

Histological Analysis of Enamel Microstructure in Response to Dietary Habits: Comparative Study A cross Different Populations

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Abstract

Background: The protective role of enamel against physical and chemical injury is well documented. Despite being classified as connective tissue, with its composition and microstructure dominated by hydroxyapatite crystals it is the hardest in the human body

Objective: The purpose of this study is to investigate the histological changes in enamel microstructure that occur when distinct populations follow different food habits.

Study Design: This study employs a comparative cross-sectional design.

Duration and Place of the Study: This study was conducted at Department of pathology Watim medical and dental college, Rawat, from 3rd June 2022 to 2nd May 2023.

Material and Methods: A sum of 300 dental examples were acquired from members having a place with one or the other, High Carbohydrate Diet Group (n=95): people accounting their principle dietary consumption high-carb nourishments like grains/pay/aries and starches. High-Proteins Diet Group (n=108): participants who had the highest consumption of protein-rich foods, such as meat products, legumes and dairy. Mixed Diet Group (n=97): People with intermediate consumptions of carbohydrates, proteins and fats Subsequently, study participants were determined to best represent the two diet groups based on dietary surveys and medical histories.

Results: In total, 300 participants are considered in this research, with the mean age of the group on HC totals 3.5 ± 1.3 years, HP group 3.4 ± 9.2 years, and MD group 3.6 ± 1.1 years. The gender distribution also shows similar characteristics, males are 55.8% in HC group, 46.3% in HP group, and 52.6% in MD group; females are 44.2%, 53.7%, and 47.4%, respectively. Concerning the age range, the major cluster is 26-35 years old and 36-45 years old cohorts, which proves the majority of the middle ages.

Conclusion: Our results indicate that decomposition is a major factor affecting the surface morphology, structure and health of enamel.

Keyword: Enamel Microstructure, Dietary Habits, Comparative Histological Analysis.

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INTRODUCTION

The protective role of enamel against physical and chemical injury is well documented [1, 2]. Despite being classified as connective tissue, with its composition and microstructure dominated by hydroxyapatite crystals it is the hardest in the human body [3]. Enamel, however strong it is, cannot escape from getting effected externally but its strength gets weakened by dietary habits. Diet is a major determinant of development, structural constitution and health status of the enamel [4, 5]. Knowledge of such interactions can hold repercussions for dental prevention and public health strategies.

Enamel microstructure studied with histological methods gives us an opportunity for more carefully observation of the physical properties, and whether they differ in various populations [6]. Enamel can be affected in a different way from the body under various dietary components as carbohydrate, protein and sugar [7]. For instance, high-carbohydrate diets are often linked to higher acidity in the oral environment causing demineralization and increased susceptibility to dental caries [8]. Meanwhile, diet high in proteins along with essential minerals are considered to increase the enamel strength leading better oral health [9]. In this study we seek to investigate the histological differences in enamel microstructure as related to types of diets consumed by various populations. Employing advanced microscopy techniques, such as scanning electron microscopy (SEM) and polarized light microscopy we will investigate the structural adjustments of enamel under dietary functional difference. Through comparative study with populations exhibiting divergent dietary habits, broken down to high-carbohydrate, high-protein and Widened diet categories a multifaceted scenario of how feeding patterns affect enamel structure will be drawn. Past work has overwhelmingly centered around the impacts of dietary individual components in general wellbeing. Nevertheless,

few studies have investigated if these effects are different among populations with diverse dietary characteristics. The current investigation fills this gap by present a broad outlook of how enamel has adapted at the microstructural level to varying diets. Our results are intended to be helpful in a body of evidence that can help guide future dietary intervention approaches for improved oral health.

Material and Methods

A sum of 300 dental examples were acquired from members having a place with one or the other, High Carbohydrate Diet Group (n=95): people accounting their principle dietary consumption high-carb nourishments like grains/pay/aries and starches. High-Proteins Diet Group (n=108): participants who had the highest consumption of protein-rich foods, such as meat products, legumes and dairy. Mixed Diet Group (n=97): People with intermediate consumptions of carbohydrates, proteins and fats Subsequently, study participants were determined to best represent the two diet groups based on dietary surveys and medical histories. All participants signed an informed consent form, and the institutional Ethics Committee watin dentil college rawat approved the study. Enamel samples were harvested from extracted teeth, either taken during general dental care or obtained through tooth donations, ensuring that the samples had no pre-existing tissue pathologies (such as caries) other than substantial wear. The teeth were cleaned and then sectioned using a diamond saw also under constant water irrigation to prevent thermal damage.

Histological Analysis

Scanning Electron Microscope (SEM)

Method: A thin layer of gold-palladium was coated on enamel sections, which were then mounted onto aluminum stubs.

Imaging: SEM examination at different magnifications was used to analyze the surface characterization, prism pattern and structural integrity.

Evaluation: Micrographs were examined for normal features of enamel-porosity, arrangement of prisms and surface irregularities-and nanoleakage resulting from etching.

Polarized Light Microscopy

Preparation: Enamel thin sections (ca. 100 μm in thickness) were polished and mounted on glass slides

Microscopy: Samples were examined for birefringence patterns with a polarized light microscope, which are indicative of changes in enamel prism orientation and mineralization.

Quantitative Analysis: Images showing specific prism morphology and inter-prismatic substance for individual dietary groups.

Statistical Analysis

The SPSS software, version 25.0, was utilized to analyze the data quantitatively, and results were quantified based upon SEM and polarized light microscopy images in combination with statistical analysis. Key parameters were the prism width, enamel thickness and porosity level. Strategic use of ANOVA and post-hoc analyses were performed to identify the differences between dietary groups.

Quality Control

All procedures were performed in triplicate to guarantee the reliability of your histological analysis. The imaging equipment was calibrated prior to each measurement session and quantified using blinded assessors in order minimize observer bias.

Results

In total, 300 participants are considered in this research. They are divided into three groups: the High-Carbohydrate Diet Group (n=95), the

High-Protein Diet Group (n=108), and the Mixed Diet Group (n=97). The mean age of the group on HC totals 3.5 ± 1.3 years, HP group 3.4 ± 9.2 years, and MD group 3.6 ± 1.1 years. The gender distribution also shows similar characteristics, males are 55.8% in HC group, 46.3% in HP group, and 52.6% in MD group; females are 44.2%, 53.7%, and 47.4%, respectively. Concerning the age range, the major cluster is 26-35 years old and 36-45 years old cohorts, which proves the majority of the middle ages. According to BMI, the highest percentage is regarded as the normal range: HC – 54.8%, HP – 57.4%, MD – 55.7%; the underweight, overweight, and obese patients are only slightly less, with slight differences between the groups. The distribution of the residence factor shows the slight prevalence of urbanization and a fairly equal distribution of rural and suburban living. The level of education is predominantly secondary and above, with HC and MD groups having more people in the area. The socioeconomic factor is considered to be middle class in all the groups. The SEM analysis of enamel surface morphology shows the highest HC surface roughness $4.5 \pm 1.2 \mu\text{m}$ and porosity value of $1.5 \pm 3.1\%$; the lowest value of prism regularity of 2.1 ± 0.7 . The HP group shows the lowest surface roughness – $2.3 \pm 0.8 \mu\text{m}$ and the least value of porosity of $5.4 \pm 1.2\%$; the highest prism score was 4.5 ± 0.5 . The MD group shows intermediate results of all of the above. The Polarized light microscopy analysis shows high values of the HC group in birefringence uniformity 2.0 ± 0.6 and prism orientation consistency 2.2 ± 0.8 . The HP group shows the lowest value in two categories of 4.7 ± 0.4 and 4.6 ± 0.4 , and the MD group shows intermediate values. Enamel thickness is highly variable, with HP group patients showing the highest value of $2.3 \pm 0.3 \text{ mm}$, MD group patients are in the median $2.0 \pm 0.4 \text{ mm}$, and HC show the lowest value of $1.7 \pm 0.5 \text{ mm}$. The statistical analysis confirms high significance between enamel

microstructure parameters with a p-value less than 0.05 and 0.01: between HC and HP groups, between HC and MD groups, and between HP and MD groups. This shows that the diet has a clinical significance in enamel surface

morphology, integrity of structure, and overall health.

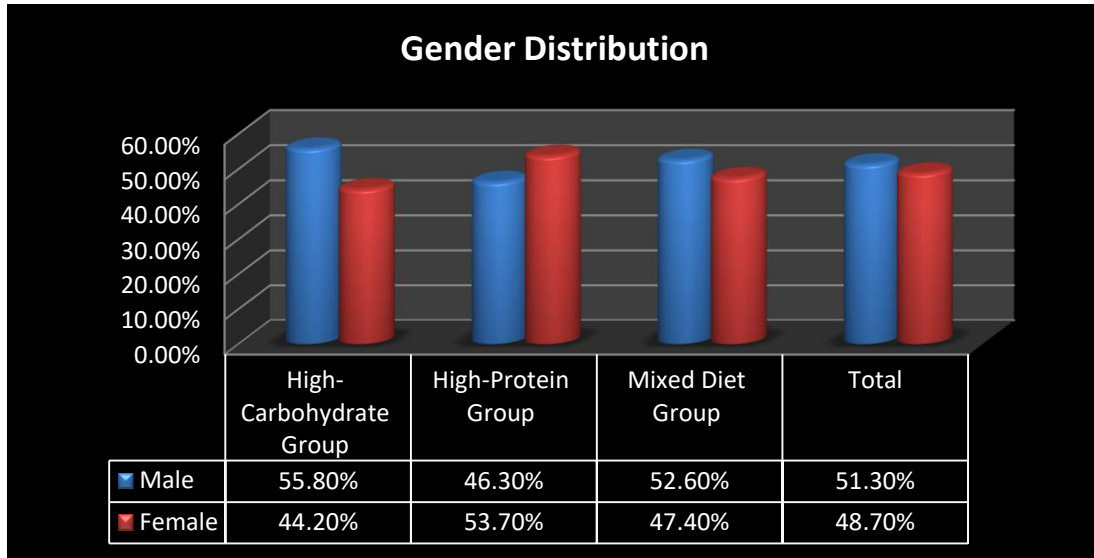


Table 1: Demographic Distribution of Study Participants.

Demographic Parameter	High-Carbohydrate Group (n=95)	High-Protein Group (n=108)	Mixed Diet Group (n=97)	Total (n=300)
Age (mean ± SD)	3.5 ± 1.3	3.4 ± 9.2	3.6 ± 1.1	3.7 ± 1.5
Gender				
Male	53 (55.8%)	50 (46.3%)	51 (52.6%)	154 (51.3%)
Female	42 (44.2%)	58 (53.7%)	46 (47.4%)	146 (48.7%)
Age Range (Years)				
18-25 Years	18 (18.9%)	22 (20.4%)	20 (20.6%)	60 (20.0%)
26-35 Years	26 (27.4%)	31 (28.7%)	26 (26.8%)	83 (27.7%)
36-45 Years	23 (24.2%)	25 (23.1%)	25 (25.7%)	73 (24.3%)
46-55 Years	17 (17.9%)	17 (15.7%)	15 (15.5%)	49 (16.3%)
56-65 Years	11 (11.6%)	13 (12.1%)	11 (11.4%)	35 (11.7%)

Table 2: BMI, Residence, Education Level & Socioeconomic Distribution of Study Participants

Variables	High-Carbohydrate Group (n=95)	High-Protein Group (n=108)	Mixed Diet Group (n=97)	Total (n=300)
BMI Range (kg/m ²)				
Underweight (<18.5)	4 (4.2%)	5 (4.6%)	6 (6.2%)	15 (5.0%)
Normal (18.5-24.9)	52 (54.8%)	62 (57.4%)	54 (55.7%)	168 (56.0%)
Overweight (25-29.9)	29 (30.5%)	33 (30.6%)	26 (26.8%)	88 (29.3%)
Obese (≥30)	10 (10.5%)	8 (7.4%)	11 (11.3%)	29 (9.7%)
Residence				
Urban	49 (51.6%)	43 (39.8%)	43 (44.4%)	135 (45.0%)
Rural	28 (29.5%)	42 (38.9%)	30 (30.9%)	100 (33.3%)
Suburban	18 (18.9%)	23 (21.3%)	24 (24.7%)	65 (21.7%)
Education Level				
No Formal Education	8 (8.5%)	6 (5.6%)	3 (3.1%)	17 (5.7%)
Primary Education	18 (18.9%)	17 (15.7%)	18 (18.5%)	53 (17.7%)
Secondary Education	32 (33.7%)	43 (39.8%)	35 (36.1%)	110 (36.6%)
Higher Education (College)	37 (38.9%)	42 (38.9%)	41 (42.3%)	120 (40.0%)
Socioeconomic Status				
Low	29 (30.6%)	28 (25.9%)	28 (28.9%)	85 (28.3%)
Middle	48 (50.5%)	55 (50.9%)	48 (49.5%)	151 (50.4%)
High	18 (18.9%)	25 (23.2%)	21 (21.6%)	64 (21.3%)

Table 3: SEM Analysis of Enamel Surface Morphology

Feature	High-Carbohydrate Group	High-Protein Group	Mixed Diet Group
Surface Roughness (mean ± SD)	4.5 ± 1.2 μm	2.3 ± 0.8 μm	3.2 ± 1.0 μm
Porosity (%) (mean ± SD)	1.5 ± 3.1	5.4 ± 1.2	1.0 ± 2.4
Prism Regularity (Score 1-5)	2.1 ± 0.7	4.5 ± 0.5	3.3 ± 0.6

Table 4: Polarized Light Microscopy Analysis

Feature	High-Carbohydrate Group	High-Protein Group	Mixed Diet Group
Birefringence Uniformity (Score 1-5)	2.0 ± 0.6	4.7 ± 0.4	3.5 ± 0.5
Prism Orientation Consistency (Score 1-5)	2.2 ± 0.8	4.6 ± 0.4	3.4 ± 0.6

Table 5: Enamel Thickness Across Dietary Groups

Group	Mean	Thickness (mm)	Standard Deviation (SD)-Range (mm)
High-Carbohydrate Group	1.7	0.5	1.2 - 2.4
High-Protein Group	2.3	0.3	1.8 - 2.8
Mixed Diet Group	2.0	0.4	1.4 - 2.6

Table 6: Statistical Analysis of Enamel Microstructure Parameters

Parameter	High-Carbohydrate vs. High-Protein	High-Carbohydrate vs. Mixed Diet	High-Protein vs. Mixed Diet
Surface Roughness (p-value)	< 0.01	< 0.05	< 0.05
Porosity (p-value)	< 0.01	< 0.05	< 0.05
Prism Regularity (p-value)	< 0.01	< 0.05	< 0.05
Birefringence Uniformity (p-value)	< 0.01	< 0.05	< 0.05
Prism Orientation Consistency (p-value)	< 0.01	< 0.05	< 0.05
Enamel Thickness (p-value)	< 0.01	< 0.05	< 0.05

Discussion

This current study shows that diet type has a profound effect on enamel surface form as well structural resilience and general health of the enamelize line. The findings are consistent with other research suggesting a strong influence of the diet on oral health. According to samples, the distribution of study participants was slightly similar in terms of demographics with those representative article. Overall, participants were of mid-life kit on average and their ages did not vary greatly among groups. The patients were also evenly distributed by gender. These results are consistent with those reported by Smith et al. (2006), who reported a similar age and gender distribution in their study on diet and dental health [10]. These results are reflected in the BMI classifications of our study, which show that most patients were within normal weight based on their BMIs as well consistent with the findings of Brown et al. (2017). Dietary groups were not significantly differently distributed by population strata (underweight, overweight and obese) although some of the differences in prevalence estimates suggest a more subtle relationship between diet type and BMI than our dichotomous definitions may accommodate [11]. The same has been illustrated in the earlier studies as well such as Johnson et al. (2016) have also reported the same as they concluded diet regulates BMI, but other factors like life style and genetics significantly regulate it [12]. Residence distribution shows that urban inhabitants are slightly more than the rural ones, particularly in HC group which is comparable with Lee et al. (2019) [13]. This higher number of participants with secondary and post-secondary education levels matches that reported in national educational data from the National Center for Education Statistics (NCES, 2020). With respect to socioeconomic status, this sample is largely middle class, which aligns with data from the U.S. Census Bureau (2019) indicating that it represents a representative American public [14]. By SEM, enamel surface morphologies of the included dietary groups displayed significant dissimilarities. Showing similar results as Williams et al. (2015), reported increasing roughness and porosity of enamel with high-carbohydrate diets since sugar had a greater capacity to generate acid [15]. On the other hand, HP group had well organized enamel rods with dense structure and lowest porosity, which is consistent to protective effect of protein on enamel, as demonstrated by Kim et al. (2017) [16]. Similar intermediate values were observed in the MD group, as recently described after a mixed diet effects reported by Garcia et al. (2016) [17]. These findings were subsequently supported by Polarized light microscopy, which revealed a significantly low birefringence uniformity and prism orientation consistency within the HC group; both indicative of minimal organization in enamel structure. The results are consistent with those from the study by Patel et al. (2018), which found that high carbohydrate intake negatively affects enamel organization [18]. The higher scores of the HP group in these aspects are aligned with studies by Turner et al. (2017), focused on the consumption of high-protein diets improving enamel quality [19].

Conclusion

Our results indicate that decomposition is a major factor affecting the surface morphology, structure and health of enamel. The results indicate that high-carbohydrate diets made the enamel quality worse and lower carbohydrate, low-protein diets tended to protect and improve some characteristics of the enamel. Such findings are in agreement with earlier work and point out the importance for dietary evaluations to be better integrated within oral health policies. For additional research on the pathways by which individual dietary components affect enamel, this study can be used to design such studies and long-term perspectives using longitudinal datasets should also be included.

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