Article

Reduced Salivary Flow Rate and Increased Caries Susceptibility in Italian Children in Remission from Hematological Malignancy

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Featured Application: Considering that saliva is the major local defense mechanism in the oral cavity, screening for hyposalivation in children in remission from hematological malignancy is suggested to provide supportive care aimed at improving oral health.

Abstract: Salivary gland dysfunction is an underestimated oral late effect of chemotherapy in childhood cancer survivors (CCS). Therefore, the aim of this cross-sectional study was to assess the salivary functioning and dental caries experience in Italian CSSs and age- and gender-matched healthy children. A total of 32 children (15 females and 17 males, age range 6–14 years) in remission from hematological malignancy and 32 healthy controls were compared for salivary parameters (stimulated whole salivary flow rate, pH, and buffer capacity) and presence of carious lesions in the primary and permanent dentition using the dmft/DMFT indexes. Significantly lower stimulated whole saliva (SWS) and pH were observed in CSS than in healthy pediatric patients (both $p < 0.001$), together with a higher prevalence of carious lesions on both the deciduous ($p = 0.002$) and permanent teeth ($p = 0.015$). SWS was more severely impaired in children treated with chemotherapy before 5 years of age ($p < 0.001$) and, in spite of the tendency to improve over time, low SWS was still observed after 5–9 years of disease remission. According to the present data, chemotherapy has a detrimental effect on salivary gland functioning, which would seem to maintain up to 9 years after antineoplastic treatment.

Keywords: childhood; chemotherapy; acute lymphoblastic leukemia; saliva; cancer survivors

1. Introduction

Despite their rarity, pediatric malignancies remain the leading cause of death among children and adolescents [1,2]. Approximately 200,000 children and adolescents are diagnosed with cancer every year worldwide. In Italy, 8031 cancers were diagnosed between 2013 and 2017 in the age range 0 to 14 years, with hematological tumors constituting approximately 40% of the pediatric neoplastic diseases [3,4]. Leukemia is the most common pediatric cancer due to the malignant transformation of stem cells whose proliferation begins in the bone marrow. The most frequent form is the acute lymphoblastic leukemia (ALL) resulting from a massive proliferation of T and B lymphoblasts, leading to bone marrow failure syndrome [5].

Today, more than 80% of children diagnosed with ALL are alive 5 years after diagnosis, owing to the increased cure rate, and the number of long-lived childhood cancer survivors (CSS) is expected to increase [6]. Therefore, for hematological cancers, the delayed side effects are becoming increasingly relevant, as they can severely impact the functional and psychological status of CSSs, and ultimately their quality of life. In Italy, over 60% of CSSs develop one or more late effects arising from cancer treatment [4].

Chemotherapy (CT) is the primary therapeutic approach used in pediatric hematological tumors [7] and has adverse effects on oral health status [8–11]. As such, high prevalence of oral dryness, mucosal ulceration, and dental decay has been reported in these children, mostly secondary to changes in saliva functioning and properties. Salivary glands and mucous membranes are highly vulnerable to the cytotoxic agents, due to their high turnover rate [8]. In particular, CT can impact the excretion flow rate of saliva, its organic and inorganic composition, as well as its immunological and antioxidant characteristics [12,13]. Changes in free radicals and antioxidant levels may play an important role in the onset of oxidative stress-related oral pathologies [12]. Decreased salivary flow in pediatric cancer patients increases their risk of being affected by infectious odontogenic processes and gingival and mucosal inflammation, as whole saliva is a fundamental biofluid for maintaining oral homeostasis [9–11]. Its complex protective role is related to mechanical cleansing functions and lubrication of mucosal surfaces, buffering capacity, maintenance of tooth integrity, facilitation of cell healing, and antibacterial activity [14]. Alterations of such biochemical properties along with the impairment of oral hygiene status, accumulated over time, may manifest as increased caries activity [15]. Unfortunately, most studies assessed salivary gland functioning only during CT with no long-term follow-up.

Derangements in the rheological characteristics of saliva have been described during the induction and consolidation phases of CT [13,16,17], and they have been positively correlated to the neutrophil counts in children suffering from ALL [16]. Conversely, other studies failed to demonstrate any adverse effect of CT on salivary glands in the early stage of cancer treatment [18–20]. Long-term data are scarce [21,22], and most of them focused on hematopoietic stem cell transplantation (HSCT) recipients [23,24]. Reduced salivary flow has been observed even 5 to 15 years after CT [21,22].

Considering that salivary gland dysfunction is an underestimated oral late effect of CT in pediatric patients, it is important to understand more about the long-term alterations in the salivary parameters and their correlation with oral health status. Changes in quantitative and qualitative characteristics of whole saliva may hamper the oral environment equilibrium, predisposing to gingival inflammation and dental caries occurrence [12,25]. Thus, the aim of this study was to evaluate the salivary parameters and caries experience in Italian CSSs of hematological tumors compared to age- and gender-matched healthy controls. We hypothesized that CT would be able to induce long-term changes in these biological parameters.

2. Materials and Methods

2.1. Study Design and Population

This cross-sectional study with a case-control design was conducted in adherence to the Declaration of Helsinki and was approved by the Institutional Ethical Committee of the "AOU Città della Salute e della Scienza" of Turin (Approval no. 0038521). The reporting followed the STROBE guidelines for observational studies [26]. The children's parents or legal guardians signed an informed consent form allowing them to participate in the study.

CCSs (cases), both sexes, were serially selected among outpatients referred to the Section of Pediatric Dentistry, University of Turin, from the Pediatric Onco-Hematology and Stem Cell Transplant Division of the Regina Margherita Children Hospital of Turin for routine dental care between September 2020 and December 2022. Only children affected by hematological cancer when younger than 10 years, treated exclusively with CT protocols, and in remission from the disease for at least 2 years were included in the study. Furthermore, all were to be in mixed or permanent dentition. Children with any concurrent disease or syndrome likely to impact negatively on oral health or salivary parameters (e.g., Sjogren's disease, diabetes mellitus, celiac disease, hepatitis C or human immunodeficiency virus/AIDS, liver/kidney failure, radiation therapy (RT), or HSCT) were excluded.

The same number of systemically healthy subjects (controls) were randomly selected from the C.I.R. Dental School, University of Turin and compared with the study patients.

They were matched referring to age and sex, and they were recruited from the same population (Turin, North Italy).

2.2. Clinical Data Collection and Dental Caries Experience

Information concerning age, sex, socioeconomic background, cancer type, age at diagnosis, chemotherapeutic type, antineoplastic treatment duration, and date of inclusion in the off-therapy list was collected from the medical records. All patients were treated according to the Italian Association of Pediatric Hematology and Oncology (AIEOP) protocols for hematological tumors.

A specialist in pediatric dentistry examined the dental conditions of both groups of children. Diagnosis of carious lesions was based on the criteria established by the World Health Organization [27]. These included the number of tooth surfaces decayed (d/D), the number of teeth missed due to caries (m/M), and the number of surfaces filled after decay (f/F). For the primary teeth, the caries parameter was scored as decayed missing filled teeth (dmft); for the permanent teeth, it was recorded as Decayed Missing Filled Teeth (DMFT).

Before the start of the study, the examiner was calibrated against a reference clinician on 18 non-study subjects (9 CCSs and 9 controls), and he was assessed for reproducibility by duplicating dental examination on the same patients one week after the initial screening. The level of agreement with the reference examiner was ≥90% and the intra-examiner reproducibility between the first and the second recording was ≥94% using the kappa test. Previous studies considered kappa coefficients of 0.75 and 0.93 as the minimum acceptable levels of inter-examiner and intra-examiner reliability, respectively, for carious lesion detection [28–30].

2.3. Saliva Collection and Measurement

Whole saliva was collected between 9 and 11 a.m., at least 2 h after tooth brushing, eating, or drinking, to minimize the influence of the circadian rhythm [31]. Saliva quantity and quality were determined using a chairside kit (Saliva Check buffer kit, GC America Inc., Alsip, IL, USA) containing wax, a measuring cup, pH paper strips, and buffer test strips.

Saliva was sampled using the saliva stimulation technique with paraffin chewing. Collection was started after chewing a paraffin wax for 30 s. The accumulated saliva during the first 10 s was discarded and then continuously swallowed for 5 min into a sterile graduate tube. The stimulated whole salivary flow rate (SWS) was measured as mL/min and scored on a scale from 1 to 3, where 1 represented normal (>1 mL/min), 2 low (between 0.7 mL/min and 1 mL/min), and 3 very low (<0.7 mL/min) salivary secretion. The salivary buffering capacity (SBC) was determined by applying a drop of the collected saliva over the three fields of a buffer test strip for 2 min. The resultant color was compared with the manufacturer's standard; very low SBC was 0 to 5, low was 6 to 9, and normal was 10 to 12. The pH was measured using a test strip. The pH strip was dipped into the saliva for 10 s and then compared with the reference values provided by the manufacturer within a measuring range from 5.0 to 7.8.

2.4. Statistical Analysis

The quantitative data were expressed as means ± standard deviation (SD), or median and interquartile range when appropriate, and the qualitative data as absolute and relative frequency values. In order to assess the effect of age at the time of CT and the effect of follow-up time on outcome variables (salivary characteristics, dmft, DMFT, and the respective subcategories), the CSS group was divided into two subgroups—under and over 5 years at the time of CT, and under and over 5 years after the end of cancer treatment.

Quantitative variables were then examined for normality with the Shapiro–Wilk test. Differences between groups in quantitative variables were analysed by means of Student's t-test or Mann–Whitney U-test for two group comparisons or by means of ANOVA or Kruskal–Wallis test for multiple comparisons (followed by post hoc testing in case of significance). The association between categorical variables was assessed using Fisher's

exact test and χ^2 test. The statistical significance of correlations among salivary parameters (SWS, pH, and SBC) and dental caries was determined using the Spearman correlation analysis. A logistic regression model was used to analyze the association between low SWS and independent variables postulated as having a potentially negative impact on this late effect. The strength of the association between the dependent and independent variables was reported using the odds ratio (OR) and 95% confidence interval (CI). The level of statistical significance was set at 5%. Analyses were performed using statistical analysis software (SPSS, v. 28.0, IBM, Chicago, IL, USA).

3. Results

3.1. Participant Characteristics

A total of 32 CCSs (15 females and 17 males) and 32 age- and gender-matched controls were consecutively enrolled. Clinical data and salivary samples were available for all the enrolled children.

All participants were Caucasian, with a mean age of 9.8 ($\pm$2.2) years in the case group and 9.9 ($\pm$2.5) years in the control group. No statistically significant differences were found for the socio-demographic status between the CSS and control groups (Table 1). CCS children had been diagnosed with hematological malignancies between the age of 1 year and 9 years (mean age 4.0 ± 2.3 years) and 18 of them (56.2%) were younger than 5 years at the time of cancer diagnosis. The majority of them were diagnosed as having acute lymphoblastic leukemia (n = 25; 78.1%). The mean follow-up time from cessation of CT was 4.3 ± 1.8 years (range 2–9 years), with 17 children (53.1%) having completed cancer treatment since 5 to 9 years.

Table 1. Characteristics of the study population.

Variables	Childhood Cancer Survivors	Controls
Male (n, %)	17 (53.1)	17 (53.1)
Age at examination (years, mean $\pm$ SD)	9.8 $\pm$ 2.2	9.9 $\pm$ 2.5
Socioeconomic level (n, %)		
Low	8 (25.0)	7 (21.9)
Intermediate	14 (43.7)	12 (37.5)
High	10 (31.2)	13 (40.6)
Age at cancer therapy (years, mean $\pm$ SD)	4.0 $\pm$ 2.3	NA
Follow-up time (years, mean $\pm$ SD)	4.3 $\pm$ 1.8	NA
Type of cancer (n, %)		
Acute lymphoblastic leukemia	25 (78.1)	NA
Acute myeloid leukemia	7 (21.9)	NA

3.2. Salivary Parameters

Table 2 shows data for salivary parameters. SWS and pH were significantly lower in the case than in the control group (both $p < 0.001$), while no statistically significant difference was detected for SBC. Seventeen of the children in the CSS group (53.1%) compared to three in the control group (9.1%) showed hyposalivation (SWS < 0.7 mL/min). SWS was decreased more in children treated with CT before 5 years of age ($p < 0.001$). Although a tendency for SWS to increase with time was evident, it was still impaired among CSSs after 5–9 years of disease remission compared to age- and gender-matched healthy controls ($p = 0.009$). With regard to pH, it was found to be lower among CSSs than among healthy children, with few differences related to the age at cancer treatment and the time of disease remission.

Table 2. Salivary parameters in the study groups (median, IQR).

Groups	Stimulated Whole Saliva Flow Rate	pH	Salivary Buffering Capacity
CCSs (n = 32)	1.0 (1.0)	6.9 (0.7)	10.0 (2.8)
Controls (n = 32)	3.0 (1.0)	7.7 (0.6)	11.0 (2.0)
p value	<0.001	<0.001	0.101
Age at cancer therapy			
<5 years (n = 18)	1.0 (1.0) ***	6.7 (0.8) ***	10.0 (2.0)
≥5 years (n = 14)	1.5 (2.0) *	7.0 (0.6) *	10.0 (3.0)
Controls (n = 32)	3.0 (1.0)	7.7 (0.6)	11.0 (2.0)
p value	<0.001	<0.001	0.195
Years of remission			
<5 years (n = 15)	1.0 (1.0) **	7.0 (0.4) **	11.0 (2.0)
≥5 years (n = 17)	2.0 (1.5) **	6.6 (0.7) **	10.0 (3.0)
Controls (n = 32)	3.0 (1.0)	7.7 (0.6)	11.0 (2.0)
p value	<0.001	<0.001	0.167

Note: Values with superscript asterisks show statistically significant differences with respect to the control group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Abbreviations: IQR = interquartile range; CCS = childhood cancers survivors.

3.3. Risk Indicator Analysis for Low Stimulated Whole Saliva

Univariate analyses including the potential factors associated with low SWS (<1 mL/min) are shown in Table 3. Age at cancer treatment and time of disease remission were found to be risk indicators for impaired SFR. In the adjusted regression model, cancer treatment before the age of 5 was the only variable that remained statistically significantly associated with reduced salivation (adjusted OR = 9.42, 95% IC: 1.75–50.84, $p = 0.009$).

Table 3. Univariate logistic regression analysis: low vs. normal stimulated salivary flow rate.

Variables	Crude Effect		
	Odds Ratio	95% Interval Confidence	*p* Value
Sex			0.679
Male	1.00		
Female	0.81	(0.29, 2.20)	
Age at dental visit (years)	0.93	(0.75, 1.15)	0.506
Cancer treatment			0.003
No	1.00		
Yes	5.22	(1.74, 15.61)	
Age at cancer therapy			
Healthy controls	1.00		
<5 years	11.69	(2.29, 59.70)	0.003
≥5 years	2.63	(0.72, 9.66)	0.145
Years of remission			
Healthy controls	1.00		
<5 years	5.85	(1.37, 24.89)	0.017
≥5 years	4.75	(1.26, 17.86)	0.021

3.4. Caries Experience

As described in Table 4, CCS subjects showed significantly higher dmft scores as compared to healthy controls ($p = 0.012$), with the number of decayed primary teeth contributing most to the overall score ($p = 0.002$). CCSs treated for hematological malignancy before the age of 5 had more deciduous teeth with active caries compared to older children ($p = 0.011$). Dental decay was also significantly more prevalent among CCSs with shorter time of disease remission ($p = 0.005$).

Table 4. Dental caries in primary teeth according to the dmft index in the study groups.

Groups	dmft		d		m		f	
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
CCSs (n = 32)	3.9 (4.4)	3.0 (7.0)	2.7 (4.1)	2.0 (4.0)	0.2 (0.5)	0.0 (0.0)	1.0 (1.5)	0.0 (2.0)
Controls (n = 32)	1.5 (2.2)	0.0 (2.0)	0.5 (1.2)	0.0 (0.0)	0.2 (05)	0.0 (0.0)	0.8 (1.4)	0.0 (1.0)
p value	0.012		0.002		0.909		0.605	
Age at cancer therapy								
<5 years (n = 17)	4.6 (5.1)	4.0 (7.0) *	3.3 (5.1)	2.0 (4.5) *	0.2 (0.6)	0.0 (0.0)	1.0 (1.1)	1.0 (2.0)
≥5 years (n = 12)	2.9 (3.0)	2.0 (6.2)	1.7 (1.9)	1.5 (3.7)	0.2 (0.4)	0.0 (0.0)	1.0 (2.0)	0.0 (1.5)
Controls (n = 32)	1.5 (2.2)	0.0 (2.0)	0.5 (1.2)	0.0 (0.0)	0.2 (05)	0.0 (0.0)	0.8 (1.4)	0.0 (1.0)
p value	0.034		0.006		0.984		0.515	
Years of remission								
<5 years (n = 13)	3.8 (5.6)	2.0 (7.0) **	3.1 (5.6)	0.0 (4.5) **	0.1 (0.3)	0.0 (0.0)	0.7 (1.7)	0.0 (0.5)
≥5 years (n = 16)	3.9 (3.3)	4.0 (6.5)	2.4 (2.5)	2.0 (4.0)	0.3 (0.6)	0.0 (0.7)	1.2 (1.3)	1.0 (2.0)
Controls (n = 32)	1.5 (2.2)	0.0 (2.0)	0.5 (1.2)	0.0 (0.0)	0.2 (05)	0.0 (0.0)	0.8 (1.4)	0.0 (1.0)
p value	0.029		0.004		0.447		0.195	

Note: Values with superscript asterisks show statistically significant differences with respect to the control group: * $p < 0.05$; ** $p < 0.01$. Abbreviations: dmft = decayed missing filled primary teeth; d = decayed primary teeth; m = missing primary teeth; f = filled primary teeth; CCS = childhood cancers survivors; SD = standard deviation; IQR = interquartile range.

With regard to the prevalence of dental caries in the permanent dentition, as described in Table 5, CCS children showed significantly higher DMFT scores compared to healthy controls ($p < 0.001$); in particular, they presented more decayed ($p = 0.015$) and filled teeth ($p < 0.001$). The DMFT values were also higher among survivors treated when older than 5 years of age ($p < 0.001$), mainly for filled ($p = 0.033$) and missing components ($p < 0.001$), compared to healthy controls. A significant difference was also found when data were stratified according to the follow-up time after the end of cancer treatment, with higher DMTF values among children in remission for at least 5 years ($p = 0.001$). Salivary pH ($\rho = -0.403$, $p = 0.001$) and buffer capacity ($\rho = -0.315$, $p = 0.11$) both showed a significant negative correlation with DMFT scores.

Table 5. Dental caries in permanent teeth according to the DMFT index in the study groups.

Groups	DMFT		D		M		F	
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
CCSs (n = 32)	2.7 (3.4)	1.5 (5.5)	1.3 (2.1)	0.0 (2.7)	0.1 (0.5)	0.0 (0.0)	1.2 (1.5)	0.0 (2.0)
Controls (n = 32)	0.4 (1.1)	0.0 (0.0)	0.3 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.7)	0.0 (0.0)
p value	<0.001		0.015		0.154		<0.001	
Age at cancer therapy								
<5 years (n = 18)	1.9 (3.2)	0.5 (2.5)	1.1 (1.9)	0.0 (2.0)	0.0 (0.0)	0.0 (0.0)	0.8 (1.4)	0.0 (2.0)
≥5 years (n = 14)	3.6 (3.6)	2.5 (6.2) ***	1.6 (2.4)	0.0 (4.0)	0.3 (0.7)	0.0 (2.0) *	1.7 (1.4)	2.0 (3.0) ***
Controls (n = 32)	0.4 (1.1)	0.0 (0.0)	0.3 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.7)	0.0 (0.0)
p value	<0.001		0.052		0.027		<0.001	
Years of remission								
<5 years (n = 15)	2.1 (3.2)	1.0 (3.0) *	1.0 (2.1)	0.0 (1.0)	0.0 (0.0)	0.0 (0.0)	0.9 (1.2)	0.0 (2.0) *

Table 5. *Cont.*

Groups	DMFT		D		M		F	
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)
≥5 years (n = 17)	3.2 (3.6)	2.0 (6.0) **	1.6 (2.2)	0.0 (3.5) *	0.3 (0.7)	0.0 (0.0) *	1.5 (1.7)	2.0 (3.0) ***
Controls (n = 32)	0.4 (1.1)	0.0 (0.0)	0.3 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.7)	0.0 (0.0)
p value		0.001		0.022		0.036		<0.001

Note: Values with superscript asterisks show statistically significant differences with respect to the control group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Abbreviations: DMFT = decayed missing filled permanent teeth; d = decayed permanent teeth; m = missing permanent teeth; F = filled permanent teeth; CCS = childhood cancers survivors; SD = standard deviation; IQR = interquartile range.

4. Discussion

Findings from the present cross-sectional study showed that survivors of pediatric hematological cancers are still at higher risk of salivary dysfunction and carious lesions after a mean remission time of 4.3 years. Thus, the null hypothesis that salivary function and cariological status would not be altered in children with previous malignant diseases treated by CT was rejected.

4.1. Salivary Parameters

The prevalence of objectively measured hyposalivation was 53% in CSSs compared to 9% among systemically healthy controls with the same age, sex, and social background. Although sialometry can be performed using different methods [32], we measured salivary functioning under stimulation with a paraffin tablet and we diagnosed hyposalivation when SWS was less than 0.7 mL/min [33]. Considering that parotid glands primarily provide stimulated salivary secretion [14], altered SWS could be suggestive of major salivary gland hypofunction [21].

Studies on this topic are sparse when compared the substantial number of publications on salivary gland sequelae induced by RT. In line with the present findings, previous studies found that stimulated salivary secretion was significantly decreased in survivors compared to controls [21,34], while unstimulated saliva did not differ significantly between them [21,35]. Cross-sectional studies with a follow-up time ranging between six months to 7 years after CT of childhood and adult solid and hematological malignancies observed decreased saliva secretion rates and xerostomia [34,36]. A recent national cross-sectional study conducted in the Netherlands with a follow-up time of 24 years reported a prevalence of hyposalivation (<0.7 mL/min) of 26% among CSSs of hematological malignancy who received CT but were not submitted to neck and head irradiation or HSCT [22].

Higher frequency of hyposalivation was also found among HSCT recipients, with percentages ranging from 24% to 70% after a 1 year follow-up [24,37] and from 8% to 54% after 4 to 7 years [23,38–40] when considering cut-off values of 0.5 mL/min. Based on SWS values less than 0.7 mL/min the percentages were 53% and 31.7% after a follow-up time of 7 and 24 years [22,23]. Before transplantation, an intense conditioning regimen using total body irradiation, high-dose CT, or both is applied to eradicate the patient's own stem cells. Since these conditioning protocols could impact the salivary gland functioning, we excluded HSCT recipients from the present study.

On the contrary, other studies did not report any alteration in SWS during, within a few months or up to several years after CT for hematological malignancies [20,21,41].

In the present study, the main risk indicators of low SWS were hematological cancer treatment performed before 5 years of age and time of disease remission. Salivary parenchymal tissue is sensitive to the cytotoxic effect of CT and pediatric patients may be more vulnerable than adults because of the immaturity of their tissues, organs, and immune system. Nonetheless, apocrine functions have been found to regenerate gradually over time, with some studies reporting complete recovery of salivary secretion rate approximately twelve months after CT is completed [41–43]. Interestingly, in spite of the tendency for

SWS to increase with time, in the current study decreased SWS was still observed in CSSs after 5–9 years of disease remission.

Notably, female gender was not identified as a risk indicator for impaired salivation. In contrast, previous studies observed a higher prevalence of hyposalivation among women compared to men after pediatric malignancies [22,37]. This finding has been explained by the smaller size of the salivary glands and the hormonal changes in women [44], whose relevance in the present study could be limited by the age of the enrolled children.

Another factor to be taken into account is the role of the dose and frequency of antineoplastic drugs [41]. Chemotherapy alone has been shown to produce damage to immortalized salivary acinar and ductal cells in vitro and nuclear degeneration, interstitial fibrosis, and necrosis of acinar and ductal cells in the animal model. Degeneration of minor salivary glands in humans has also been reported [45]. Clinical studies also support the additive effect of cisplatin in enhancing radiation-induced salivary gland dysfunction by the mechanism of DNA cross-linkage and possibly by blockage of aquaporin expression in the acinar cells [46]. It has been also reported that methotrexate and etoposide are secreted in saliva favoring oral toxicity. Agents such as Adriamycin, cyclophosphamide, and fluorouracil can cause salivary flow reduction leading to xerostomia and taste disturbances [41,47,48]. These differing observations could be the result of a small number of patients, the multiple drug combinations used, and variable time intervals between the end of CT and evaluation of salivary function. In our study, however, due to the use of multiagent chemotherapeutic regimens, it was not possible to discriminate the contribution of each individual cytostatic drug.

Besides low SWS values, we observed a reduction in both salivary buffering capacity and pH, but only the latter reached statistical significance. Wang et al. observed lower salivary flow rate but comparable buffer properties of whole saliva of ALL children submitted to CT compared to healthy controls [25]. Other studies reported the tendency of saliva to be more acidic in children who were either under treatment or had completed CT for leukemia 1–8 years ago with respect to healthy counterparts [13,14,16,49].

4.2. Caries Experience

As secondary outcome, we explored the caries experience in relation to the salivary parameters. The considerably greater prevalence of dental decays observed in the CCS group in both the deciduous and permanent dentition is consistent with previous data [8,25,34,50]. It is known that saliva is the major defense mechanism of the oral cavity, protects from infections, and maintains local homeostasis [14]. Thus, changes in salivary parameters may lead to acute and late adverse effects on the oral and dental structures [8,9,15]. Indeed, both salivary pH and buffer capacity showed a significant negative correlation with DMFT scores, which may be suggestive of perturbations of the ecological stability of the oral environment contributing to bacterial dysbiosis and enrichment of caries-related species [18,25].

The higher DMFT index was attributed to the significantly greater prevalence of active carious lesions and more filled teeth compared to the age- and gender-matched healthy controls. A significant difference was also found when data were stratified according to the follow-up time after the completion of cancer treatment, with higher DMTF values among CSSs in remission for at least 5 years. These findings cannot be precisely compared to the studies published up to now because other investigators examined children in different age groups. Age is a relevant factor to take into account when assessing the oral health status within a social background. Alpaslan et al. [51] found a mean DMFT of 5 in a population of 5- to 14-year-olds, where the subjects had mixed dentition. Avsar et al. [34] examined children with an average age of 6.4 years and obtained a mean DMFT of 7.75. Oguz et al. [52] examined a group of 10-year-olds (who had conditions similar to those of the patients included in the present study) and found a mean DMFT of 6.25.

Besides the impaired salivary functioning, the influence of both home oral hygiene and professional dental care frequency during and after CT on caries occurrence should not be neglected [8]. Indeed, CT children tend to consume more often mouth moistening, which is

sometimes done by sugar-rich soft drinks to relieve oral dryness. However, no differences in oral health parameters were found when children receiving regular preventive protocols during CT were compared to healthy controls [53].

4.3. Strengths and Limitations

This study brings new insights into the late effects of CT on salivary gland functioning as no firm conclusion has been reached in the literature. No data are available for Italy to our knowledge.

This study has some limitations. First, due to the cross-sectional design it was not possible to establish baseline levels for salivary parameters with which to compare subsequent measurements. It has been reported that patients having low salivary secretion before cancer treatment are more likely to develop hyposalivation following CT. Furthermore, we did not collect information on the subjective feeling of dry mouth. This may be a relevant issue, because CCSs who experience hyposalivation at a young age may not appreciate their oral mouth as abnormal and thus they may not complain of xerostomia [22]. We could not assess the drug influence on salivary parameters due to the multiagent CT regimens administered to the enrolled children. Finally, the results of this study cannot be generalized to other hematological malignancies. Further studies with larger sample sizes should be encouraged to tease out the confounding effects of tumor stage, pre-existing dental status, and salivary functioning, and other comorbidities; the long-term effect of CT on saliva production needs further investigation.

4.4. Clinical Significance

Due to high prevalence of hyposalivation and the poor cariological profile of CSSs, regular oral examinations and screening of salivary gland dysfunction during long-term follow-ups are highly recommended in order to provide individualized oral supportive care and dietary interventions aimed to improve oral health. The use of artificial saliva based on carboxymethylcellulose or stimulation of salivary secretion via simple dietary measures such as chewing sugar-free gums or using pilocarpine can promote salivary functioning [11,54]. It is essential to educate the healthcare provider/s and child/children about the significance of oral health care to minimize discomfort and maximize the chances for a long-term successful outcome. Cooperation between pediatric and dental clinics should be encouraged to improve long-term oral health and quality of life of children in remission from hematological malignancy.

5. Conclusions

After a mean remission time of 4 years, children with acute leukemia treated with CT showed higher prevalence of hyposalivation and active carious lesions compared to age- and gender-matched healthy counterparts, which might reveal an unfavorable future oral health status. This greater caries experience might be related to the decreased SWS and pH, and such salivary changes could be considered as long-term side effects of CT and they would seem to maintain up to 9 years after antineoplastic treatment.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of "AOU Città della Salute e della Scienza" of Turin (Italy) (protocol code 0038526, date of approval 9 April 2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study or their parents or legal guardians.

Data Availability Statement: The dataset used and analyzed during the current study is available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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