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Comparison of the Toilet Training Features between Children with Normal Development and Children with Down Syndrome at the Ages of 3–6 Years

Vahide Özdemir[1],
Hatice Yıldırım Sarı[2]

[1]T.R. Ministry of Health Kütahya University of Health Sciences Evliya Çelebi Training and Research Hospital, Kütahya, Türkiye
[2]Department of Nursing, Department of Child Health and Diseases Nursing, İzmir Katip Çelebi University, Faculty of Health Sciences, İzmir, Türkiye

Abstract

Background: It was indicated that children with Down syndrome complete toilet training later than children with normal development.

Aim: This study was conducted to compare the ages at which toilet training starts and finishes, how long it takes to complete the training, and the materials and methods used in children with normal development and Down syndrome between the ages of 3–6.

Methods: A case-control type study was conducted with the families of 40 children with Down syndrome and 40 children with normal development aged 3–6 who attended special education facilities and kindergartens in the provinces of Kütahya, İzmir, and Eskişehir between May 2019 and December 2019. The data were collected by the researcher using the closed envelope method with the control group, and a face-to-face interview with the parents of the case group using the Sociodemographic Feature Form and Toilet Training Questionnaire. To analyze the data, Descriptive statistics and the Mann–Whitney U test were used.

Results: In children with Down Syndrome, the mean age of starting toilet training was 41.12 ± 14.16 months, the mean age to complete toilet training was 51.75 ± 13.83 months, the mean time to complete toilet training was 41.40 ± 35.51 weeks, the mean age to ditch the diaper at nights was 50.43 ± 14.26 months, and the mean age to ditch the diaper in daytime was 43.23 ± 15.40 months. In children with normal development, the mean age to start toilet training was 30.35 ± 9.57 months, the mean age to complete toilet training was 32.80 ± 11.16 months, the mean time to complete toilet training was 13.10 ± 13.74 weeks, the mean age to ditch the diaper at nights was 32.73 ± 11.14 months, and the mean age to ditch the diaper in daytime was 30.35 ± 9.58 months.

Conclusion: Children with Down syndrome start and complete toilet training later than children with normal development, between the ages of 3 and 6. Toilet training should be done specifically for each child's developmental characteristics because each child's developmental process and traits vary.

Keywords: Down syndrome, nursing, toilet training

Introduction

Hypotonia and intellectual impairment are two of Down syndrome's most notable characteristics in children. It was suggested that children with Down Syndrome start toilet training later than children with normal development, due to the delayed motor functions associated with hypotonia.[1-3] Powers et al.[4] stated that children with Down Syndrome start toilet training at the mean age of 5.5, whereas children with normal development start it at the mean age of 2.2. It was also added that the incontinence rate was 46% in children with Down Syndrome and 24.5% in children with normal development.

Independently performing the defecation process is a critical vital skill. Incontinence is one of the major barriers to independent living, and toilet training is an essential skill for independent living in children with developmental disabilities. The ability to undertake defecation and eating independently was identified as the first self-care skill in the study of Konarski and Diorio.[5] Incontinence is a significant issue for people with developmental disabilities because it hinders them from actively participating in social life. Incontinence negatively affects the quality of life of the child due to many reasons such as inadequate hygiene, stigma, irritation and discomfort in the genital area, decreased self-confidence, and restriction of daily activities.[6] For a toddler to be healthy

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Corresponding author: Vahide Özdemir
E-mail: vahide342@gmail.com

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and successfully toilet trained, she/he must be able to perceive the urge to go to the toilet, understand what the feeling of being taken short means, and be able to express that s/he needs someone's help to go to the toilet.[7] Toilet training, which is one of the most important issues of childhood, is a very important step in terms of the child's self-control over his/her own life, and the failure of the process can have serious psychological consequences.[8]

Sphincter control in children with normal development begins to develop in the months of 9–12. Usually, 18–24 months are suitable for starting toilet training, and effective and independent toilet use behavior is expected during the months of 18–36.[8] According to a study, 68.5% of children who started to be given toilet training gain urine control at the age of 2 years, and 55.8% gain control of the defecation at the age of 3. According to the American Academy of Pediatrics Toilet Training Guidelines, boys' toilet training can be delayed by about 6 months compared to girls.[9] The factors affecting the success of the training given can be listed as; the activity level of the children, their physical and mental development, the age to start training, the methods and materials used, the education level of the people who give toilet training, their working status, experience, and socioeconomic factors. In their study, Boyraz et al.[8] found that the age to start toilet training is affected by circumstances such as socioeconomic factors, working mother and/or father, living in rural/urban areas, and toilet training is longer in children who start training earlier than the 18th month and later than the 30th month. The readiness of the family and the child to start toilet training in both normally developing and Down Syndrome children are the most important elements of training (and even affects the entire training process). It is not enough for the family to be ready to give training on its own. Some signs that show the child being ready and the ability of the family to observe, facilitate this process. First of all, the child must have the strength and ability to go to the toilet as part of his physical readiness. Within the scope of psychological readiness, which is another element, s/he should be able to concentrate and control before, during, and after urination and defecation. This situation, which is essential in all children, shows some changes in children with special needs such as Down Syndrome.[10] Children with Down Syndrome may experience some difficulties in urination and defecation due to reasons such as learning, language and teeth development, delayed speech development,[11] and hypotonia in the abdominal muscles. Babies with Down Syndrome with significant hypotonia may take longer to reach normal muscle tone than children with normal development, thus it may delay the time when they are ready for toilet training.[2] After receiving acupuncture treatment, the frequency of enuresis in children with Down syndrome aged 7–12 decreased by 70% in Indonesia.[12] It was argued that cultural characteristics rather than scientific data serve as the foundation for the social conventions around toilet training. This study aims to compare the toilet training characteristics between children with Down Syndrome and those with normal development in the 3 and 6 age period in Turkish society.

Research Questions: Are there any differences between children with normal development and children with Down Syndrome at the ages of 3–6 years in terms of:

1. The age to start toilet training
2. The age to complete toilet training
3. Period to complete toilet training
4. Materials and methods used in toilet training?

Method

Type of the Research

The case–control method was used in the research design of this study.

Place of the Research

The research was carried out between May 2019 and December 2019. To collect data on children with Down syndrome, interviews were conducted in the city centers of Kütahya and Eskişehir with the families of the children who attended the Special Education and Rehabilitation Centers, which educate kids with intellectual and developmental difficulties. Children with Down Syndrome, Autism, and Intellectual Disabilities receive special care and training in these special education and rehabilitation centers. The data on children with normal development were collected from families of children aged 3–6 who were educated in private kindergartens in the same provinces.

Sample of the Research

Forty children with Down syndrome and 40 children with normal development participated in this study. The research power was 0.98 according to the power analysis results of the mean age to start toilet training, and 0.99 to complete toilet training.

Inclusion Criteria (Case and Control)

- Turkish literacy
- Not having any diagnosed psychiatric disease
- Volunteering to participate in the research
- The child should have completed the toilet training.

Case Group

Having a child with Down Syndrome at the age of 3–6 years.

Control Group

Having a child between the ages of 3–6 years.

Exclusion Criteria (Case and Control)

- The child with a urogenital disorder
- Untreated hypothyroidism in the child
- The child with diseases that affect the function of the sphincter or bladder.

Data Collection Tools

Research data were collected by sociodemographic characteristics form and toilet training questionnaire.

Sociodemographic Characteristics Form

The form that was developed by the researchers included questions about the child's age, gender, health status, age of parents, education level of the parents, income status, and family characteristics.[1,4,6-9]

Toilet Training Questionnaire

The following items were all covered in the questionnaire form that the researcher created in accordance with the literature: the age to start toilet training, the time to complete toilet training, the age to finish toilet training, the materials and methods used in toilet training, how toilet training was decided to be given, who gave the training, the status of getting support, the resources benefitted in toilet training, and the problems encountered in toilet training. Expert opinion was taken for the preparation of the questionnaire form.

Data Collection

The researcher collected the data through face-to-face interviews with mothers who enrolled their children in special education and rehabilitation centers. The face-to-face interview lasted about 10 min. In kindergartens/daycare homes, the questionnaire forms were distributed to the families in a closed envelope by means of the school administrations, and the filled forms were collected back from them. The case group's data were initially obtained and included in the research data. The forms were given to the control group which was chosen at random, and they were the same age and gender as the case group.

Data Analysis

The analysis of the research data was made with the SPSS 25.0 program, and significance was sought at the $P < 0.05$ level. Chi-square analysis was used to compare numerical variables between case and control groups. The Mann-Whitney U test was used to compare the mean values in the case-control group since the data did not show a normal distribution in terms of age and gender ($P=0.000$).

Ethical Considerations

After receiving approval from İzmir Kâtip Çelebi University's Non-Interventional Research Ethics Committee, permission was obtained from the institutions where the research was done to carry it out (Approval Number: 148, Date: 27.03.2019). Verbal informed consent was taken from the families participating in the study. The research was conducted in line with the principles of the Helsinki Declaration.

Results

The study included 17 girls (42.5%), and 23 (57.5%) boys in the group with Down Syndrome, 17 girls (42.5%) and 23 (57.5%) boys in the group without Down Syndrome. A total of 34 girls and 46 boys were included in the study. There were equal numbers of children from both genders in both groups ($P > 0.05$). The mean age of children with Down Syndrome was 66.45 ± 12.55 months, and the mean age of children without Down Syndrome was 66.45 ± 12.55, and there was no significant difference between the two groups (t=0.000, $P > 0.05$).

In addition, 35% of children with Down Syndrome had a chronic disease (Heart disease and hypothyroidism: 16.5%, n=12, epilepsy: 1.4%, n=1, and hearing difficulties: 1.4%, n=1). None of the children without Down Syndrome had a chronic disease. The children with Down Syndrome (42.5%) were the second child of the family. When the number of children in the family of children with and without Down Syndrome was compared, a significant difference was found between the groups ($P < 0.05$). The difference in Bonferroni correction was due to the 1st child and 3rd child groups.

Thirty percent of mothers of children with Down Syndrome were primary school graduates and 30% were high school graduates, while 40% of mothers of children without Down Syndrome were high school graduates and 27.5% were secondary school graduates. In addition, 45% of fathers of children with Down Syndrome and 47.5% of fathers of children without Down Syndrome were high school graduates. The rate of families of children with Down Syndrome whose income was less than the expenditure was 35%, and 12.5% in families of children without Down Syndrome. A significant difference was found between the two groups in terms of family income status ($P < 0.05$), and the difference in family income status was due to the group with income less than expenses and income more than expenses. Six percent of families of children with Down Syndrome lived in metropolitan cities, while 65% of families of children without Down Syndrome lived in the cities. A significant difference was found between the groups, and according to Bonferroni's analysis, the difference was between families living in the District, Province, and Metropolitan cities. Descriptive features are shown in Table 1.

As shown in Table 2, the mean age to start toilet training (months) was found to be 41.12 ± 14.16 months in children with Down Syndrome and 30.35 ± 9.57 months in children without Down Syndrome. It was determined that there was a significant difference between the two groups ($P=0.000$) in the age to start toilet training (months). The mean age to complete toilet training (months) was 51.75 ± 13.83 months in children with Down Syndrome and 32.80 ± 11.16 months in children without Down Syndrome. It was found that there was a significant difference between the two groups in the age to complete toilet training (months) ($P=0.000$). The mean time to complete toilet training (weeks) was 41.40 ± 35.51 weeks in children with Down Syndrome, and 13.10 ± 13.74 weeks in children without Down Syndrome. It was identified that there was a significant difference between the two groups in the periods to complete toilet training (weeks) ($P = 0.000$). The mean age to ditch the diaper at night (months) was 50.43 ± 14.26 months in children with Down Syndrome, and 32.73 ± 11.14 months in children without Down Syndrome. It was seen that there was a significant difference between the two groups ($P = 0.000$) in the age to ditch the diaper at night (months). The mean age to ditch the diaper in the daytime (months) was found to be 43.23 ± 15.40 months in children with Down Syndrome and 30.35 ± 9.58 months in children without Down Syndrome. It was determined that there was a significant difference between the two groups in the age to ditch the diaper in the daytime (months) ($P=0.000$).

As shown in Table 3, the age at which toilet training began and ended, the length of time it took to complete, and the age at which children could stop wearing diapers during the day and at night were all examined. In the analyzes performed, a significant difference was found between the gender of children with Down Syndrome and the age at which they completed toilet training ($P=0.016$). It was found that girls with Down Syndrome completed toilet training later than boys with Down Syndrome.

There was no significant difference between the genders of the children participating in the study and the age at which they started toilet training, the period of toilet training, and the age to ditch the diaper in the daytime/at night.

Even though it was not mentioned in the table, no significant difference was found between the groups that participated in the toilet training of children with and without Down Syndrome, such as mothers, fathers, elders, caregivers, and others (siblings). The nursery/teacher participation rate was found to be 17.5% in children with Down Syndrome, while nursery/teachers did not participate in the toilet training of the children without Down Syndrome. The difference was statistically significant ($P=0.012$).

Thirty percent (n=12) of mothers of children with Down Syndrome, and 18.8% (n=15) of mothers of children without Down Syndrome responded to the question as "I stopped using the diaper at once and never tied it again." Sixty percent (n=24) of mothers of children with Down Syndrome and 57.5% (n=23) of mothers of children

Table 1. Comparison of sociodemographic attributes of families and children

	With down syndrome		Without down syndrome		Total	
Sociodemographic attributes	n	%	n	%	n	%
Education level of mother						
Literate	2	5.0	0	0	2	2.5
Primary School	12	30.0	3	7.5	15	18.8
Secondary School	5	12.5	11	27.5	16	20.0
High School	12	30.0	16	40.0	28	35.0
University	9	22.5	10	25.0	28	35.0
Education Level of Father						
Literate	1	2.5	0	0	1	1.3
Primary School	5	12.5	1	2.5	6	7.5
Secondary School	6	15.0	10	25.0	16	25.0
High School	18	45.0	19	47.5	37	46.3
University	10	25.0	10	25.0	20	25.0
Does the mother work full-time?						
Yes	8	20.0	16	40.0	24	30.0
No	32	80.0	24	60.0	56	70.0
Pearson Chi-square analysis: 3.810, P=.087						
Mother's Occupation						
Government Official	5	12.5	10	25.0	15	18.8
Worker	2	5.0	7	17.5	9	11.3
Her Own Workplace	2	5.0	2	5.0	4	5.0
Housewife	31	77.5	21	52.5	52	65.0
Pearson Chi-square analysis: 6.368, P=.095						
Father's Occupation						
Government Official	13	32.5	18	45.0	31	38.8
Worker	20	50.0	10	25.0	30	37.5
Retired	2	5.0	0	0	2	2.5
His Own Workplace	5	12.5	12	30.0	17	21.3
Family's Income Status						
Income Less Than Expenses	14	35.0	5	12.5	19	23.8
Income Equal to Expenses	22	55.0	22	55.0	44	55.0
Income More Than Expenses	4	10.0	13	32.5	17	21.3
Pearson Chi-square analysis: 9.028, P=.011						
Living Place						
Village	1	2.5	1	2.5	2	2.5
District	3	7.5	11	27.5	14	17.5
City	12	30.0	26	65.0	38	47.5
Metropolitan	24	60.0	2	5.0	26	32.5
Pearson Chi-square analysis: 28.345, P=.000						
Chronic Disease						
Yes	14	35.0	0	0	14	17.5
No	26	65.0	40	100.0	66	82.5
Fisher Chi-square analysis: 16.970, *P*<0.001						
Birth Order of the Child						
1	11	27.5	23	57.5	34	42.5
2	17	42.5	13	32.5	30	37.5
3	11	27.5	4	10.0	15	18.8
4+	1	2.5	0	0	1	1.3

Pearson Chi-Square Analysis: 9.035, P=.029
*Analyzes were indicated in the bottom line of the variables.

Table 2. Comparison of starting toilet training months for children with and without down syndrome

	With down syndrome		Without down syndrome			
Features	Mean	SD	Mean	SD	Mann-whitney U	*P*-value
Starting time of toilet training (months)	41.12	14.16	30.35	9.57	419.000	**0.000**
Completion time of toilet training (months)	51.75	13.83	32.80	11.16	217.000	**0.000**
Toilet training period (weeks)	41.40	35.51	13.10	13.74	323.500	**0.000**
Time of ditching the diaper at night (months)	50.43	14.26	32.73	11.14	255.000	**0.000**
Time of ditching the diaper in the daytime (months)	43.23	15.40	30.35	9.58	383.000	**0.000**

without Down Syndrome responded to the question as "I took off the diaper for the daytime and only put it on at night." Mothers using a *notice system* were 7.5% (n=3) in the group of children with Down Syndrome, and 15% (n=6) in the group of children without Down Syndrome. In the "other" option with open-ended answers, 2.5% of the parents of children with Down Syndrome responded as "the teacher taught him/her in the nursery," 2.5% "dryness test," and 2.5% "I often took him/her to the toilet." While the rate of potty use was found to be 72.5% in children with Down Syndrome, this rate was 90% in children without Down Syndrome, and there was a statistically significant difference (P = 0.042). European-style toilet use was found to be 47.5% in children with Down Syndrome, while it was 15% in children without Down Syndrome and it was statistically significant (P=0.02) (Table 4).

The rate of receiving medical advice during the toilet training process of children with Down Syndrome was determined as 12.5%, while it was 2.5% for children without Down Syndrome. There was no significant difference between the groups with and without Down Syndrome in terms of getting medical advice in toilet training (P=0.090).

In the comparison of the seasons in which toilet training was given, 40% of the children with Down Syndrome were trained in the "Spring" season and 45% in the "Summer" season, while 30% of the children without Down Syndrome were trained in the "Spring" season and 47.5% in the "Summer" season. There was no significant difference between the seasons in which children with and without Down Syndrome were toilet trained (P > 0.05).

Discussion

The age of starting and finishing toilet training was shown to be later in children with Down Syndrome than in children without Down Syndrome in this study, which investigated the characteristics of toilet training in children with and without Down Syndrome. In support of the research findings, it was shown in other studies that the age of starting and completing toilet training in children with Down Syndrome was later than that of children with normal development.[1,4] The results of different studies revealed varying findings about the period to start toilet training in children with normal development.[13-18] This suggests that toilet training practices are affected by cultural characteristics. In addition, as each child's development is unique

Table 3. Comparison of toilet training in children with and without down syndrome by gender

	With down syndrome		Without down syndrome	
Features	Girl Mean±SD	Boy Mean±SD	Girl Mean±SD	Boy Mean±SD
Starting time of toilet training (months)	47.29±12.998	41.81±13.006	30.71±9.666	29.23±8.728
Analysis	*MWU: 480.000	P = 0.054	MWU = 619.000	P = 0.712
Completion time of toilet training (months)	80.714±30.714	63.483±26.097	33.523±9.885	31.161±10.668
Analysis	MWU:**437.000**	P = **0.016**	MWU = 558.500	P = 0.2089
Toilet training period (weeks)	132.738±103.236	84.032±80.337	11.833±10.534	12.161±13.909
Analysis	MWU:492.500	P = 0.076	MWU = 635.000	P = 0.857
Time of ditching the diaper at night (months)	61.360±24.814	58.970±25.837	32.790±10.223	31.320±10.616
Analysis	MWU:606.500	P = 0.618	MWU = 597.500	P = 0.545
Time of ditching the diaper in the daytime (months)	49.980±15.231	44.970±16.117	30.900±10.043	29.260±8.513
Analysis	MWU:505.000	P = 0.103	MWU = 612.500	P = 0.658

*MWU=Mann Whitney U

Table 4. Comparison of equipment used in toilet training in children with and without Down syndrome

	With down syndrome		Without down syndrome		Total		
Equipment used in toilet training	n	%	n	%	n	%	Statistical evaluation
Potty							
Yes	29	72.5	36	90.0	65	81.3	***Fisher's Exact: 4.021. P=0.042***
No	11	27.5	4	10.0	15	18.8	
European-style toilet							
Yes	19	47.5	6	15.0	25	31.3	***Fisher's Exact: 9.833. P=0.02***
No	21	52.5	34	85.0	55	68.8	
Alla turca toilet							
Yes	3	7.5	5	12.5	8	10.0	Fisher's Exact: 0.556. *P*>0.05
No	37	92.5	35	87.5	72	90.0	
By holding and sitting in the Alla Turca toilet							
Yes	2	5.0	4	10.0	6	7.5	Fisher Exact: 0.721. *P*>0.05
No	38	95.0	36	90.0	74	92.5	
By using the toilet seat that is placed on the potty							
Yes	6	15.0	2	5.0	8	10.0	Fisher's Exact: 2.222. *P*>0.05
No	34	85.0	38	95.0	72	90.0	
Toilet seat was placed on the potty and stool support was placed under the feet							
Yes	4	10.0	2	5.0	6	7.5	Fisher Exact: 0.721. *P*>0.05
No	36	90.0	38	95.0	74	92.5	

and the preparedness of the child to begin toilet training is considered, the findings of the study on the best timing to begin toilet training may vary.

This study determined that the toilet training period of children with Down Syndrome took longer than children without Down Syndrome. Even though a study[19] found no correlation between the age at which toilet training began and the length of time it took in children with normal development, a subsequent study discovered that the training period for children who started between 19 and 24 months took less time compared to other age groups.[13] Blum et al.[20] found that children who started toilet training at 17–19 months completed their toilet training in 2 months. Moreover, the duration of toilet training is extended if it is started early or late, thus it could lead to negative consequences such as urinary incontinence and defecation refusal. Similar to these studies, in the study conducted by Koc et al.,[21] it was determined that the training period of children, who started toilet training at 18 months or less, took longer. These differences in toilet training periods may have varied due to reasons such as the developmental characteristics of the child, the education level of the family, and economic factors.

In this study, the age of ditching the diapers of the children with Down Syndrome was also later than the children without Down Syndrome. According to the study by Schum et al.[22] conducted with 126 girls and 141 boys with normal development, the age of staying dry all day long for girls was 32.5 months, while the age for staying dry for boys was 35 months. According to Özkul's study,[16] it was carried out with 620 children aged 4–6 years with normal development, the rate of daytime dryness at the age of 2 years was 63.3%, and the rate of dryness at night at the age of 3 years was 52.7%.

In this study, toilet training of children with and without Down Syndrome was mostly provided by the mother. While a nursery/teacher assisted in toilet training for children with Down Syndrome, there was no nursery/teacher assistance for children without Down Syndrome. This may be because children with Down Syndrome complete their toilet training later and are enrolled at special education institutions in this process. In the study of Nunes and Dupas,[23] it was found that mothers were generally responsible for the care of children with Down Syndrome, and toilet training was mostly given by the mother. According to Özkul's study,[16] it was concluded that 88.4% of the mothers were responsible for toilet training.

The most preferred method of accustoming the child to toilet use is similar for children with and without Down Syndrome. In Özkul's study,[16] it was seen that 28.2% of the families took off diapers during the day and put them on at night, and 50.4% took them to the toilet every 1–2 h.

The equipment used in the toilet training of children with and without Down Syndrome was mostly "potty." The equipment preferred in toilet training was similar to the literature.[16,19] In both groups, while the potty was mostly used "only in the toilet and/or bathroom," this rate was lower in children with Down Syndrome than in children without Down Syndrome. It was assumed that this difference might be due

to the longer duration of toilet training for families with children with Down Syndrome, the desire of the family to teach it to the child in any way and wherever, and the child's refusal to go to the toilet. In Tatari's study[24] with normally developing children, parents stated that they mostly kept the potty in the bathroom.

Families that had children with Down Syndrome received medical advice during the toilet training process. This rate was lower in children without Down Syndrome. In the study of Mrad et al.,[1] the rate of families who received medical advice was found to be higher in children with and without Down Syndrome compared to this study. In his study, Ünsal[25] found the rate of parents of normally developing children receiving medical advice as 1%. In the study of Boyraz et al.,[8] this rate was found to be 15.9%. There can be several factors that affect families to seek medical advice. It may be useful to provide education on toilet training by nurses who carry out follow-ups in early childhood and to create accessible training materials for families.

Limitations of the Research

It was challenging to reach the families of children with Down syndrome in the 3–6 age range in private educational institutions, despite the study's strong capacity to reach a larger sample size. Data from the study were lost because children over the age of six who had not finished toilet training could not be included. The results of the study may have been impacted by the children with Down syndrome who had additional chronic disorders, the number of children in the family, and the different income status of the families of the children in both groups.

Conclusion

In this study, it was seen that children with Down Syndrome started and completed toilet training later than children with normal development. While toilet training was mostly provided by the mother in children with and without Down Syndrome, special education and kindergarten teachers were also involved in toilet training in children with Down Syndrome. According to the research's findings, it is important to emphasize that first the child, and then the family members should be ready for training, the process of toilet training should be maintained according to the preparedness of the child, and family training should be given to support the readiness of the families. Nurses can administer training programs for toilet training for children with Down syndrome at Family Health Centers, healthy children's outpatient clinics, and other clinics. It is possible to conduct research to find out, whether toilet training that is carried out with the participation of special education teachers affects the duration of toilet training. The effect of structured training programs on the age of completion of toilet training can be examined.

Ethics Committee Approval: After receiving approval from İzmir Kâtip Çelebi University's Non-Interventional Research Ethics Committee, permission was obtained from the institutions where the research was done to carry it out (Approval Number: 148, Date: 27.03.2019).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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