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Evaluation of the Children with Black Tooth Stain According to the Severity of the Stain and the Number of Affected Primary Teeth: A Cross-Sectional Study

Siyah Diş Renklenmesi Olan Çocukların Renklenme Şiddetine ve Etkilenen Süt Dişi Sayısına Göre Değerlendirilmesi: Bir Kesitsel Çalışma

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ABSTRACT Objective: The present study aimed to evaluate a group of Turkish children with black tooth stain (BTS) according to the severity of stains (SBST) and number of black stained primary teeth (NBST). **Material and Methods:** The children with BTS aged 4-6 years who met the inclusion criteria were selected. Teeth were examined for SBST, NBST, caries and plaque index. The demographic factors, medical and birth history, nutritional status, habits in infancy, using of iron supplements, oral hygiene habits, presence of BTS in parents/siblings, and dietary habits were compared according to SBST and NBST. The data were analyzed with the SPSS program. **Results:** A total of 120 patients (53 females, 67 males) were included. It was found that NBST increased with age. NBST and severe BTS were found to be higher in children born by cesarean section compared to those born by vaginal delivery (p=0.014; p=0.004). The children who fed with nursing bottles less than 6 months have lower NBST (p<0.001). The mild BTS was more common in children who used no iron supplements (81%). Children with plaque index score 0 and 1 did not have severe BTS, while 94.4% of children with plaque index 3 had severe BTS (p<0.001). The difference between decayed, missing, and filled teeth according to SBTS was not found significant (p=0.232). **Conclusion:** In conclusion, it was found that both the NBST and SBST were differed according to age, type of birth, breastfeeding, using nursing bottles, using iron supplements, consumption of cariogenic foods, and presence of BTS in parents/siblings.

Keywords: Tooth discoloration; child; oral hygiene

ÖZET Amaç: Bu çalışmada, siyah diş renklenmesi [black tooth stain (BTS)] olan bir grup Türk çocuğunun renklenme şiddeti ve siyah renklenme görülen süt dişi sayısına göre değerlendirilmesi amaçlanmıştır. **Gereç ve Yöntemler:** Çalışma için dâhil edilme kriterlerini karşılayan 4-6 yaş arası BTS'si olan çocuklar seçildi. Hastalar, siyah renklenme görülen süt dişi sayısı, dişlerin renklenme şiddeti, çürük ve plak indeksi açısından muayene edildi. Demografik faktörler, tıbbi ve doğum öyküsü, beslenme durumu, bebeklik dönemindeki alışkanlıklar, demir takviyesi kullanımı, oral hijyen alışkanlıkları, ebeveynlerde/kardeşlerde BTS varlığı ve beslenme alışkanlıkları, renklenme şiddeti ve siyah renklenme görülen süt dişi sayısına göre karşılaştırıldı. Veriler SPSS programı ile analiz edildi. **Bulgular:** Çalışmaya, toplam 120 (53 kadın, 67 erkek) hasta dâhil edildi. Siyah renklenme görülen süt dişi sayısının yaşla birlikte arttığı bulundu. Sezaryen ile doğan çocuklarda siyah renklenme görülen süt dişi sayısı ve şiddetli BTS'si, vajinal doğumla doğanlara göre daha yüksek bulundu (p=0,014; p=0,004). Altı aydan daha az biberonla beslenen çocukların siyah renklenme görülen süt dişi sayısı daha düşük bulundu (p<0,001). Hafif BTS, demir takviyesi kullanmayan çocuklarda daha yaygındı (%81). Plak indeksi skoru 0 ve 1 olan çocuklarda şiddetli BTS görülmezken, plak indeksi 3 olan çocukların %94,4'ünde şiddetli BTS vardı (p<0,001). BTS'nin şiddeti ile çürük, eksik ve dolgulu dişler arasındaki fark anlamlı bulunmadı (p=0,232). **Sonuç:** Sonuç olarak, hem siyah renklenmenin şiddetinin hem de BTS görülen süt dişi sayısının, yaş, doğum şekli, anne sütü alımı, biberon kullanma, demir takviyesi kullanma, karyojenik gıda tüketimi ve ebeveyn/kardeşlerde BTS varlığına göre farklılık gösterdiği bulundu.

Anahtar Kelimeler: Dişte renk değişikliği; çocuk; ağız hijyeni

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Black tooth stain (BTS) is the most common specific type of extrinsic discoloration among children.[1] BTS can be seen in both primary and permanent teeth, but they are more common in childhood than adolescence and adulthood.[2,3] The prevalence of BTS in the primary dentition ranges from 2.4% to 12.4% in previous studies.[4-9]

The etiology of BTS is not entirely understood. It is formed as a result of the occurrence of insoluble ferric compounds.[10] BTS has higher concentrations of iron salts, calcium and phosphate.[2,10] A positive correlation was found between BTS and consumption of iron supplements, chromogenic diet (drinks, tea, soy sauce), fruits and vegetables with high iron content.[7,11,12] In the study of Chen et al., BTS was associated with age, parental factors, usage of nursing bottle, oral hygiene, and dental caries.[7] Microbiological formation of BTS is associated with the colonization of chromogenic bacteria such as *Porphyromonas, Prevotella* and *Actinomyces* on the Nasmyth membrane, which require vitamin K and hemin for their growth.[13,14]

BTS can cause social and psychological problems in children, causing a loss of self-confidence. The disturbance of parents about children's appearance increase the importance of aesthetic treatments. Due to its high mineral content, black stain is tightly attached to the tooth so, it needs professional scaling.[9,15] It can be removed professionally, but tends to reappear a few months after removal.[1,16]

While most studies in the available literature evaluate prevalance and various factors associated with the presence of BTS, studies of its severity or extension are limited.[5,7,9,11,12,17-19] In the study of Gasparetto et al., authors evaluated the extension of the tooth surface area affected by the black stain in Brazilian children aged between 6-12 years.[18] They classified into three groups according to severity and the most commonly found score was 3 (41%), followed by score 2 (30.8%) and score 1 (28.2%), respectively. In another study, Zhang et al. reported the prevalence of BTS among Chinese preschool children as 12.4% with a mean of 13.7 black stained teeth.[9] They emphasized that most of the children with BTS had more than eight black stained teeth.

In Türkiye, the studies about BTS were very limited and best of authors' knowledge there was no study evaluated the severity or extension of BTS.[20,21] The present study aimed to evaluate a group of Turkish children with BTS according to the severity of stains and number of black stained primary teeth.

MATERIAL AND METHODS

This cross-sectional study ethical approval was taken from the Ethics Committee of Afyonkarahisar Health Science University (date: May 3, 2019, no: 2019/170). The study was conducted in accordance with ethical principles of the Declaration of Helsinki.

Minimum sample size was calculated according to the study of Chen et al. (the prevalence of BTS 9.9%, confidence interval level 95%, margin of error was 5% or less).[7] The minimum sample size to satisfy the requirements was estimated to be 98 children. It was decided to include 120 children with BTS by increasing the number of subjects by 20%.

A two-stage sampling technique was used for the inclusion of the children. First, all the children aged between 4-6 years who visit the pediatric department of university hospital for any reason between 2019 to 2021 years were examined. Children with BTS were diagnosed according to Koch et al.'s criteria and recorded by an experienced pediatric dentist (B.G.T.). The intraoral photograph of a 5 year-old patient with BTS was shown in Figure 1.[22] These criteria consist in visual inspection of dark dots (<0.5 mm in diameter), forming linear stains parallel to the gingival margin at the smooth surfaces of at least two different teeth without cavitation of the enamel surface.[22] Secondly, all children who met the inclusion criteria were re-invited to the clinic.

The inclusion criteria included:

1. Children aged between 4 and 6 years

2. Systemically healthy children

3. Children with primary dentition

The exclusion criteria included:

1. Children who do not accept to participate in the study or whose questionnaires are not filled in completely



FIGURE 1: The intraoral photograph of a child patient with black tooth stains. Note: The mean severity of black stained tooth of the patient was 2, the number of black stained tooth was 18. The 9 teeth of the patient were recorded as score 1 (FDI #51, #54, #62, #64, #71, #72, #73, #82, #83), the 6 teeth of the patient were recorded as score 2 (FDI #55, #65, #74, #75, #84, #85), and the 3 teeth of the patient were were recorded as score 3 (FDI #52, #53, #63).

2. Children who have systemic, genetic or syndromic disease

3. Children born premature or underweight

4. Non-cooperative children during oral examination

Initially, all parents were informed about the aim of the study and a written consent was obtained for the participation of each child. A detailed anamnesis form was filled by parents with the assistance of a researcher (M.T.). Then, children underwent an oral examination by a calibrated and blinded pediatric dentist (E.G.M.).

The anamnesis form comprises demographic factors, medical history, birth history, nutritional status (breastfeeding, nursing bottles) and habits (using pacifiers) in infancy, periods the using of iron supplements, oral hygiene habits (frequency of toothbrushing, type of toothpaste), presence of BTS in parents or siblings, and dietary habits. The frequently consumed foods are then classified and cariogenic (snacks, sweets, honey, carbonated drinks, juices, etc.) and coloring (fruit juices, tea, coffee, turnip, soy sauce, iron rich vegetables such as spinach) foods were recorded.

Oral examination were performed under dental light reflector with a probe and mirror for the severity of black stained tooth (SBST), number of black stained tooth (NBST), dental caries examinations were performed by the same dentist according to World Health Organization criteria.[23] Caries scores were composed of the number of decayed teeth (d), number of missed teeth (m) and number of filled teeth (f). The plaque index of the children were examined according to Sillness-Löe criteria.[24]

The percentage ratio of black stained teeth to the total number of included teeth (teeth with caries free buccal/lingual surfaces) was calculated and recorded as NBST. Teeth with crown restoration or fixed space maintainers were excluded. For determination of SBST, each stained tooth was scored according to the study of Gasparetto et al., then divided to NBST, the score was rounded to the next digit after the decimal point.[18] If mean SBST was 1, it was recorded as mild, 2 as moderate, 3 as severe.

The criteria of Gasparetto et al. were as described;[18]

Score 1: The presence of stained dots/ thin lines with incomplete coalescence which is parallel to gingival margin.

Score 2: The continuous stained lines, limited to half of the cervical 1/3 of the tooth surface.

Score 3: The presence of stained lines more than half of the cervical 1/3 of the tooth surface.

The 10% of the children with BTS (n=12) were randomly selected and the teeth were re-measured by the same researcher one week after the first examination. The intra-examiner reliability of the researcher who did oral examination (E.G.M.) was found almost perfect for NBST and SBST (Cohen's kappa values were 0.96 and 0.88, respectively).

STATISTICAL ANALYSIS

The SPSS program (Version 23, IBM SPSS Statistics, IBM Corporation, NY, USA) was used for analysing the data of the study. The continuous data were presented as mean and standard deviation (SD), median (minimum-maximum) values. The categorical data were presented as number (n) and percentage (%). Normality of the data were tested using the Kolmogorov-Smirnov test. Descriptive statistical analyses of continuous data (NBST) were performed

with Mann-Whitney U for two groups and Kruskal-Wallis H test for three and more groups. The pairwise comparisons were done with post hoc Dunn test. The Pearson's chi-squared test was used to investigate the differences in the prevalance of categorical factors among the different severity of BTS. The post hoc comparisons were done with Bonferroni corrections. The level of significance was set at $p<0.05$.

RESULTS

A total of 120 patients (53 females, 67 males) were included. The mean age of the children was 5.3±0.88 (minimum 4-maximum 6). The mean NBST of all children with BTS was 54.97±21.61. In the study, 32.5% of the children have mild stains, 40.8% moderate stains, and 26.7% severe stains. The NBST was higher in children with severe SBST.

In the study, NBST was not statistically different among males and females (p=0.764). Besides that, nearly half of the females (50.9%) have mild stains. It was found that NBST increased with age. While severe BTS was not observed in 4-year-old children, severe BTS (44.3%) was observed more commonly in 6-year-old children (p<0.001).

According to mode of delivery, severe BTS was found more commonly in children born by cesarean section (p=0.004). NBST was found to be lower in children who were breastfed for <6 months, and severe BTS was not observed in these children. The children who fed with nursing bottles less than 6 months have lower NBST than others (p<0.001). The severity of BTS was not associated with using a pacifier in early childhood (p=0.402). But, NBST was higher in children who used pacifiers in early childhood compared to those who did not (p=0.001).

While mild BTS was more common in children who never used iron supplements (81%), severe BTS was more common in those who used iron for more than 12 months (50%). NBST was found to be the lowest in children who use no iron supplements (p=0.01). NBST and SBST were not found to be associated with chromogenic food consumption (p>0.05). Children who frequently consumed cariogenic foods had lower NBST (50%) and lower percentages (12%) of severe BTS than those who did (p=0.003; p<0.001).

Tooth brushing frequency or toothpaste type did not differ in terms of NBST (p>0.05). Children who brush their teeth irregularly have more severe BTS than others (p=0.001). Children with plaque index score 0 and 1 did not have severe BTS, while 94.4% of children with plaque index 3 had severe BTS (p<0.001).

It has been found that children whose parents or siblings do not have BTS have fewer NBST (50%). Also, mild BTS (55.9%) was more common in children whose parents or siblings did not have BTS (p<0.001). The distribution of the parameters according to severity of BTS was shown in Table 1, and their distribution according to NBST was shown in Table 2.

The mean dmft of all children with BTS was 2.52±2.722. The mean dmft of children with mild BTS was 1.74±1.044, moderate BTS was 2.94±3.556, and severe BTS was 2.81±2.533. The difference between dmft according to severity of BTS was not found statistically significant (p=0.232).

DISCUSSION

Since BTS causes aesthetic anxiety in children and parents, it has been the subject of many studies, from past to present. These studies carried out in different populations mostly focused on the prevalence, etiological factors, microbial and chemical structure of BTS.[6,10,20-22,25] The severity of BTS or the number of stained teeth has been discussed in a limited number of studies.[7,9,18,19] In this cross-sectional study, we evaluated a group of Turkish children with BTS according to the severity of stains and the number of stained teeth. Of the 120 children included in the study, the mean NBST was 54.97±21.61, and 32.5% of the children have mild stains, 40.8% moderate stains, and 26.7% severe stains.

In the current study, NBST was not different among males and females, in agreement with most previous studies.[4,6,7,22] However, according to SBST, females have commonly mild stains. Most of the previous studies did not find an association between age and BTS.[4,6,22,25] Only Chen et al. reported that children more than 5 years old had almost three times NBST than those less than 4 years old.[7] Similarly, in

TABLE 1: The distribution of the parameters according to severity of black tooth stain.

Parameters		Mild (score 1) % (n)	Moderate (score 2) % (n)	Severe (score 3) % (n)	p value
Sex	Female (n=53)	50.9 (27)[a]	20.8 (11)[b]	28.3 (15)[ab]	**<0.001**
	Male (n=67)	17.9 (12)[a]	56.7 (38)[b]	25.4 (17)[ab]	
Age	4 years old (n=34)	32.4 (11)[a]	67.6 (23)[a]	0 (0)[b]	**<0.001**
	5 years old (n=16)	18.8 (3)[ab]	75 (12)[b]	6.3 (1)[a]	
	6 years old (n=70)	35.7 (25)a	20 (14)b	44.3 (31)c	
Type of birth	Vaginal (n=81)	29.6 (24)[ab]	50.6 (41)[b]	19.8 (16)[a]	**0.004**
	Cesarean (n=39)	38.5 (15)[ab]	20.5 (8)[b]	41 (16)[a]	
Periods of breastfeeding	<6 months (n=31)	51.6 (16)[a]	48.4 (15)[a]	0 (0)[b]	**<0.001**
	6-12 months (n=31)	12.9 (4)[a]	35.5 (11)[a]	51.6 (16)[b]	
	12 months < (n=58)	32.8 (19)[a]	39.7 (23)[a]	27.6 (16)[a]	
Periods of using nursing bottle	None (n=45)	22.2 (10)[a]	24.4 (11)[a]	53.3 (24)[b]	**<0.001**
	<6 months (n=23)	52.2 (12)[a]	47.8 (11)[a]	0 (0)[b]	
	6-12 months (n=15)	6.7 (1)[a]	40 (6)[ab]	53.3 (8)[b]	
	12 months < (n=37)	43.2 (16)[a]	56.8 (21)[a]	0 (0)[b]	
Using pacifiers	Absence (n=48)	29.2 (14)	37.5 (18)	33.3 (16)	0.402
	Presence (n=72)	34.7 (25)	43.1 (31)	22.2 (16)	
Use of iron supplements	None (n=21)	81 (17)[a]	14.3 (3)[b]	4.8 (1)[b]	**<0.001**
	<6 months (n=25)	44 (11)[a]	52 (13)[a]	4 (1)[b]	
	6-12 months (n=50)	20 (10)[a]	44 (22)[ab]	36 (18)[b]	
	12 months < (n=24)	4.2 (1)[a]	45.8 (11)[b]	50 (12)[b]	
Consumption of chromogenic foods	Absence (n=55)	36.4 (20)	32.7 (18)	30.9 (17)	0.248
	Presence (n=65)	29.2 (19)	47.7 (31)	23.1 (15)	
Consumption of cariogenic foods	Absence (n=45)	28.9 (13)[a]	20 (9)[a]	51.1 (23)[b]	**<0.001**
	Presence (n=75)	34.7 (26)[a]	53.3 (40)[a]	12 (9)[b]	
Tooth brushing	Irregular (n=26)	19.2 (5)[a]	19.2 (5)[a]	61.5 (16)[b]	**<0.001**
	Once a day (n=47)	34 (16)[a]	63.8 (30)[a]	2.1 (1)[b]	
	Twice a day (n=47)	38.3 (18)[a]	29.8 (14)[a]	31.9 (15)[a]	
Toothpaste	Without toothpaste (n=4)	100 (4)	0 (0)	0 (0)	0.024
	With fluoride toothpaste (n=56)	23.2 (13)	48.2 (27)	28.6 (16)	
	With flouride-free toothpaste (n=60)	36.7 (22)	36.7 (22)	26.7 (16)	
Plaque Index(Silness & Loe)	Score 0 (n=27)	44.4 (12)[a]	55.6 (15)[a]	0 (0)[b]	**<0.001**
	Score 1 (n=21)	71.4 (15)[a]	28.6 (6)[b]	0 (0)[b]	
	Score 2 (n=54)	22.2 (12)[a]	50 (27)[a]	27.8 (15)[a]	
	Score 3 (n=18)	0 (0)[a]	5.6 (1)[a]	94.4 (17)[b]	
BTS in parents/siblings	Absence (n=59)	55.9 (33)[a]	25.4 (15)[b]	18.6 (11)[b]	**<0.001**
	Presence (n=61)	9.8 (6)[a]	55.7 (34)[b]	34.4 (21)[b]	

Bold numbers indicates significant p values (p<0.05); [a-b]Each subscript letter denotes a subset of group categories whose column properties do not differ significantly from each other at the 0.05 level; BTS: Black tooth stain.

our study, both the frequency of severe BTS and the NBST were higher in children aged 6 years than children aged 4 years.

One of the interesting results in this study was that the NBST was found to be higher in children with cesarean section than in children with vaginal delivery. The frequency of severe BTS in children with vaginal delivery was lower than in others. Considering the results, it can be thought that vaginal delivery reduces susceptibility to BTS. However, since there is no study evaluated the relationship between BTS and mode of delivery in the available literature, a definite conclusion could not be reached on this issue. This result can be related with the competition of different oral bacteria for colonization. In the oral cavity, mutans streptococci were detected more frequently in children delivered by cesarean section.[26] General opinion was the mode of delivery may lead

TABLE 2: The distribution of the parameters according to number of black stained tooth.

Parameters		$\bar{X}$±SD NBST	Median (minimum-maximum) NBST	Test statistics	p value
Sex	Female (n=53)	56.7±22.99	50 (20-100)	1719	0.764*
	Male (n=67)	53.6±20.52	53 (20-100)		
Age	4 years old (n=34)	43.82±12.8	40 (30-60)[a]	9.533	**0.009#**
	5 years old (n=16)	61.94±33.18	51.5 (20-100)[ab]		
	6 years old (n=70)	58.8±20.01	53 (20-95)[b]		
Type of birth	Vaginal (n=81)	50.98±18.8	50 (20-100)	2013.5	**0.014***
	Cesarean (n=39)	63.26±24.76	78 (20-95)		
Periods of breastfeeding	<6 months (n=31)	36.77±7.48	40 (30-50)[a]	39.461	**<0.001#**
	6-12 months (n=31)	55.81±15.2	53 (20-95)[b]		
	12 months < (n=58)	64.24±23.57	60 (20-100)[b]		
Periods of using nursing bottle	None (n=45)	59.73±21.92	53 (20-100)[a]	33.359	**<0.001#**
	<6 months (n=23)	35±6.97	33 (30-50)[b]		
	6-12 months (n=15)	73.13±28.37	86 (25-100)[a]		
	12 months < (n=37)	54.22±13.86	58 (30-95)[a]		
Using pacifiers	Absence (n=48)	60.68±23.44	60 (25-100)	1108.5	**0.001***
	Presence (n=72)	46.4±15.06	50 (20-87)		
Use of iron supplements	None (n=21)	44±14.16	40 (20-78)[a]	11.298	**0.01#**
	<6 months (n=25)	51.64±20.04	50 (30-100)[ab]		
	6-12 months (n=50)	56.04±21.46	53 (30-95)[ab]		
	12 months < (n=24)	65.79±24.38	60 (30-100)[b]		
Consumption of chromogenic foods	Absence (n=55)	58.82±26.66	50 (20-100)	1938	0.425*
	Presence (n=65)	50.42±12.18	53 (20-83)		
Consumption of cariogenic foods	Absence (n=45)	49.73±18.73	50 (20-100)	1147	**0.003#**
	Presence (n=75)	63.69±23.41	53 (30-100)		
Tooth brushing	Irregular (n=26)	48.5±11.34	53 (30-78)	3.455	0.178#
	Once a day (n=47)	59±22.2	60 (20-100)		
	Twice a day (n=47)	54.51±24.59	40 (20-87)		
Toothpaste	Without toothpaste (n=4)	50±0	50 (50-50)	0.348	0.840#
	With fluoride toothpaste (n=56)	57.84±26.28	56.5 (20-100)		
	With flouride-free toothpaste(n=60)	52.62±16.82	53 (20-100)		
Plaque Index (Silness & Loe)	Score 0 (n=27)	54.81±17.9	60 (33-100)	4.816	0.186#
	Score 1 (n=21)	46.43±19.36	50 (20-100)		
	Score 2 (n=54)	58.94±26.07	54 (30-100)		
	Score 3 (n=18)	53.22±8.6	53 (30-80)		
Black tooth stain in parents/siblings	Absence (n=59)	51.49±21.07	50 (20-100)	1389.5	**0.03***
	Presence (n=61)	58.33±21.76	55 (30-100)		
Severity of black tooth stain	Mild (n=39)	42.31±12.24	40 (20-80)[a]	28.949	**<0.001#**
	Moderate (n=49)	55.67±24.35	60 (30-100)[b]		
	Severe (n=32)	69.31±16.61	53 (20-100)[c]		

*Mann-Whitney U test; #Kruskal-Wallis H test; bold numbers indicates significant p values (p<0.05); a-cEach subscript letter denotes a subset of group categories whose column properties do not differ significantly from each other at the 0.05 level; SD: Standard deviation; NBST: Number of black stained tooth.

to differences in the microbial acquisition pattern in the oral cavity but, other factors could modify its effect.[27,28] Therefore, this result should be supported by future microbiological studies about BTS and mode of delivery.

In this study, it was observed that all children with BTS were breastfed for varying periods of time. While mild scores were more common in children who were breastfed for less than 6 months, severe scores were more common in children who were fed

between 6 and 12 months. The NBST was also higher in children who were breastfed for more than 6 months. This result can be supported by studies reporting less *Streptococcus mutans* colonization in children who were breastfed up to 12 months.[29,30] It can be thought that the low colonization of cariogenic bacteria increases the concentration of chromogenic bacteria in the oral flora. These bacteria compete for the location sites and reducing the potential adhesion.[22] Besides that, children who were never fed with nursing bottle had more severe stains than those who were fed for more than 12 months. This result is in line with previous studies reporting nursing bottle use as a risk factor for BTS.[6,7] The using of pacifiers was not associated with SBST but, children who used pacifiers at least 6 months have higher NBST than who did not. The result can be explained with the study which reported that pacifiers constitute a preferential site for the growth of oral biofilm.[31]

In the present study, when children were compared according to tooth brushing frequency, SBST and NBST were similar as in previous studies.[7,8] Chen et al. found that the mean visible plaque index of patients with BTS was lower than in the control group.[7] When the plaque index according to Silness and Loe was compared among children with BTS, mild stains were more commonly observed in children with lower plaque index, while SBST was more severe in children with high plaque index. Additionally, no difference was found in terms of NBST. The differences in the studies may be due to differences in the sample group and the study method.[24]

The relationship between BTS and fluoride has been controversial in previous studies. Most of the authors have reported that children who use fluoride toothpaste or mouthwash more frequently are exposed to BTS, and the authors explain this result as fluoride reduces the acidogenic capacity of dental plaque and the higher pH of saliva promotes BTS formation.[2,6,12] However, in one of the study, correlation between BTS and the type of toothpaste was not found significant.[7] Similarly, in this study, no significant difference was found in terms of SBST or NBST between children who used and did not use fluoride toothpaste. The differences in the frequency of brushing, the variable amount of fluoride in the toothpastes, and the unknown intake of fluoride from other sources are the limitations of the study and may explain the differences between the results of previous studies.

Iron supplements have been reported to increase the occurrence of BTS by increasing the level of iron in the saliva.[32] In this study, severe SBST was more common in children who used iron supplements for more than 12 months. NBST was found to be higher in children who used iron supplements for 6 months or longer compared to children who did not use iron supplements. This result is in agreement with studies which found that iron supplementation during pregnancy and in childhood was associated with BTS development.[6,7,33]

In this study, children who have BTS in at least one of their family members had more stained teeth and more severe scores than those without. This result may be associated with maternal or family transmission as an important colonization route in children, intra-familial transmission of bacteria such as *Provetella intermedia* and *Porphyromonas gingivalis*, or similar dietary habits of family members.[34,35]

Most of the authors suggested that there is an inverse ratio between BTS and dental caries, lower dmft index was found in children with BTS. In the study of Gasparetto et al., children with severe BTS had less caries in their permanent teeth.[18] Various factors such as chemical composition of saliva, bacterial adhesion competition and buffering capacity were related with this result.[2,3,5,7,8] In the present study, children who frequently consumed cariogenic foods had lower NBST (50%) and lower percentages (12%) of severe BTS than those who did. But, dmft scores of children with different stain scores were found similar in the present study. The difference between the results of previous studies can be thought to be caused by the differences in the age of the study group and method. While in previous studies, children were evaluated as having BTS or not, in our study, all children had BTS and children were classified according to the severity of BTS. Therefore, it can be thought that caries affect the presence of BTS but, not its severity. However, further studies are

needed as more definitive conclusions can be made in larger populations.

This present study has some limitations because of its cross-sectional design and not including some factors (demographic feature and socioeconomic level of parents, analysis of saliva, microbiological analysis of oral flora) that may affect severity and extension of BTS. Further longitudinal clinical studies and microbiological studies are needed to fully understand the relationship between factors which influence the severity and extension of BTS.

CONCLUSION

In conclusion, in the present study it was found that both the number of black stained teeth and severity of black stains were differed according to age, type of birth, periods of breastfeeding, periods of using nursing bottles, using iron supplements, consumption of cariogenic foods, and BTS in parents/siblings. Understanding the different factors which influence the severity and extension of BTS in primary teeth will support to better management approaches and quality of esthetic treatments, and improving children's quality of life.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Burcu Güçyetmez Topal, Mehmet Ünal;* ***Design:*** *Burcu Güçyetmez Topal, Tuğba Yiğit;* ***Control/Supervision:*** *Burcu Güçyetmez Topal, Tuğba Yiğit;* ***Data Collection and/or Processing:*** *Edanur Gökçe Meydan, Melike Tıraş;* ***Analysis and/or Interpretation:*** *Burcu Güçyetmez Topal, Tuğba Yiğit;* ***Literature Review:*** *Edanur Gökçe Meydan, Melike Tıraş;* ***Writing the Article:*** *Burcu Güçyetmez Topal, Melike Tıraş;* ***Critical Review:*** *Burcu Güçyetmez Topal, Tuğba Yiğit;* ***References and Fundings:*** *Mehmet Ünal, Melike Tıraş;* ***Materials:*** *Burcu Güçyetmez Topal, Melike Tıraş, Edanur Gökçe Meydan.*

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