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Influence of age, sex, and presence of cleft lip and palate on the quantity and quality of saliva-derived DNA

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ABSTRACT

The quantity and quality of DNA are crucial factors in determining polymorphisms in samples from individuals with and without pathologies such as cleft lip and palate (CL/P). Saliva is increasingly being evaluated as a collection method. This study aimed to compare saliva DNA concentrations from individuals of different ages with and without CL/P.

Saliva samples were collected, DNA was extracted and the quantification was performed by spectrophotometry. Kruskal-Wallis test was used to compare frequencies between groups, and Spearman's rho for correlations.

We obtained saliva DNA from 314 individuals: 107 with CL/P (mean age: 12.7 ± 6.5 years), 103 parents of children with CL/P (mean age: 41.1 ± 9.3 years), 52 individuals without CL/P under 18 years old (controls), and 52 individuals without CL/P over 18 years old (controls). The sample comprised 32.2 % females and 67.8 % males.

Statistically significant differences in DNA concentrations were found between individuals under and over 18 years old (12.38 and 21.3 ng/μl, respectively, $p = 0.0001$), but no differences were observed between males and females. Individuals with CL/P had lower DNA concentrations (12.45 ng/μl) compared to their parents and controls (21.95 and 21.15 ng/μl, respectively). Our results showed a direct correlation between age and DNA concentration in individuals with CL/P under 18 years old ($R = 0.328$).

1. Introduction

Genetic polymorphism studies require simple, non-invasive methods to obtain high-quality DNA in sufficient quantities. Traditionally, genomic DNA extraction from peripheral blood samples has been the most common approach. However, this method is costly, invasive, and often causes discomfort, leading to reduced participation, especially among children and adolescents. Furthermore, blood sampling necessitates experienced personnel and specialized laboratory infrastructure for processing and storage.[1]

Saliva collection has emerged as an alternative method for DNA sampling.[2] Its advantages include lower cost, ease of collection without the need for expert personnel, greater acceptability among study subjects, and the ability to store samples at room temperature for extended periods.[3] Some studies have suggested potential drawbacks of saliva-derived DNA, such as lower DNA yield and possible bacterial DNA contamination.[4–7] However, comparative studies between blood- and saliva-derived DNA have shown that saliva provides sufficient DNA of adequate quality for genetic analysis and specific SNP determination.[5,6]

The influence of factors such as age, sex, or pathologies like oral clefts on the quantity of DNA obtained from saliva samples remains unclear. Therefore, this study aimed to investigate variations in the quantity and quality of saliva-derived DNA based on age, sex, and the presence of cleft lip and palate (CL/P).

2. Methodology

2.1. Determination of DNA quantity and quality from saliva samples

This study was approved by the institutional ethics committee (Acta N°004). Written informed consent was obtained from all participants and/or their parents. Whole saliva samples were collected from 314

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Table 1
Demographic data of the subjects.

		n	%	Average age
Group	CLP	107	34.1 %	12.7 ± 6.5
	Parents	103	32.8 %	41.1 ± 9.3
	Controls >18 years	52	16.5 %	14.5 ± 8.6
	Controls <18 years	52	16.5 %	38.6 ± 7.7
Age	>18 years	155	49.3 %	13.6 ± 7.5
	<18 years	159	50.6 %	39.8 ± 8.5
Sex	Female	101	32.2 %	
	Male	213	67.8 %	

subjects receiving multidisciplinary care at the dental clinics of Universidad El Bosque and Universidad de Cartagena, Colombia, between 2023 and 2024.

The study population comprised four groups: 1. Individuals with cleft lip and palate (CL/P) (n = 107). 2. Parents of individuals with CL/P (n = 103). 3. Individuals without CL/P under 18 years old (n = 52). 4. Unrelated individuals without CL/P over 18 years old (controls) (n = 52)

Saliva samples (2 mL) were collected from each participant using the Oragene DNA Sample Collection Kit (OG-500, DNA Self-Collection Kit, Genotek, Ottawa, Ontario, Canada).

The mean age of individuals with CL/P was 12.7 ± 6.5 years; parents, 41.1 ± 9.3 years; controls over 18 years old, 39.3 ± 8.7 years; and controls under 18 years old, 13.8 ± 4.3 years. As shown in Table 1, 98 subjects were over 18 years of age, and 166 were under 18 years of age. The sex distribution comprised 80 females and 184 males.

DNA was extracted from 200 µl of saliva samples using the Qiaamp DNA blood mini kit (Qiagen, Germantown, MD, USA)), taking into account the manufacturer's recommendations. DNA concentration was evaluated at 260 nm, and the 260/280 nm ratio was used to estimate DNA purity by spectrophotometry using NanoDrop 2000c. Genomic DNA integrity was assessed by 0.8 % agarose gel electrophoresis, followed by ethidium bromide staining visualization.

To determine the quality of DNA obtained from saliva samples, two genes associated with CL/P formation were analyzed. The promoter region of the OSR2 gene was amplified by real-time qPCR, and the amplicons were sequenced using the Sanger method. Additionally, saliva-derived DNA was used to detect polymorphisms in BMP2 using restriction enzymes such as Bsr1.

2.2. Statistical analysis

Data were processed using IBM SPSS Statistics V22 software. Descriptive statistics (median and interquartile range) were used for age and DNA concentration. Categorical variables were characterized by absolute and relative frequencies. Kruskal-Wallis test with post hoc Dunn's test was used to compare mean values between study groups. Mann-Whitney *U* test was used to compare DNA concentration between age groups and sexes. All tests were performed with a significance level of 5 %. The correlation between DNA quantifications and age by study groups was assessed using Spearman's rho (two-tailed).

3. Results

3.1. Evaluation of DNA quality from saliva samples

The DNA obtained from saliva samples exhibited a 260/280 ratio between 1.8 and 2.0, indicating high purity. To assess DNA quality, we amplified and sequenced the promoter region of the OSR2 gene to detect SNPs in individuals with CL/P. The resulting chromatograms were of good quality across all analyzed samples. Furthermore, the saliva-derived DNA was suitable for determining polymorphisms in the BMP2 gene associated with CL/P using the restriction enzyme BSR1 (Fig. 1).

3.2. Comparison of DNA Yield from saliva samples by age, sex, and presence of CL/P

When evaluating DNA yield according to age, we found significantly less DNA in individuals younger than 18 years. The average DNA concentration in individuals younger than 18 years was 12.38 ng/µl, while in those older than 18 years, it was 21.3 ng/µl. This difference was statistically significant (p = 0.0001) (Fig. 2).

Comparing DNA yield between groups, individuals with CL/P had a

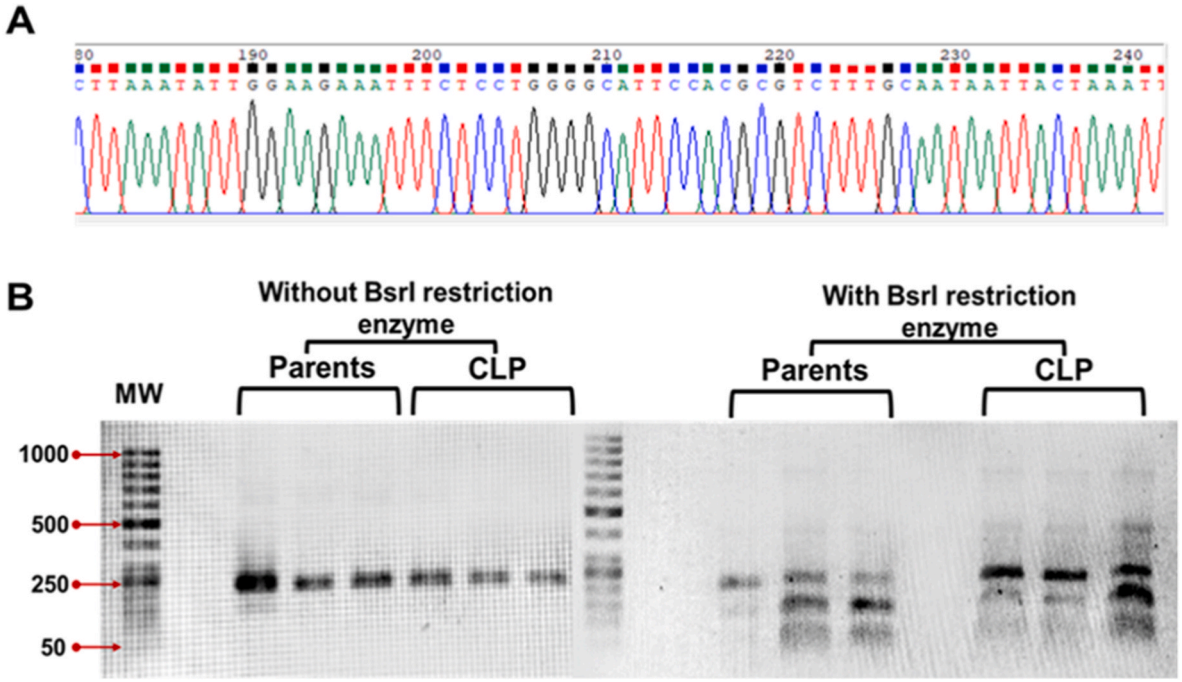

Fig. 1. Assessment of DNA quality obtained from saliva samples. The high-quality DNA enabled (A) amplification and sequencing of the OSR2 gene promoter region and (B) evaluation of SNPs in exon 3 of the BMP2 gene in individuals with cleft lip and palate (CL/P).

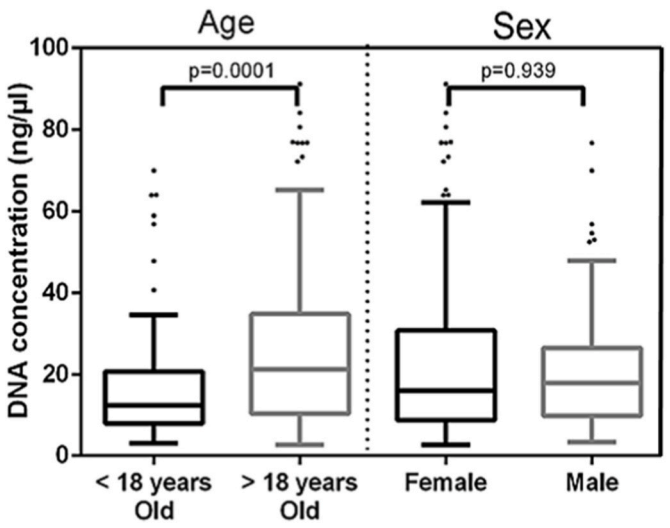

Fig. 2. Comparison of DNA concentration by age and sex. Box plots showing the distribution of DNA concentrations (ng/µl) for different age groups and sexes. Statistically significant differences are indicated.

lower average concentration (12.45 ng/µl) compared to their parents (21.95 ng/µl) and control individuals over 18 years old (21.15 ng/µl). This difference was statistically significant ($p = 0.01$). No significant differences were found in DNA yield between sexes or between parents and controls over 18 years old (Fig. 2).

We observed a significant positive correlation between age and DNA concentration (Rho = 0.24, $p = 0.0001$), with younger individuals showing lower DNA concentrations than adults (Fig. 3A). When analyzing this correlation within groups, individuals with CL/P showed a significant correlation between age and DNA concentration ($R = 0.32$, $p = 0.001$), while no correlation was found in the other groups (Fig. 3B).

To control sample size differences, we analyzed DNA concentration with matched sample sizes between individuals with CL/P, their parents, and control groups (under and over 18 years without CL/P). This analysis confirmed that the DNA yield in individuals with CL/P was 45 % lower than that obtained from their parents and controls over 18 years of age ($p = 0.0001$) (Table 2). However, no significant differences were observed between the CL/P group and controls under 18 years of age.

4. Discussion

Saliva collection has been previously reported as a non-invasive and useful method for obtaining DNA.[8] While the inclusion of exogenous material, such as oral microbial DNA, has been cited as a potential disadvantage affecting sample quality and analysis results,[4–6] previous studies have demonstrated that saliva samples provide sufficient DNA of optimal quality for genetic analysis and determination of specific SNPs.[9–12] Some studies even suggest that a greater amount of DNA can be obtained from saliva compared to whole blood samples of the same volume.[5]

Genetic studies of individuals with cleft lip and palate (CL/P) often require DNA samples from children and adolescents, making easier and painless methods like saliva collection preferable to blood sampling.[13] This approach can facilitate participation and increase sample sizes in such studies. In our study, the DNA obtained was successfully used for sequencing and restriction enzyme analysis to determine polymorphisms in CL/P-associated genes, confirming adequate quantity and quality for genetic evaluations.

We observed significant variation in DNA yield among individual saliva samples (Table 2), consistent with previous reports attributing this variability to differences in oral salivary content before collection, washing, and the extraction method used.[4,5]

Our results revealed significant differences in DNA yield based on age, with less DNA obtained from individuals under 18 years compared to adults. Individuals with CL/P yielded 45 % less DNA than their parents and adult controls. When analyzing matched group sizes and including a control group of individuals under 18 without CL/P, we consistently found lower DNA yields from younger individuals,

Table 2
Comparison of age, sex, and DNA concentrations between children with CL/P, parents, and controls under and over 18 years old.

	CL/P	Controls Under 18 years old	Parents	Controls Over 18 years old
	n = 52	*n = 52*	*n = 52*	*n = 52*
Age (years)				
Mean (SD)	11,2 ± 5,6[a]	12,8 ± 4,7[a]	38,1 ± 8,8	38,1 ± 8,8
Gender				
Female *n (%)*	26 (50)[a]	26 (50)[a]	46 (88,5)	46 (88,5)
Male *n* (%)	26 (50)[a]	26 (50)[a]	6 (11,5)	6 (11,5)
DNA Concentration (ng/µl)				
Median (IQR)	13 (7,25 - 25,4)[a]	14 (6,34-22,3)[a]	23,88 (10,73 - 41,6)	20,85 (9,35 - 32,53)

[a] $p < 0.05$ statistically significant differences between CL/P and Control under 18 years old with respect to Parents and Controls over 18 years old. No differences were found between CL/P and controls under 18 years old. Analysis performed with ANOVA of repeated measures, Friedman test and chi^2 test.

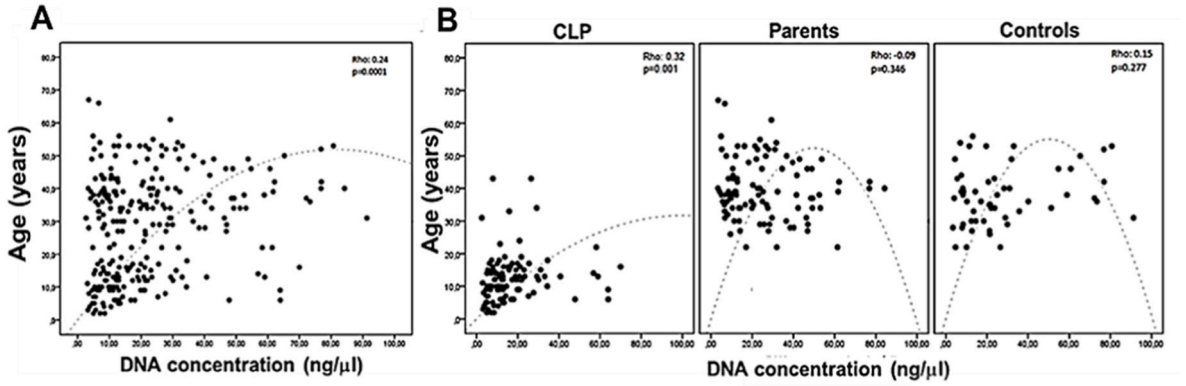

Fig. 3. Correlation between age and DNA concentration. (A) Overall direct correlation between age and DNA concentration, with younger individuals showing lower DNA concentrations. (B) Group-specific correlations, demonstrating a significant direct correlation between age and DNA concentration in individuals with CL/P ($R = 0.328$). No significant correlations were observed in other groups.

regardless of CL/P status. These findings align with previous studies by Gasso et al. (2014)[14] and Fitzsimons et al. (2021),[15] which reported that DNA concentrations increased proportionally with age and that sample quality decreased in children under 12, possibly due to low DNA concentration.

Unlike Gasso et al.[14], our study analyzed the 260/280 nm absorbance ratio, finding values between 1.8 and 2.0 across all age groups, indicating high purity. The lower DNA yield in younger individuals may be attributed to decreased epithelial desquamation of the oral mucosa in this age group, resulting in fewer cells available for DNA extraction. However, the exact cause of these differences remains unknown.

Future studies should investigate the relationship between DNA yield per ml of saliva collected and different ages tested, as well as examine how genetic analysis success rates vary with collection method, A260/A280 ratio, and DNA yield.

In summary, our results demonstrate that sufficient DNA can be obtained from saliva samples of individuals both over and under 18 years of age. The quantity and quality of DNA extracted from saliva samples are generally adequate for genetic analysis. However, age is a significant factor contributing to variability in DNA concentration. Our findings indicate that approximately 40 % less DNA is obtained from the same volume of saliva in individuals under 18 years of age, with or without cleft lip and palate (CL/P), compared to those over 18 years. To address this variability, researchers may need to adjust collection volumes or modify extraction protocols for younger participants. This age-related variability should be taken into account when designing methodologies for population studies, particularly those including children with conditions such as CL/P, to ensure sufficient DNA yield for analysis.

Author statement

Conceptualization, Methodology, Validation: L.M.E., Z.B. Formal analysis, Investigation: D.A., D.D.B., A.P. Resources, Data Curation: M.C. G., L.M.E. Writing - Original Draft, Writing - Review and editing: L.M.E., F.G.M., D.A. Visualization, Supervision, and Project administration: A. P., F.G. M., M.C.G. All authors have read and agreed on the current version of the manuscript to be published.

Ethical approval and consent to participate

This project was approved by the ethics committee of the Universidad de Cartagena, Colombia (Acta N°004). Written informed consent was obtained from all subjects and/or their parents.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Küchler EC, Tannure PN, Falagan-Lotsch P, Lopes TS, Granjeiro JM, Amorim LM. Buccal cells DNA extraction to obtain high quality human genomic DNA suitable for polymorphism genotyping by PCR-RFLP and Real-Time PCR. *J Appl Oral Sci*. Jul-Aug. 2012;20(4):467–471. https://doi.org/10.1590/s1678-77572012000400013.
2. Looi ML, Zakaria H, Osman J, Jamal R. Quantity and quality assessment of DNA extracted from saliva and blood. *Clin Lab*. 2012;58(3-4):307–312.
3. Nunes AP, Oliveira IO, Santos BR, et al. Quality of DNA extracted from saliva samples collected with the Oragene™ DNA self-collection kit. *BMC Med Res Methodol*. May. 2012;12:65. https://doi.org/10.1186/1471-2288-12-65.
4. Abraham JE, Maranian MJ, Spiteri I, et al. Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping. *BMC Med Genomics*. May. 2012;5:19. https://doi.org/10.1186/1755-8794-5-19.
5. Bruinsma FJ, Joo JE, Wong EM, Giles GG, Southey MC. The utility of DNA extracted from saliva for genome-wide molecular research platforms. *BMC Res Notes*. Jan. 2018;11(1):8. https://doi.org/10.1186/s13104-017-3110-y.
6. Gudiseva HV, Hansen M, Gutierrez L, et al. Saliva DNA quality and genotyping efficiency in a predominantly elderly population. *BMC Med Genomics*. Apr. 2016;9:17. https://doi.org/10.1186/s12920-016-0172-y.
7. James G, McMullin MF, Duncombe AS, Clarke M, Anderson LA. The MOSAICC study: assessing feasibility for biological sample collection in epidemiology studies and comparison of DNA yields from saliva and whole blood samples. *Annals of human genetics*. Mar. 2018;82(2):114–118. https://doi.org/10.1111/ahg.12227.
8. Koni AC, Scott RA, Wang G, et al. DNA yield and quality of saliva samples and suitability for large-scale epidemiological studies in children. *Int J Obes (Lond)*. *Apr.* 2011;35(Suppl 1):S113–S118. https://doi.org/10.1038/ijo.2011.43.
9. Bahlo M, Stankovich J, Danoy P, et al. Saliva-derived DNA performs well in large-scale, high-density single-nucleotide polymorphism microarray studies. *Cancer Epidemiol Biomarkers Prev*. Mar. 2010;19(3):794–798. https://doi.org/10.1158/1055-9965.EPI-09-0812.
10. Gudiseva HV, Hansen M, Gutierrez L, et al. Saliva DNA quality and genotyping efficiency in a predominantly elderly population. *BMC Med Genomics*. Apr. 2016;9:17. https://doi.org/10.1186/s12920-016-0172-y.
11. Hu Y, Ehli EA, Nelson K, et al. Genotyping performance between saliva and blood-derived genomic DNAs on the DMET array: a comparison. *PLoS One*. Mar. 2012;7(3), e33968. https://doi.org/10.1371/journal.pone.0033968.
12. Philibert RA, Zadorozhnyaya O, Beach SR, Brody GH. Comparison of the genotyping results using DNA obtained from blood and saliva. *Psychiatr Genet*. 2008;18:275–281.
13. Rylander-Rudqvist T, Håkansson N, Tybring G, Wolk A. Quality and quantity of saliva DNA obtained from the self-administrated oragene method–a pilot study on the cohort of Swedish men. *Cancer Epidemiol Biomarkers Prev*. Dec. 2006;15(9):1742–1745. https://doi.org/10.1158/1055-9965.EPI-05-0706.
14. Gassó P, Pagerols M, Flamarique I, et al. The effect of age on DNA concentration from whole saliva: implications for the standard isolation method. *Am J Hum Biol*. Nov-Dec. 2014;26(6):859–862. https://doi.org/10.1002/ajhb.22593.
15. Fitzsimons E, Moulton V, Hughes DA, et al. Collection of genetic data at scale for a nationally representative population: the UK Millennium Cohort Study. *Longit Life Course Stud*. Jul. 2021;13(1):169–187. https://doi.org/10.1332/175795921X16223668101602.