited. Each child was asked to reach as far as possible during standing in four directions using a self-selected movement pattern. The movement patterns were classified by two experienced pediatric physical therapists. Results: The reach distance in children with DS aged 7-9 years old was significantly shorter than TD children aged 7-9 years old for the forward and backward directions. Also, the reach distance in DS children aged 7-9 years old was significantly smaller than that of TD children aged 10-12 years old for all directions. For children with DS aged 10-12 years old, the reach distance was significantly less than that of TD children only in the backward direction. All children with DS in this study adopt a hip and mixed strategy during forward and backward reaching. In contrast, TD children adopt an adult-like movement pattern. Conclusion: The boundary of stability in an anteroposterior (AP) direction of children with DS aged 7-12 years old was lesser than the matched TD children, especially for the backward direction. These findings may assist therapists in detecting postural control and balance problems in children with DS.


Keywords: Down syndrome; limits of stability; Multi-Directional Reach Test; movement patterns.

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## Introduction

Down syndrome (DS) is the chromosome genetic disorder which most commonly causes mental impairment and developmental disability. ${ }^{1}$ Children with DS show a deficiency of static and dynamic balance tasks, difficulty acquiring the movement skills and have a slowness of movements described as clumsy. ${ }^{2,3}$ A standing reach is one of the essential components in functional balance and is necessary in performing daily activities. ${ }^{2}$ Ineffective postural control and poor reaching performance during standing reaching were found in school-age ( $7-12$ years old) children with DS. They demonstrated significantly decreased anteroposterior (AP) center of pressure (COP) displacement and forward momentum during forward reaching. ${ }^{4}$ Moreover, the consequences of balance deficits lead to falls which relate to lacking confidence to performing daily activities, and activity restrictions. ${ }^{5}$

At present, several of the available clinical balance measurement tools for children with or without disabilities in any age group have been developed. Balance assessment tools are helpful in identifying whether a balance problem exists and determining the effectiveness of balance interventions. ${ }^{6}$ Reach tests include the Functional Reach Test (FRT), the Pediatric Reach Test (PRT) and the Multi-Directional Reach Test (MDRT) which commonly assess risk of falling and the maximum limits of stability (LOS) in elderly and pediatric populations. ${ }^{7,8}$ The variability of movements with immature nervous and musculoskeletal systems can increase the probability to fall among children. ${ }^{9}$ Falls occur neither exactly forward nor exactly in a lateral direction. A previous study reported that school age children have a high risk of injury when falling backward. ${ }^{10}$ Although most daily activities are performed on the anterior side of our body, leaning backwards could occur during daily routine. The task of leaning backwards is more difficult as it requires a lot of effort to control the body without falling. ${ }^{11}$ It may prove more sensitive for identifying the balance problems. The MDRT is a single task test which evaluates the voluntary postural responses of upper limb and indirectly assesses the LOS in forward, backward, and lateral directions. ${ }^{12}$ The MDRT is more challenging when compared with the FRT and the PRT as it is conducted on the subject in the backwards direction. In addition, the MDRT is easy for children to perform and can be interpreted and therefore yield results quickly. ${ }^{11}$ The test
demonstrated high reliability and validity with other balance measurement tools. In healthy adult and the elderly, the MDRT has excellent reliability ( $\mathrm{ICC}=0.93$ to 0.95 ) and fair to moderate concurrent validity (concurrent validity of the MDRT with Timed Up and Go (TUG) $r=0.26$ to 0.44 and Berg Balance Scale (BBS) $r=0.36$ to 0.48). ${ }^{13,14}$ In school-age typical development (TD) children, the MDRT has high inter-rater reliability (ICC $=0.80-0.86) \quad$ and intra-rater reliability (ICC $=0.89-0.97)$ in all directions. ${ }^{11}$ Various clinical balance tests such as FRT, four square step test (FSST), TUG are available for children with DS. ${ }^{15-17}$ However, no study has examined dynamic standing balance using the MDRT in this population. Possibly, children with DS would demonstrate differences in their LOS in other directions compared with TD children. Hence, the MDRT in children with DS would help to examine the boundary of stability in four directions.

Therefore, the purpose of this study was to evaluate the LOS by applying the MDRT and compare the reach distances between children with DS and TD aged 7-12 years old. Exploration of the MDRT in children with DS indicated the difference of the boundary of stability and the movement patterns used for reaching between children with DS and TD. This study might be useful for detecting balance problems and providing rehabilitation programs to improve postural performance in children with DS.

## Materials and Methods

A total of 65 children with DS $(n=35)$ and age and gender matched TD children $(n=30)$, aged 7 to 12 years. The calculation of the sample size was based on the pilot study which evaluated the MDRT in 10 children with DS aged $7-12$ years compared with aged and gender matched TD children. The sample size was calculated by G * Power program version 3.1.9.2. A power analysis (power, $95 \% ; \alpha=0.05$; effect sizes $=0.48$ ) determined a total of 58 children required for this study. Children with DS were recruited from special education schools in Bangkok and the metropolitan region. Five children with DS aged 7-9 years were not cooperative during testing. Data was obtained from 30 children with DS and 30 age and gender matched TD children. The convenient sampling technique was used to recruit children who were eligible based on the criteria. Children with DS and

TD were divided into 4 groups: DS aged $7-9$ years, DS aged 10-12 years, TD aged 7-9 years, TD aged $10-12$ years. The division of groups was based on the development of postural control. Differences in the maturation of sensory systems affected the postural control between the two age groups. The $7-9$ years group required to properly solve the conflict of sensory information and motor experiences for their complete motor development and postural control. On the other hand, the maturation of the integrated motor and sensory systems and the postural control strategies similar to adults were observed in the 10-12 years group. ${ }^{18}$ Children with DS were recruited through interviewing the parents or guardians. The inclusion criteria for children with DS was presence of trisomy-21, aged $7-12$ years, able to stand and walk independently, and able to follow instructions. The exclusion criteria included impairment of hearing or uncorrected visual, musculoskeletal problems that might affect standing. The study protocol was approved by the University Ethics Review Committee for Research Involving Human Research Subjects. Informed consent to participate in the research was obtained from parents and children prior to data collection.

Anthropometric data including height and weight were measured and recorded before performing the MDRT. The MDRT tool in this study consisted of a yardstick and a bubble level was used
to ensure that the level of the yardstick was horizontal to the floor. A slide pad was used to evaluate reach distance (Fig. 1). The MDRT was administered in a private room without noise and disturbance. The order of reaching directions was randomized by computer program. At the beginning of measuring the MDRT, a child was asked to stand barefoot on a sheet of paper which was fixed on the floor. Stance width was equal to shoulder width. Foot position was traced in order to prevent any starting point change. The testing procedures were explained and demonstrated by the same rater. Each child outstretched an arm to shoulder height, made a fist with the thumb in palm. All children used their dominant arm for forward and backward reaching and used right and left arms for lateral reaching. The child was instructed to "reach to the (direction given) as far as possible, without lifting the feet, stepping forward or losing their balance and to maintain that position for two seconds", and "lean backwards as far as possible" for the backward direction. The reach distances were measured between starting and ending position. The child was guarded during testing. For all directions each child had one practice trial and three trials of the test. Ten-second and one-minute rests were given between trials and directions, respectively. The best performance of the three successful trials was used for analysis. In addition, the children's height was a covariate in analyses.


Fig. 1. MDRT tool consisted of a yardstick, a bubble level and a slide pad.


Fig. 2. The movement patterns during forward and backward reaching.

A reliability evaluation was performed in a pilot study with 10 children with DS aged between 7 and 12 years. They were evaluated by two pediatric physical therapists who had more than two years of clinical experience. For the intra-rater reliability of the MDRT, in the first session, the reach distance in each direction for each child was measured three trials by the first rater, and then again 10 min later by the second rater. In the second session, the whole procedure was performed by the same two raters with the children again 8 hours after the first session. The intra-rater reliability was moderate to excellent (the 1st rater, $\mathrm{ICC}=0.72-0.90$ and the 2nd rater, $\mathrm{ICC}=0.70-0.82$ ).

The inter-rater reliability of the MDRT was assessed by two raters. The first rater explained and then evaluated the reach distances in each direction three trials for individual child, who then took a 10 min seated rest. The second rater then carried out an evaluation using the same testing procedure. The inter-rater reliability of the MDRT estimate varied from fair to excellent (ICC $=0.47-$ 0.915 ) in all directions. Regarding the low estimates, this may be due to the rater's experience. It was probably that the rater who had longer experience in applying the MDRT might have greater self-efficacy in using the test. The self-efficacy of the rater is associated with their confidence, proper task performance, and making the appropriate decisions in any situation. ${ }^{17}$ Nevertheless, all children were given the similar testing set-up and testing protocol that included standardized verbal instructions and visual demonstrations.

Later, the movement patterns during reaching considered and classified by the same two raters who conducted the MDRT. All children were allowed to reach in a self-selected movement pattern. The movement patterns during reaching were
analyzed using the video recorded by camera. Movement patterns were classified for the best performance trial for each subject. The raters watched the videos separately. Inter-rater agreement between the two raters was high ( $95 \%$ confidence intervals for kappa coefficients ranged from 0.76 to 0.89 and 0.70 to 0.86 ) for forward and backward directions. Concerning classification, the movement patterns were divided into three strategies based on the study by Liao and $\operatorname{Lin}^{19}$ as shown in Fig. 2. First, the hip strategy, whereby the body moves as a double-segment inverted pendulum, while the upper part of the trunk moves in the opposite direction from the lower limbs during the reaching. Second, an ankle strategy, the primary movement occurring at the ankle joint and the whole body moving as a single-segment inverted pendulum. Third, the mixed strategy is executed by the ankle joint moving at the beginning and then the trunk forward or backward bending with hip flexion or extension. The mixed strategy is achieved through hip flexing or extending at the hip, without the lower limbs backward or forward.

## Statistical Analysis

The data analysis was performed using the IBM SPSS Statistics (version 22.0) software. The normality of all variables was checked by Kolmogorov-Smirnov test. Descriptive statistics were applied to the characteristics data and reach distances. The independent sample $t$-test was used to determine the between-group differences in the participants' characteristics. The effects of group and direction on all the calculated variables were evaluated using 4 (groups: DS aged 7-9 years, DS aged 10-12 years, TD aged 7-9 years, TD aged $10-12$ years) $\times 4$ (directions: forward, backward,
leftward, rightward) two-way mixed analysis of variance (ANOVA) for repeated measures. A Tukey post-hoc test was performed to assess differences between groups. The level of statistically significant differences was set at $p$-value $<0.05$.

## Results

The characteristics of children with DS and TD children are presented in Table 1. Regarding the weight, there were no significant differences between children with DS and TD children. The heights of the children with DS were shorter than the TD children ( $p<0.001$ ). The reach distances in each direction for all children with DS and TD is shown in Table 2. There were interactions between group and direction effects for the reaching distance ( $F_{9,168}=2.06, p=0.036$ ). There was significant difference in forward, backward, leftward and rightward directions between groups ( $F_{3,56}=13.10, p=0.001$ ). Children with DS aged $7-9$ years old had significantly smaller reach
distances than TD children aged 7-9 years old in both forward and backward directions ( $p<0.05$ ). In children with DS and TD children aged 10-12 years old, the reach distances only showed significant differences in the backward direction $(p<0.05)$. In addition, the reach distances for all directions in children with DS aged 7-9 years old demonstrated significantly shorter distances when compared with TD children aged 10-12 years old ( $p<0.05$ ). The direction effects were found in all groups $\left(F_{3,168}=143.89, p=0.0001\right)$. The reach distance in the forward direction was the highest value while the reach distance in the backward direction was the lowest value in all direction for all four groups ( $p<0.001$ ). Lateral reach distances were symmetrical, and no significant difference was found between leftward and rightward for all groups ( $p>0.05$ ).

The movement patterns during forward and backward reaching in each group are included in Figs. 3 and 4. Almost all children with DS adopted hip and mixed strategies. In the DS aged 7-9

Table 1. Characteristics of the participants.

|  | DS 7-9 years <br> $(n=15)$ <br> $($ mean $\pm \mathrm{SD})$ | TD 7-9 years <br> $(n=15)$ <br> $($ mean $\pm \mathrm{SD)}$ | $p$ value |  | DS 10-12 years <br> $(n=15)$ <br> $($ mean $\pm \mathrm{SD)}$ | TD 10-12 years <br> $(n=15)$ <br> $($ mean $\pm \mathrm{SD)})$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| Age (years) | $8.29 \pm 0.59$ | $8.32 \pm 0.64$ | 0.880 | $10.76 \pm 1.08$ | $10.71 \pm 1.02$ | 0.906 |
| Gender (male: female) | $4: 11$ | $4: 11$ | - | $7: 8$ | $7: 8$ | - |
| Weight (kg) | $25.87 \pm 8.22$ | $28.38 \pm 5.43$ | 0.330 | $34.13 \pm 9.86$ | $35.55 \pm 5.71$ | 0.636 |
| Height $(\mathrm{cm})$ | $121.37 \pm 6.72$ | $128.61 \pm 4.89$ | $0.002^{*}$ | $129.6 \pm 6.76$ | $143.93 \pm 8.03$ | $0.001^{* *}$ |

Notes: SD: standard deviation; ${ }^{*}$ statistically significant, $p<0.05 ;{ }^{* *}$ statistically significant, $p<0.001$.

Table 2. Group comparison of the reach distance in all directions.

| Group | Forward $(\mathrm{cm})$ <br> $($ mean $\pm$ SD $)$ | Backward $(\mathrm{cm})$ <br> $($ mean +SD$)$ | Leftward $(\mathrm{cm})$ <br> $($ mean $\pm$ SD $)$ | Rightward $(\mathrm{cm})$ <br> $($ mean $\pm$ SD $)$ | Main effects |
| :--- | :---: | :---: | :---: | :---: | :---: |
| DS 7-9 years $(n=15)$ | $16.2 \pm 2.3$ | $8.1 \pm 2.1^{\mathrm{a}}$ | $12.2 \pm 1.6^{\mathrm{a}, \mathrm{b}}$ | $12.4 \pm 1.3^{\mathrm{a}, \mathrm{b}}$ | $F_{9,168}=2.06$ <br> $p=0.036$ |
| TD 7-9 years $(n=15)$ | $20.7 \pm 3.9^{*}$ | $10.9 \pm 1.7^{*, \mathrm{a}}$ | $14.1 \pm 2.9^{\mathrm{a}, \mathrm{b}}$ | $14.4 \pm 2.6^{\mathrm{a}, \mathrm{b}}$ | $F_{3,56}=13.10$ <br> $p=0.001$ |
| DS 10-12 years $(n=15)$ | $18.9 \pm 4.8$ | $8.6 \pm 2.6^{\mathrm{a}}$ | $13.3 \pm 2.4^{\mathrm{a}, \mathrm{b}}$ | $13.5 \pm 3.5^{\mathrm{a}, \mathrm{b}}$ | $F_{3,168}=143.89$ <br> $=0.0001$ |
| TD 10-12 years $(n=15)$ | $21.2 \pm 3.9^{*}$ | $11.8 \pm 0.9^{*, \pm, \mathrm{a}}$ | $15.3 \pm 3.1^{*, \mathrm{a}, \mathrm{b}}$ | $15.4 \pm 1.5^{*, \mathrm{a}, \mathrm{b}}$ |  |

[^1]

Fig. 3. Frequency distribution of each strategy in children with DS and TD children aged 7-9 years old.
years, the hip strategy was the dominant movement pattern during forward and backward reaching. The mixed strategy was the dominant movement pattern in the DS aged 10-12 years. In contrast, the TD children adopted hip, mixed, and ankle strategies when given the same instructions. In the TD aged $7-9$ years, the mixed strategy was the dominant pattern during forward and backward reaching. Conversely, the ankle strategy was the dominant pattern during forward reaching in the TD aged $10-12$ years. The mixed strategy was the dominant pattern during backward reaching.


Fig. 4. Frequency distribution of each strategy in children with DS and TD children aged 10-12 years old.

## Discussion

This is the first study to evaluate children with DS aged $7-12$ years using the MDRT. We investigated dynamic balance ability from the reach distance and movement pattern in children with DS. Children in each group used different movement patterns during forward and backward reaching.

This study found that children with DS in both $7-9$ and $10-12$ years groups had significantly shorter reach distance in the backward direction, compared to TD children. Backward reaching is the most challenging task, requiring a shift in the body weight toward the rear which is unable to depend on visual information. ${ }^{8}$ The ability to lean backward was limited to the biomechanical arrangement of the ankle and foot which allows greater ability for forward movement than backward movement, ${ }^{8}$ sensorimotor deficiency and fear of falling. ${ }^{13,20}$ It is possible that individuals with DS demonstrated deficits in sensorimotor, including poor sensory organization, delayed activation of postural muscles, inefficient postural control strategies. ${ }^{21}$ In addition, children with DS were unfamiliar with backward reaching. Mostly daily activities are commonly performed in front of the body. ${ }^{11}$ These might affect their confidence to perform the movement in the backward direction. Therefore, backward reaching is the most restrictive when compared to TD children. Moreover, the lowest reaching distance was observed in the backward direction for both children with DS and TD. This may be due to the great effort to control the body without falling, which is required when leaning backward. Furthermore, the reason for the backward reaching distance being lower than the forward reaching distance could be because the angle obtained from the hip extension is much smaller than the hip flexion angle. ${ }^{11}$

Focusing on the forward direction, there were significant differences between children with DS and TD in aged 7-9 years old, and the children with DS aged 7-9 years old and the TD aged 10-12 years old. This result shows that children with DS aged 7-9 years old had a restrictive boundary of stability in the forward direction. These results correspond with previous studies ${ }^{4,22}$ whereby children with DS had significantly smaller AP COP displacement than TD children in the forward reaching phase and during the forward dynamic tasks. The smaller value was probably a result of a trunk stiffening strategy which exhibited trunk
stabilization and less movement to compensate for their impairments. The body's degrees of freedom adjustment are required to maintain postural stability and perform the tasks. ${ }^{7}$ During reaching, adopting the stiff trunk decreases the degree of freedom and simplifies inter-segmental coordination in order to complete a task. ${ }^{4,7}$

Children with DS exhibited reliance on hip strategy during both forward and backward reaching. Liao and Lin ${ }^{19}$ reported that older adults and patients who had balance deficits adopt a hip strategy during the FRT. This may be due to compensatory postural adjustment in order to return the COG to the center of BOS. ${ }^{23}$ Possibly, children with DS also need to keep the COG within the BOS in order to maintain stability. The findings in this study are consistent with the reporting of a previous study, that children with cerebral palsy (CP) moved their COP less in the AP direction compared with TD children while reaching forward. They used this strategy to compensate for their poor ankle control. ${ }^{24}$ However, half of children with DS in younger group also exhibited the mixed strategy. According to the previous study, researchers showed that the mixed strategy was the second highest in frequency during the FRT motion in healthy older adults. In this pattern, the forward reaching is executed by bending the trunk forward with hip flexion and without moving the lower limbs in opposite directions, the forward weight shift is controlled by a contraction of ankle plantarflexors. ${ }^{23}$ The magnitude of ankle plantarflexor torque was higher than the magnitude of the hip flexor torque in the mixed strategy which was associated with larger COG displacements that indicated better balance control. The mixed strategy contains the hip and ankle strategies which are transient features of an adaptive process in order to produce an optimal postural response. ${ }^{25}$ This implies that some children with DS who adopted the mixed strategy might have greater postural control than children with DS who adopted the hip strategy.

Interestingly, the ankle strategy was not observed in all children with DS. The ankle strategy in forward and backward reaching is controlled by the eccentric contraction of the surrounding muscles of the lower limbs especially the ankle plantarflexors in forward reaching, dorsiflexors in backward reaching, followed by activation of the trunk muscles. ${ }^{23,25}$ It requires precise somatosensory information. ${ }^{26}$ Children with DS presented a significant
decrease in plantar flexor moments, the muscle power and higher hypotonia and ligament laxity at the ankle joint. ${ }^{27,28}$ These impairments may result in the decrease of the proprioceptive input ${ }^{29}$ that partially causes their inability to adopt the ankle strategy. In contrast, the ankle strategy was used by TD children and this increased with age. It indicated that dynamic balance and adult-like movement pattern improved within TD children as they grew older. This finding demonstrates that children in all groups used different strategies to maintain their balance in the AP direction. Children with DS preferred to adopted hip and mixed strategies in order to compensate for the insufficient control of the ankle and in the attempt to keep the COG within the BOS.

Additionally, there was no significant difference in the forward reach distance between children with DS aged 10-12 years and TD peers. This result reveals that children with DS could improve the ability to move the COG's body toward the LOS in the forward direction. A previous study found that children with DS aged 10-11 years spent more time in moderate-to-vigorous physical activity compared with other age groups. ${ }^{30}$ They might have more experience performing activities in daily life such as physical education, sport, etc. Another possible explanation could be connected to the different movement patterns used by the DS and TD children. The reach distances obtained from the ankle strategy adopted by TD children were less than the hip strategy in children with DS. According to the previous study, the reach distances in ankle strategy were significantly less than the hip strategy. ${ }^{19}$ Thus, there was no significant difference in the reach distance between DS and TD children due to the increasing of TD children aged $10-12$ years who adopted the ankle strategy.

The lateral reach distances in the children with DS showing no significant difference from TD children in similar age may be due to familiarity with preserving mediolateral (ML) stability in children with DS. The wider step width is one of the typical features of school-age children with DS during walking. ${ }^{31}$ The increase in the step width conduces to ML stability by exhibiting a wider BOS. This characteristic is due to the compensatory strategies of balance preservation. ${ }^{32}$ In addition, during the quiet standing and dynamic task movement, individuals with DS demonstrated a significant increase in the ML COP displacement compared to normal subjects. ${ }^{22,27}$ Their characteristics
of having a wider BOS and the COP within their BOS seems adequate in providing greater stability in this position. Moreover, the efficacy of ankle joint control is not enough to maintain standing stability, a balance strategy involving the hip occurs instead. ${ }^{33}$ Therefore, they familiarized themselves with the using hip in order to control stability. The balance in ML direction is the responsibility of the hip muscles. ${ }^{34}$ The greater hipgenerated work possibly leads to a more stable posture during lateral reaching. This result revealed that lateral reaching is not challenging enough for children with DS when compared with similar age TD children. However, children with DS aged 7-9 years old demonstrated significantly smaller lateral reach distance than TD children aged $10-12$ years old. By 10 years of age, children with TD present a mature postural control that is similar to that of adults. ${ }^{35}$ This implies that the balance in children with DS aged 7-9 years is not yet fully completed.

This result indicates that children with DS aged $7-9$ years demonstrated both insufficient forward and backward reaching, while insufficient backward reaching was observed in children with DS aged $10-12$ years. This may increase the risk of falling forwards and backwards. Therefore, restoring their ability to shift the COG in the stability area for the forward and backward directions should be considered as important part of a dynamic balance rehabilitation program for children with DS aged $7-12$ years. Our results suggest that the MDRT may be useful and more sensitive to identifying the standing balance deficit and discrepancy of the LOS especially in the backward direction in children with DS and TD aged 7-12 years old. However, this requires a laboratory measurement tool to discriminate different balance impairment. Moreover, the MDRT may be limited to children who have the ability to understand and follow instructions. A limitation of this study was that information on the participation of children with DS in any rehabilitation program or extracurricular activities was not collected. This information may confound the significant findings between children with DS and TD. Thus, generalizations of this study findings should be made cautiously. Further study needs to collect information on the physical activities of the participants and investigate different populations who have poor balance performance, such as autism, CP, developmental coordination disorder, etc.

## Conclusion

The decreasing of the boundary of stability in an AP direction indicated inefficient postural balance in children with DS. Moreover, movement patterns during reaching in children with DS were different from TD that may be associated with the difference of the ability to maintain postural stability. This finding may be helpful for physical therapists to detect balance deficits and promote the balance rehabilitation program for children with DS.

## Conflict of Interest

There are no conflicts of interest for any author of this paper.

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## Author Contributions

Data collection, data analysis, and manuscript writing were carried out by Promsorn. Data analysis, critical discussion, revising manuscript, and management of the study were carried out by Taweetanalarp.

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[^1]:    Notes: *Significant difference between group when compare with DS 7-9 years ( $p<0.05$ ).
    $\pm$ Significant difference between group when compare with DS $10-12$ years ( $p<0.05$ ).
    ${ }^{\text {a }}$ Significant difference within group when compare with forward direction ( $p<0.001$ ).
    ${ }^{\mathrm{b}}$ Significant difference within group when compare with backward direction ( $p<0.001$ ).

