Age Estimation in Children with History of Acute Lymphoblastic Leukemia and Chemotherapy

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THESIS

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1. INTRODUCTION

1.1 Childhood cancer and Acute Lymphoblastic Leukemia

Diagnosis with cancer is a life-altering event for children and adolescents as well as their families. Although it's promising that the overall 5-year survival rates for childhood cancer have been enhanced due to improved diagnosis and treatment, cancer is still the second leading cause of death in children aged 5 to 14 years[1–3]. According to the latest American Cancer Society estimate (2016), cancers that are most common in children are leukemia (30%), brain and central nervous system (CNS) tumors (26%), neuroblastoma (6%), and non- Hodgkin lymphoma (NHL) (5%)[1]. Among leukemia, Acute Lymphocytic Leukemia (ALL) is the most common one in children[1]. Moreover, ALL is the most common diagnosed cancer in children and adolescents under the age of 15, representing 25% of cancer diagnoses[1,2]. Fortunately, ALL has one of the highest cure rates of all childhood cancers[1,2,4–6]. Around 98% of children with ALL achieve remission, and 85% of children with newly diagnosed ALL treated on current therapies are anticipated to be long-term event-free survivors, with over 90% surviving rate at 5 years[5,6]. Childhood ALL is a type of cancer that originates in the T and B lymphoblasts in the bone marrow in which the bone marrow makes too many immature lymphocytes (a type of white blood cell) that do not mature correctly[5,7]. The role of normal and healthy lymphocytes is to identify and destroy foreign proteins in the body, such as bacteria and viruses[1,5,7]. As a result, children with ALL are more prone to infections, fever

and easy bruising[6,7].

A child born in the United States has a 0.24% chance of developing cancer before age 15 years and a 0.35% chance of developing cancer before age 20 years; this is equivalent to 1 in 408 children being diagnosed with cancer before age 15 years and 1 in 285 children being diagnosed with cancer before the age of 20 years[1,2]. ALL is more common in industrialized countries than in developing countries[1,2]. In industrialized countries, there is a sharp peak in ALL incidence rates at ages 2 to 4 years; however, similar peak is not apparent among children in developing countries[1,2]. The characteristic age peak for ALL in the United States is striking for white and Hispanic children, but less so for black children[2]. In the United States, ALL is more common in boys than in girls and Hispanic and white children than in black children[2]. The most commonly reported risk factors for ALL include: prenatal exposure to x-rays, previous treatment with chemotherapy, and genetic condition such as Down syndrome and Neurofibromatosis[2,6,7]. Other studies reported an association between paternal smoking before conception or during pregnancy and the increased risk of childhood ALL[8,9].

Factors that may influence the overall prognosis of ALL include: age at the time of diagnosis, White Blood Cell (WBC) count at diagnosis, the involvement of Central Nervous System (CNS), patient's sex, race and ethnicity, patient weight at diagnosis and during treatment[4–6]. In general; children who are older than 1 and less than 10 years of age at the time of diagnosis have a better survival rate than children older than 10 years, adolescents, and infants less than 1 years of age[5]. With

regard to the WBC count, a WBC count of 50,000/μL is common operational cut-off point between favorable and poorer prognoses[4–6]. An increased count of WBC at diagnosis increases the risk of treatment failure compared to low WBC count[5]. Moreover, Children with ALL who had CNS involvement at diagnosis have poorer prognosis compared to children with no CNS involvement[5]. Among black and Hispanic children with ALL, the survival rates have been lower than the rates in white children with ALL[2,5,10].

Children diagnosed with childhood cancer often undergo therapies including radiation, transplantation, immunotherapy and/or chemotherapy[1,4–6]. The percentage of patients diagnosed with a childhood cancer that were treated with chemotherapy alone increased during the 3 decades from 18% during the 1970s to 54% during the 1990-1999[10]. On the contrary, the percentage of children with cancer treated with any radiation therapy (with or without chemotherapy) declined from 77% during the 1970-1979 to 36% during the 1990s[10]. For children diagnosed with ALL, 83 % of them were treated with a combination of chemotherapy and radiotherapy during 1970 to 1979[10]. This treatment protocol changed during the 1990s to chemotherapy alone for 78% of children diagnosed with ALL[10].

I.2 <u>Chemotherapy</u>

Currently, chemotherapy is the main treatment modality for children diagnosed with ALL[4,6]. It is usually consist of three main phases: i) induction, ii) consolidation, and iii) maintenance[4–6]. The average length of chemotherapy treatment protocol is about two to three years[4]. The treatment is more intense in

the first few months of treatment[4]. Types and doses of chemotherapy given to the child diagnosed with ALL depend on their risk group (low risk, standard risk, high risk, very high risk)[4,6]. Classifying a child into one of these groups depends on the prognostic factors mentioned earlier[4,5]. Treatment duration and intensity may increase or decrease based on the classified risk group[4,5].

1.2.1 Induction:

The main purpose of induction chemotherapy is to attain a remission[4,6]. Remission is a stage where leukemia cells are no longer found in the sampled bone marrow, a return of normal bone marrow cells, and normal blood counts[4,6]. Remission does not necessarily mean that patient is cured[5]. About 95% of children with Acute Lymphoblastic Leukemia reach the remission stage whithin1 month of induction treatment[4]. This first month of induction treatment is intense and requires lengthy hospital stays and more frequent visits to the doctor[4].

Children with standard-risk ALL usually treated with 3 drugs for the first month of induction[4,6]. These drugs include 2 chemotherapy drugs: L-asparaginase and vincristine, and a steroid drug such as dexamethasone[6]. Children with high-risk ALL will typically receive an additional fourth chemotherapy drug in the anthracycline class, most often daunorubicin[4,6]. Other therapeutic agents that may be given during the early stages of chemotherapy are methotrexate and/or 6-mercaptopurine[6].

1.2.2 Consolidation:

This is the second phase of chemotherapy, and it is usually more intense than induction phase[4]. This phase of chemotherapy starts once the leukemia reaches the remission stage and typically lasts for several months[4,6]. This is considered the most affected phase by the risk stratification[6]. For instance, low-risk ALL children will usually receive less intensive consolidation compared to high-risk individuals[6]. This phase further lowers the number of leukemia cells that remain in the body[4,6,7]. Several chemotherapy agents are combined to aid preventing the remaining leukemia cells from developing resistance[4]. This phase of chemotherapy may conducted over 4 to 6 cycles of therapy and in some patients, this may occur over duration of up to 8 months[6]. In this phase, standard-risk ALL Children are usually treated with drugs such as methotrexate, 6- mercaptopurine (6-MP), vincristine, L-asparaginase, and/or prednisone[4,6]. However, these treatment regimens may vary among cancer centers[4]. For children with high-risk leukemia, they usually receive more intense chemotherapy regimen[5]. Additional drugs such as L-asparaginase, doxorubicin, etoposide, cyclophosphamide, and cytarabine are frequently used, and dexamethasone is replaced for prednisone[4].

1.2.3 Maintenance:

This phase of therapy starts when leukemia remains in remission after induction and consolidation[4]. The main objective of maintenance phase

is to prevent disease relapse after induction and consolidation therapy[6]. Most treatment protocols include the daily use of 6-mercaptopurine (6-MP) and weekly use of methotrexate, along with vincristine and a steroid[4,6]. Other medications may be needed depending on the severity of ALL and the risk of recurrence[4].

These therapies and medications provided to children during cancer therapy, have been known to cause many complications and side effects[2,6,7,11,12]. A study that was conducted on mice showed that chemotherapeutic agents such as doxorubicin that are commonly used in treatment of children with ALL contribute to the reduction of the longitudinal bone growth in adult survivors of ALL[13]. Moreover, the use of multiple chemotherapy drugs will likely synergizes to cause further reduction in this longitudinal growth[13]. Furthermore, a significant reduction in trabecular bone volume, trabecular bone number, and trabecular thickness was reported after exposure to chemotherapy drugs such as doxorubicin[13]. A significant reduction in cortical thickness, cortical marrow area, and cortical area was also reported, even after only single dose of chemotherapy[13]. These changes in bone structure can contribute to increased bone fragility[13]. Combination chemotherapy is believed to cause additional reduction in these bone parameters, leading to further increase in the fragility of the bone[13].

Oral and dental complications such as increased caries risk and severity, dental developmental abnormalities including agenesis, dental hypoplasia, root stunting, and enamel defects were also reported in children diagnosed with cancer

following chemotherapy and radiation therapy[11,14–16]. Survivors also have a higher prevalence of xerostomia and cariogenic microflora, which have been linked to risk of periodontal disease[11,14–16]. Cetiner et al conducted research regarding oral and dental alterations and growth disruption in long-term survivors of childhood cancer following chemotherapy[14]. They found that antineoplastic therapy for childhood cancer increases the prevalence of numerous malformations in teeth, such as root malformation, microdontia, and tooth agenesis[14]. However, no difference in craniofacial growth and development was observed in this study[14]. Also, the earlier the chemotherapy started, the most severe the dental defects were, in line with the theory that immature teeth are at increased risk for developmental disturbances[14].

Several studies have reported on the effect of chemotherapy on tooth development; however, few studies reported on the impact of chemotherapy on the dental age of these patients.

1.3 Dental age:

During initial and recall wellness dental visits, dentists assess the "dental age" (the typical age of a child with a similar amount of dental development) of their pediatric patients to formulate a personalized treatment plan[17]. Abnormalities in dental eruption patterns, whether those abnormalities are changes in the timing or sequence of tooth eruption, have significant impacts on treatment decisions[17]. Intraoral exams and dental radiographs are necessary for comprehensive evaluation and to accurately assess the pediatric patient[17].

Additionally, identification and age estimation of children using radiographs is important to the field of forensics[18–20]. Details about the unknown remains of a child are elicited using dental radiographs to compare antemortem and postmortem records if restorations, pathology, or other dental anatomic differences are noted[20]. Tooth development and eruption patterns have been shown to be relatively consistent; therefore, the dentition is the most accurate way to estimate age in sub-adult remains in complex forensic cases or in age disputed cases, especially when determining if the individual is a minor or an adult[21]. Although permanent teeth eruption is under significant genetic control, various general factors such as gender, socioeconomic status, craniofacial morphology, and body composition can influence this process[22,23]. Most significant disturbances in teeth emergence are caused by systemic diseases and syndromes[21,23]. Tooth formation, rather than tooth eruption, is a more consistent indicator of dental age[19,21,24]. This is because tooth eruption is greatly influenced by environmental factors such as availability of space in the dental arch, premature loss of primary teeth due to extraction, teeth tipping, ectopic eruption and or impaction[24,25]. More information is needed on what other factors impact eruption, which will in turn facilitate the ability to accurately assess the age of the remains by the forensic odontologist especially for children living in conflict or war zones[20,22]. The data from the United Nations Annual Reports of the Secretary General on Children and Armed Conflict (CAAC) as well as new research by the Peace Research Institute Oslo (PRIO) indicate that there are approximately 420 million children living in areas affected by conflict today with more than 1,000 battle related deaths in a year[25,26]. Others risk their lives in an effort of relocating to

a safer place and find death where their bodies remain unnamed and unclaimed[25,26]. Age estimation is an important tool used in many scenarios when individuals are un-identifiable[21,22,24]. Multiple studies reported that in female children, teeth developed earlier than males[24]. Moreover, mandibular permanent dentition develops and erupts earlier than maxillary permanent teeth[19,24].

1.4 Methods for age estimation:

There are multiple methods and charts developed for age estimation based on tooth development and/or eruption, including Schour and Massler, Ubelaker, and the London Atlas[24,27,28]. All three methods tend to under-estimate actual chronological age; however, age estimated by London Atlas was closer to chronological age when compared to the other two methods[24]. According to the London Atlas, females precede males in tooth development between the age 6 and 14 years [18]. After the age of 15, maturation of third molars is more advanced in males than females[18,19]. In addition, third molar development showed the most variation among the subjects in the same age group[18]. While the AlQahtani London Atlas is supplemented with Moorrees' stage descriptions and diagrams that are used for identifying the developmental stages of teeth and root resorption of single and multi-rooted teeth, the Schour and Massler chart and the Blenkin and Taylor chart lack the presence of a written criteria for staging tooth development[29]. Other studies comparing the Cameriere's European formula and the London Atlas on a multi-ethnic American population revealed that London Atlas estimated age more accurately than the European formula[30]. When comparing the London Atlas with

Smith's method of dental age estimation, both showed comparable results[3132]. However, Smith's method requires more mathematical calculations[31].

On the other hand, London Atlas considered simpler than other methods of age estimation, including Smith's method[31,33]. London Atlas involves only visual comparison of panoramic radiographs[31]. In Addition, London Atlas includes the use of a free, web-based, software program that further facilitates the use of the atlas[31]. It has also been reported that the London Atlas is easier to use and less time consuming than other methods that use linear measurements[33]. In comparison to other methods, teeth in both sides of the maxillary and mandibular jaws can be used in London Atlas[33]. Moreover, both permanent and primary dentition can be used in London Atlas, while many dental age estimation methods focus on the permanent dentition[33].

I.5 Study Objectives

One objective of this study is to determine whether children with ALL and a history of chemotherapy have delayed dental development in comparison to the control group using the London Atlas. Second objective is to determine whether children with ALL and a history of chemotherapy show abnormal tooth formation. In addition, the study aims to evaluate the accuracy of the London Atlas in age estimation of children who have received chemotherapy at a young age for the treatment of ALL.

I.6 **Hypotheses**

H01: There is no difference in timing of dental development between children with ALL who have received chemotherapy and control subjects who have not been diagnosed with ALL nor treated with chemotherapy using the London Atlas

H02: there is no difference in frequency of dental anomalies between children with ALL who have received chemotherapy and control subjects.

H03: The London Dental Atlas does not achieve an acceptable level of accuracy in estimating dental age in children with ALL who have received chemotherapy

2. MATERIALS AND METHODS

2.1 Study Approval

This study was approved for exemption by the Institutional Review Board of the University of Illinois at Chicago (Protocol # 2019-0655) Chicago, IL (Appendix A). No funding was required for this project.

2.2 Study Criteria

A retrospective review of records of pediatric patients between the ages six to sixteen years, who had digital panoramic radiograph images taken at the University of Illinois, College of Dentistry Department of Pediatric Dentistry as part of their treatment between January 1, 2003 and May 30th, 2019. The 36 studied subjects include 24 healthy patients (Control group) and 12 patients who were diagnosed with ALL and received chemotherapy prior to the age of six (Studied group). The 36 subjects (Control & studied group) will be randomly selected from all eligible charts.

Inclusion Criteria:

- Subjects age 6 to 16 years old at the time of radiographs were obtained.
- Healthy children for the control group.
- For the Studied group: History of Acute Lymphoblastic Leukemia and chemotherapy received before the age of 6.

- Has panoramic radiograph obtained between January 1, 2003 and May 30th, 2019.

Exclusion Criteria:

- Unclear and/or grossly distorted radiographs.
- Patients who had previous orthodontic treatment and/or severe malocclusion, hyperdontia, and gross pathology (e.g., taurodontism, microdontia, amelogenesis imperfecta, dentinogenesis imperfecta, tumors, abscesses, and/or fractures).
- Presence of syndrome, craniofacial defects, or other systemic disease.

2.3 Methodology

Data was obtained from the UIC College of Dentistry electronic patient database. A report was generated that included all patients, ages 6-16 years, who had a panoramic radiograph taken between January 2003 and May 2019. The principal investigator (PI) reviewed the list for subjects that fit the inclusion and exclusion criteria. Those records that fit the criteria were then exported to a separate encrypted file in the password-protected computer and assigned an identification number. Only the PI had to the report. The document containing the report will be destroyed at the end of the study following the official policy of the Department of Pediatric Dentistry for disposing of confidential information. The identification number was linked to the panoramic radiograph; the subject's health status, sex, and age at the time of radiograph were recorded. The chronologic age of

the subjects was calculated by subtracting the subject's birthdate from the date the radiograph was obtained. The principal investigator carried out assessment of dental development and dental age estimation after training with an expert (AlQahtani). The PI analyzed the panoramic radiographs and calculated the dental age by assessing the Dental developmental stages of the subjects using the London Atlas method (27,28) following the technique described by Alqahtani et al (2010). In addition, The PI examined the radiographs for the presence of pathology or dental anomaly such as tooth agenesis, supernumerary teeth, macrodontia, microdontia, short roots. Tooth agenesis is defined as the congenital absence of at least one permanent tooth[34]. Supernumerary tooth is any tooth in excess of normal number of 32 in the permanent dentition [35]. Macrodontia is a tooth or set of teeth that is larger than the accepted normal range of teeth size[35]. On the other, Microdontia is defined as a condition where the tooth size is smaller than the accepted normal range[35]. Short roots is a condition in which the root is very short, with crown to root ratio equal to or less than 1:1[36].

2.4 Intra-examiner reliability

Prior to the beginning of data collection, the PI calibrated with an expert examiner by examining 5 randomly selected panoramic radiographs of healthy patients. Once an acceptable level of agreement was achieved between PI and the expert examiner (0.8), the PI examined the same panoramic radiographs again in one-week interval to evaluate intra-examiner reliability. Intra-examiner reliability was 0.8 using the Cohen's kappa.

2.5 Statistical Analysis

Radiographs of subjects' dentition was evaluated to determine subjects' developmental and eruption stages according to the technique described by AlQahtani et al[18]. The PI entered each stage on a table and the London Atlas software (https://atlas.dentistry.qmul.ac.uk/?app=1), and generated the estimated age. The software has options for mixed sex diagrams and for sex specific diagrams. The latter was used in this study. The IBM SPSS (Version 22.0, IBM SPSS Statistics, Armonk, NY, USA) was used for all statistical analysis. Estimated age was compared with chronological age for each subject. The chronological age was subtracted from the estimated age; a positive result indicates an overestimation and a negative result an underestimation. This difference, as well as the absolute difference for each radiograph, was tabulated. The mean deviation between chronological and estimated ages will be compared between patients who have a medical history of ALL and control group. The chronological and estimated ages for the entire sample were compared using t-test with p-value < .05 considered statistically significant.

3. RESULTS

Out of 64 patient records who had chemotherapy or diagnosed with cancer, 23 were diagnosed with ALL and had history of chemotherapy treatment. Three of these patients were excluded due to the presence of Down syndrome. Two charts were excluded because the panoramic radiographs were taken after the age of 16. Another six records were excluded due to the absence of panoramic radiographs. Only 12 records ultimately met the inclusion criteria. The control group was randomly selected and age and gender matched with the ALL group. The average age at diagnoses with ALL was around 4 years of age, with the lowest age of diagnosis being at 8 months old. The average chronological age at time of radiograph was 10.40 years in the ALL group and 11.08 years in the control group.

Table I. Demographic Statistics

	Total	**ALL**	**Non-ALL (control)**
	N (%)	**N (%)**	**N (%)**
Sex			
Male	24 (66.7)	8 (66.7)	16 (66.7)
Female	12 (33.3)	4 (33.3)	8 (33.3)
Age (in years)			
7	6 (16.6)	2 (16.6)	4 (16.6)
8	2 (5.5)	1 (8.3)	1 (4.1)
9	9 (25)	3 (25)	6 (25)
10	2 (5.5)	0 (0)	2 (8.3)
11	7 (19.4)	3 (25)	4 (16.6)
12	4 (11.1)	1 (8.3)	3 (12.5)
13	3 (8.3)	1 (8.3)	2 (8.3)
14	3 (8.3)	1 (8.3)	2 (8.3)
Ancestry			
African American	4 (11.1)	2 (16.6)	2 (8.3)
Euro-American	5 (13.8)	1 (8.3)	4 (16.6)
Hispanic	21 (58.3)	9 (75)	12 (50)
Asian	1 (2.7)	0 (0)	1 (4.1)
Declined to Report	5 (13.8)	0 (0)	5 (20.8)

About two third of both groups were male subjects (Table I). Unpaired t-test was used, and no difference was found in age estimation between sexes (*p= 0.43*). Age among ALL and control group ranged from seven to fourteen years of age.

The majority of the studied population of pediatric patients identified themselves as Hispanic (58%) (Table I). However, almost 14% of the patients declined to report their race/ethnicity (Table I). Among the ALL population, 75% were Hispanics (Table I), while 50% of the control group identified as Hispanic. Using Chi-square test, no statistically significant difference was found in age estimation based on race in the studied population *(p= 0.49*).

In term of dental developmental disturbances, five patients (41.6%) of the ALL group showed at least one developmental disturbance in the panoramic radiograph. Three subjects had both microdontia and short roots; while the other 2 had only microdontia. Teeth affected by microdontia were maxillary 3rd molars, maxillary 2nd molars, and maxillary lateral incisors. Short roots were noted in maxillary and mandibular 1st and 2nd molars. Three out of those five subjects with dental anomalies (60%) were diagnosed with ALL before or at the age of four. On the other hand, only one patient (4%) of the control group showed a dental development anomaly (microdontia).

The mean age difference (Estimated dental age – Chronological age) in the control group was 0.25 (SD: 1.26). In comparison, the mean age difference in the ALL group was 0.27 (SD: 0.65). To test the null hypothesis

that there is no difference in timing of dental development between children with ALL who have received chemotherapy and control subjects who have not been diagnosed with ALL nor treated with chemotherapy using the London Atlas, Unpaired t-test was used. The difference in age estimation between children with history of ALL and chemotherapy and non-ALL based on tooth development using London Atlas was not statistically significant ($t(34)= -0.53$, $p= 0.06$); however, it was approaching significance. Figure 1 illustrates the means of difference in age estimation per age group in the ALL and the control group. No clear pattern was detected regarding over or under estimation between the two groups.

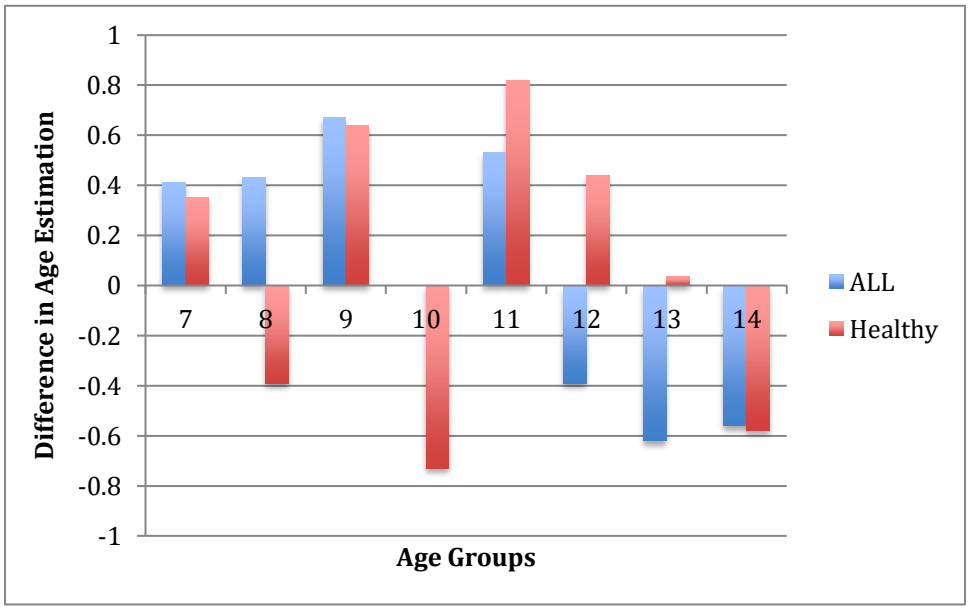

Figure 1. Means of difference in age estimation per age group.

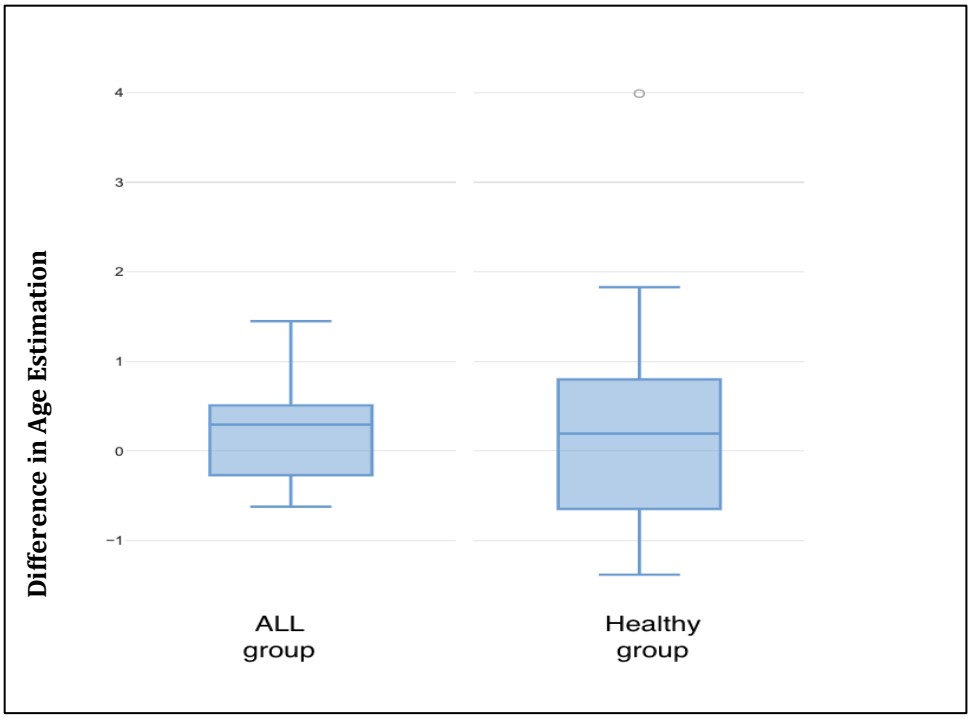

Figure 2. Distribution of the difference between the chronological age and estimated age.

Figure 2. Boxplot shows the distribution of the difference between the chronological age and estimated age. It shows that median of difference in age estimation in the healthy (control) group was closer to chronological age than the ALL group. However, the spread of estimated ages from the median in the healthy (control) group is much larger than ALL group.

4. DISCUSSION

4.1 Dental development in children with ALL

Disruption to the dental development of a growing child may occur as consequence to several factors including: body height and weight, genetics, hormonal factors, nutrition, preterm birth, craniofacial morphology, socioeconomic factors, and various systemic diseases[23]. With the exception of diabetes, the majority of these systemic diseases have been correlated with delayed tooth eruption. Amongst children, cancer is the second most frequent cause of death, where acute lymphoblastic leukemia (ALL) is considered the most common childhood malignancy especially in children under five years of age. Disturbances to the development of the permanent dentition is an expected long term sequela to cancer therapy. In 2018, Kang et al. studied 196 Childhood cancer survivors where 36% of them were diagnosed with ALL and almost the majority of these children received chemotherapy (99.5%) with or without radiotherapy or hematopoietic stem cell transplant (HSCT)[37]. Higher risk for dental developmental disturbances when they receive head and neck radiation therapy, HSCT, cancer therapy at a young age and the use of 4 classes of chemotherapeutic agents[37]. Kang et al reported that 55% of children with childhood cancer have at least one dental anomaly, where the most common dental anomalies were microdontia (30.6%), tooth agenesis (20.4%), and V-shaped roots (14.8%)[37]. The most common teeth to go into agenesis were mandibular and maxillary second premolars[37]. Microdontia was observed more in maxillary second premolar, followed by maxillary second permanent molar[37]. In

addition, there is an increase in dental anomalies in the youngest group (<3 years of age at diagnosis), with 42.2% of them showing tooth agenesis or microdontia[37]. Similar findings were reported in our study, with five subjects (41.6%) of the ALL group showed at least one developmental disturbance. Three of them were found to have both microdontia and short roots, the other 2 had only microdontia. However, Microdontia was noted more in maxillary 2nd and 3rd molar in our study. In addition, three out of the five children (60%) with dental anomalies were diagnosed with ALL earlier at life, before or at the age of four. No tooth agenesis was detected among the ALL group. This finding may be attributed to the type of treatment they received where none of the studied group received radiotherapy. Based on how the London Atlas function to estimate dental age, microdontia could have no impact on estimated age. In general, tooth formation, rather than tooth eruption, is a more consistent indicator of dental age[21,24]. However, many factors and conditions have been reported to potentially influence dental development, resulting in an impact on dental age and dental maturation[18,21,23]. This general delay of teeth development and eruption can impact diagnosis and treatment decisions, such as timing of orthodontic and restorative treatment [38]. On the other hand, dental age was not significantly affected in survivors of non-Hodgkin's Lymphoma treated with chemotherapy who had developmental dental disturbance such as enamel discoloration and tooth agenesis , and root malformation [39].

4.2 Dental age in children with ALL

Limited studies have assessed dental age in pediatric survivors of ALL who were treated with chemotherapy. In this study, dental age assessment was measured using the London Atlas to detect any discrepancy between the dental age and chronological age. The London Atlas has been one of the most commonly used method for age estimation using dental panoramic radiographs. Most studies that have tested the validity and accuracy of London Atlas were conducted on healthy population. However, A 2018 study conducted in Italy aimed to validate age estimation in syndromic children utilizing London Atlas[40]. Most reported syndrome win this study was Down syndrome (124 children) [40]. The study showed that no significant difference in age estimation using London Atlas between children with Down syndrome and healthy population[40]. They conclude that no difference in dental maturation between syndromic and healthy children using the London Atlas[40]. In regard to patients with cancer, previous reports by Dahllo¨f et al found no relationship between dental and chronological age when comparing patients with cancer treated with chemotherapy to control group[41]. In addition, ajari et al. found using the Demirjian method that children with cancer who received chemotherapy have an advanced average dental age rather than delayed when compared to the control group[42]. Similar to the later study, our findings suggest that chemotherapy has no significant impact on dental age; however, children with history of ALL and chemotherapy were more likely to have an advanced average dental age when compared to their healthy (Non-ALL) counterparts.

Multiple studies reported the negative effect of chemotherapeutic agent used in treatment of ALL children on dental and skeletal growth and development. chemotherapy might contribute to the long-term effect of bone growth and bone pattern in ALL children receiving chemotherapy[13]. Changes such as reduction in longitudinal growth and reduction in trabecular bone volume and thickness, were observed[13]. It was suggested that, except of L-asparaginase, all chemotherapeutic agents used in the treatment of pediatric ALL could cause significant effects on the developing bone[13]. This could be because dental age was not as severely affected by chemotherapy as bone development. It has been reported that growth stunting has no influence on dental development, especially among preadolescent population[43]. Also, the relationship between dental development and skeletal growth is considered to be moderate at most, suggesting that using dental development as proxy for skeletal growth or vice versa may not be reliable and could be misleading[44].

4.3 Age estimation and ALL

This study examined the accuracy of the London Atlas in estimating the age of children with history of ALL whom received chemotherapy. In our sample, London Atlas showed high accuracy in age prediction in children with history ALL and treatment with chemotherapy. In both groups, London Atlas over-estimated age by an average of three months and one week in the ALL group, and by three months in the control group. This overestimation tendency was also reported by McCloe et.al, with a mean difference of +0.35 years in age estimation reported in their study[45]. In

addition, there was no difference in age estimation between sexes in both groups in our study using the London Atlas. This was consistent with findings reported by McCloe et.al who reported no significant difference in age estimation between males and females when using the London Atlas among similar population[45]. Our sample had more male than female patients, with male being 66.7% of the sample and female being 33.3%. This male Tendency in children with ALL was consistent with the national and state findings. In 2016, Illinois reported 205 new ALL cases. Among these cases, 127 were males (62%)[46].

Among the studied population, race and ethnicity did not show an effect on age estimation. The ancestry of the patients was self-reported when registering them. Other studies reported similar findings indicating no significant difference in age estimation among different ethnicities when using The London Atlas[18,45].

In general, The London Atlas method was an accurate predictor of chronological age for our sample. Therefore, London Atlas can be a useful method for age estimation age for our studied population. Our results suggest that children who have undergone chemotherapy may not have delayed dental development and therefore can be treated on same timeline as other children. However, this study confirms prior research showing that children who have undergone chemotherapy have a higher prevalence of certain dental anomalies[37].

4.4: Limitation of the study

One of the limitations of this study is the small sample size of the ALL group, which may decrease the statistical power and resulting in type II error. In addition, children with ALL who received more intense chemotherapy or had increased treatment duration may show more severe dental developmental disturbance that could effect their dental age[15,39,42]. In our study, the duration and protocol of chemotherapy treatment that the children with ALL received was not reported. Other limitation is the age at diagnosis and treatment for ALL was not the same in the ALL group. Early diagnosis and chemotherapy treatment could cause more dental developmental defects that might impact the age estimation using panoramic radiographs[14,37,39]. Lastly, studies have reported that high BMI is correlated with advancement in dental development. That could result in overestimation of their age using London Atlas[47]. The height and weight and BMI was not calculated for both ALL and the control group.

4.5: Future Considerations

Future studies should investigate the impact of different chemotherapy protocols on the development of the dentition in the growing child. In addition, a larger sample size is needed to detect any significant effect of chemotherapy on dental development. Perhaps a similar study to ours can be carried out at an oncology center where other external factors can be controlled and more patients with similar treatment protocols can be studied. Lastly, future studies may also consider evaluating the accuracy of other age estimation methods in children with ALL.

5. CONCLUSION

According to the results of this study, the London Atlas accurately estimates dental age in patients with ALL who undergone chemotherapy. There was no significant difference in age estimation based on tooth development using London Atlas between children with history of ALL who had chemotherapy and non-ALL children. We interpret these results to indicate: 1) that the London Atlas is an accurate method for assessing age in children with ALL and a history of chemotherapy and 2) that children with ALL and a history of chemotherapy do not show delayed dental development. On average, The London Atlas was a good method for age estimation in children with history of ALL who had chemotherapy with an accuracy that is similar to the general population. We also confirmed prior studies which have indicated a greater frequency of dental anomalies among subjects who have undergone chemotherapy. These results have important implications for treatment of children with ALL, such as timing of orthodontic treatment and the decision of whether to place space maintainer or not.

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Appendix A

Exemption Granted

June 18, 2019

Mohamad Alhadlaq
Pediatric Dentistry

RE: **Protocol # 2019-0655**
"Age Estimation in Children with History of Acute Lymphocytic Leukemia and Chemotherapy Treatment"

Dear Mohamad Alhadlaq:

Your application was reviewed on **June 18, 2019** and it was determined that your research meets the criteria for exemption as defined in the U.S. Department of Health and Human Services Regulations for the Protection of Human Subjects [45 CFR 46.104(d)]. You may now begin your research.

Exemption Granted Date: June 18, 2019
Sponsor: **None**

The specific exemption category under 45 CFR 46.104(d) is: 4 Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

You are reminded that investigators whose research involving human subjects is determined to be exempt from the federal regulations for the protection of human subjects still have responsibilities for the ethical conduct of the research under state law and UIC policy.

Please remember to:

- → Use your research protocol number (2019-0655) on any documents or correspondence with the IRB concerning your research protocol.
- → Review and comply with the policies of the UIC Human Subjects Protection Program (HSPP) and the guidance ***Investigator Responsibilities***.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact me at (312) 413-9680 or the OPRS office at (312) 996-1711. Please send any correspondence about this protocol to OPRS via OPRS Live.

UNIVERSITY OF ILLINOIS AT CHICAGO
Office for the Protection of Research Subjects
201 AOB (MC 672)
1737 West Polk Street
Chicago, Illinois 60612
Phone (312) 996-1711

Sincerely,

Jovana Ljuboje, MPA
IRB Coordinator, IRB #7
Office for the Protection of Research Subjects

cc: Marcio Da. Fonseca, Pediatric Dentistry, M/C 850
Sahar Alrayyes, Faculty Sponsor

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VITA:

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2014: Bachelor degree of dental surgery (BDS) from College of Dentistry, King Saud University, Riyadh, Saudi Arabia

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Poster Presentation entitled "Age Estimation in Children with Acute Lymphoblastic Leukemia and Chemotherapy" at the American Academy of Pediatric Dentistry (AAPD) Annual Session in Nashville, TN, May 2020

Poster Presentation entitled " Bonding Resin – Composites to Enamel Following Application of Remineralizing Agents" at the CED-IADR/ NOF Oral Health Research Congress in Vienna, Austria, 2017.

Poster Presentation entitled: " Patterns of Dental Services Utilization Among Health Care Professionals" at the AADR/CADR General session and exhibition in Los Angeles, Calif. USA, 2016.

Publications: - A. Hamoud Al-Badr, A. Hassan Al-Jameel, H. Suleman Halawany, Y. Hussein Al-Jazairy, M. Khalid Alhadlaq, N. Saleh Al-Maflehi, J. Adnan Al-Sharif, V. Jacob, N. Abraham, Dental CariesPrevalence among Type 1 Diabetes Mellitus (T1DM) among 6- to 12-Year-Old Children in Riyadh, Kingdom ofSaudi Arabia Compared to Non-Diabetic Children, The Saudi Dental Journal (2020), doi: https://doi.org/10.1016/j.sdentj.2020.03.005

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