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Relationship of Caries Color to International Caries Detection and Assessment Score and its Microbial Load in Primary Molars.

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ABSTRACT:
Background: The difference between colors of caries may give a primary diagnosis of the effected tooth as long as it is related to the ICDAS score and its microbial load. **Aims:** This study was conducted to investigate the relationship between the caries color to ICDAS score and its microbial load. **Methods:** Eighty children aged 4–7 years had caries primary molars were selected from the Pediatric Dental clinic, Faculty of Dentistry, Mansoura University. They were randomly divided into four groups based on color of caries of primary molars. Group A: Yellow color of caries, Group B: Light brown color of caries, Group C: Dark brown color of caries, Group D: Black color of caries. The classification was done by photographs illustrating the caries color, the eighty colors were classified into four main groups at the Faculty of fine art, to evaluate the relationship between International caries detection and assessment (ICDAS) and the microbial load according to caries color on the effected teeth. The data were collected, tabulated and statistically analysed.
Results: As regard, it was found that there is a significant correlation between ICDAS score and microbial load in cases with different caries color, it was found that among cases with black colored caries, there are a strong positive correlation between ICDAS score and Total Colony-Forming Unit CFU/ml for anaerobic media (*lactobacilli and S. mutans*) (r = 0.76 and 0.94, respectively), and significant correlation (p value <0.05) in cases (*Streptococcus mutans*).
Conclusions: Caries color is not related to the ICDAS score, nor is it related to its microbial load.

Keywords: Dental caries, caries color, ICDAS, microbial load.

Introduction

Childhood dental caries, a widespread chronic infectious condition, arises from the interplay of bacteria, particularly Streptococcus mutans, and sugary food items on tooth enamel. [1] This bacterial activity leads to acid production, gradually eroding the tooth structure. [2]

Tracing back to 5000 BC, the belief in "tooth worms" was associated with dental caries. The term "dental caries" emerged around 1634 from the Latin word "caries," signifying decay, initially referring to holes in teeth. Dental caries, an ancient and widespread human disease, results from tooth-adherent cariogenic bacteria that break down sugars into acid, causing the tooth structure to demineralize over time. [2]

The term "dental caries" encompasses both the disease and the resulting damage. Caries development takes place within the biofilm, continuously responding to pH changes, and ultimately leads to the formation of lesions in the dental hard tissues. [3]

Dental caries happens when the oral biofilm's microorganisms, which typically maintain a balanced state, transform into acid-producing, acid-tolerant, and tooth-destructive species due to frequent sugar intake. [4] This shift may cause either invisible changes or mineral loss within the tooth's hard tissues, leading to a visible, carious lesion. The caries process can occur without a visible lesion as well. [3]

In routine dental practice, the primary techniques for identifying caries involve visual and tactile assessments. [5] For visual examination, the color of a lesion is compared to a standardized guide featuring four shades (yellow, light brown, dark brown, and black) derived from photographs of primary dental caries under normal daylight conditions. [6]

The International Caries Detection and Assessment System (ICDAS) serves as a valuable aid in identifying and documenting tooth decay. This method categorizes carious lesions into six groups, with a higher score indicating a more advanced stage of decay. ICDAS monitors the development of carious lesions and is both accurate and consistently reproducible, providing a reliable system for caries classification. [7]

Cavities are associated with a higher prevalence of specific bacterial species, such as *Streptococcus mutans*, Streptococcus sobrinus, and *Lactobacilli*, commonly found in advanced

tooth decay. [8] The specific plaque hypothesis stemmed from this observation, suggesting that only certain bacteria cause the disease. [9] However, while *Streptococcus mutans* plays a significant role in tooth decay, cavities can still develop in areas where this bacteria is not present. [10]

This observation led to the development of the nonspecific plaque hypothesis, which posits that tooth decay arises from the collective metabolic activity of the microorganisms within the biofilm. Although the cause of dental caries is not exclusively tied to specific bacteria, it is crucial to note that certain types of bacteria are consistently found in higher numbers in affected areas. [11]

The ecological plaque hypothesis suggests that tooth decay is not caused by a single specific microorganism but rather results from a shift in the dental biofilm's microbial composition towards more tooth-decay-promoting species. Frequent sugar consumption creates acidic conditions in the mouth, which favors the survival of bacteria that increase in such environments and eliminates the more benign species that cannot tolerate these conditions. [10]

The severity of dental caries can be assessed by the treatment needed, which varies from invasive procedures like caries removal and restoration for lesions with higher microbial colony forming unit (CFU) counts to less invasive options such as topical fluoride applications or no treatment for those with lower CFU counts. [12, 13]

Materials and Methods

This study was cross sectional descriptive study with analytic data, and it was conducted over one year at the Pediatric Dental Clinic, Faculty of Dentistry, Mansoura University. Ethical approval was obtained from the Research Ethics Committee, Faculty of Dentistry, Mansoura University, with the code number A26020822.

Before the commencement of the study, parents were informed about the study intervention, including a detailed explanation. Following this, informed written consent was obtained from parents, approving of their children in this study, 80 children of both genders were included in this study. The inclusion criteria specified that children aged 4-7 years, first

or second primary molar with intact proximal surface, normal furcation and normal periapical bone structure.

For sample size calculation, the power analysis was performed using G power software by F **tests –** (ANOVA), and the sample size calculator was based on Larry Hon et al. (2019). [(6)] The criteria for significance were set at 0.05 (type I error) and a total power of 90%. The effect size was set at 0.44, and the total sample size required was 80.

Clinical Procedures:

After the administration of anesthesia, rubber dam isolation of the selected primary molar (first or second molar) was done. Initial cleaning for food debris was done by water and air ship syringe, and the patient sat on a chair in a natural head position to take a photo of the isolated tooth. An assistant held the mirror, and a Canon 250 DSLR camera (Canon, Japan) with a 105 mm SIGMA lens was used. The camera's settings are 1/200s, f/29, ISO 100, YONGNUO flash 1/2 power and the lens set to 1:1.5 magnification. The camera's angulation was perpendicular to the occlusal surface of the molar via mirror, using the same unit with closed curtains under the light of the clinic. The infected caries was excavated using a sterile, sharp dental excavator and collected into a disposable sterile specimen collection swab with a tube filled with 1cm of transport medium (nutrient broth solution). The sterilized tube was labeled with the patient's name and tooth number for microbiological analyses on the same day within 2 hours. A round carbide bur attached to a high-speed handpiece, with ample water irrigation, was utilized to access the carious lesions and then remove the infected carious from the pulpal floor by a sharp dental excavator. If the dental pulp remained intact, the tooth was restored with amalgam or composite. If the pulp was exposed, the tooth was treated according to the standardized methods. The ICDAD score was recorded visually and by using the WHO probe, and we applied the score to the personal data sheet.

Color Assessment:

The picture was sent from the camera to the laptop, and then the dental caries color photo was determined by using Adobe Photoshop as a tool to copy the color as a hexadecimal value. The color picker window displayed the selected color and its code. After that, the eighty colors were classified into four main groups by three doctors from the Faculty of fine art, Mansoura University.

Laboratory procedures:

After the collection of the samples, they were placed in a sterilized tube that contained nutrient broth solution as a carrier solution and transferred to the oral microbiological lab, Faculty of Medicine, Mansoura University, to evaluate the CFUs (colony-forming units) of *Streptococci mutans* and *Lactobacilli* for caries biopsy.

The microbial load of *Streptococcus mutans* and *Lactobacilli* in dental caries was determined by these laboratory steps (Figure 1-5)

1. Insert the tube into the vortex mixer and mix the sample for 2 minutes at 2800 rpm in order to provide a fully homogenous sample in the test tube.
2. Preparation of 4% of blood agar and dispense it into sterile plates while it was liquid.
3. Insert the pipette in the tube, taking (10 microliters) from the sample, and apply it to blood agar petri plates for anaerobic microbes.
4. Sterilize the loop with a flame, and then wait 10 seconds for it to cool. After that, the loop was placed in contact with the inoculum on the surface of the plate and positioned to allow the inoculum to run evenly along the length of the loop. The inoculum was distributed to allow it to be absorbed into the blood agar plate.
5. For the anaerobic bacteria, the agar plate was incubated in the sealed anaerobic jar, which contained AnaeroPack-Anaero, at 37°C for 48 hours. AnaeroPack-Anaero is a disposable oxygen-absorbing and carbon dioxide-generating agent for use in anaerobic jars.
6. After incubation of the bacteria, the growth of colonies was observed on the plates, and the colony counting was done under a colony counter, and the number of CFU was multiplied by (10 mL) of the sample that was diluted.
7. Identification of *Streptococcus mutans* and *Lactobacilli* by their morphological characteristics, such as size, shape, and color.
8. The light intensity of the compound light microscope was adjusted to the appropriate level using a high magnification level (100 x), and then a drop of immersion oil was placed between the slide and the microscope objective to reduce the light refraction and improve image clarity.
9. Finally, the findings were documented and reported, including the numbers and the presence or absence of the bacteria.

Figure 1- A photograph shows the steralized tubes inserted into the vortex mixer.

Figure 2- A photograph shows inserting the sample by the pipette into the plate.

Figure 3- A photograph shows distributing the inoculum on the plate by the sterilized loop

Figure 4- A photograph shows the sealed anaerobic jar for the anaerobic bacteria.

Figure 5- A photograph shows the AnaeroPack-Anaero.

Color chart validity:

Three dental experts measured the validity of the chart color, and they were asked to evaluate the caries color of the teeth. Data were collected and further kappa analyzed, and the results were > 0.8, indicting chart validity.

Statistical analysis:

The data were statistically analysed by the Statistical package of Social Science (SPSS) program for windows (Standard version 26). Monte Carlo test were used to calculate the difference between qualitative variables as indicated. Quantitative data were expressed as median and minimum and maximum for non-parametric data. Kruskal-Wallis tests were used to calculate the difference between quantitative variables in two groups for non-parametric variables, the correlation coefficient was evaluated by the using of Spearman rank test. The significance level was set at the probability value of 0.05.

Results

This is a clinical study carried out to investigate the relation of caries color (which is grouped as yellow, light brown, dark brown, and black) to the ICDAS score and the microbial load of *Streptococcus mutans* and *Lactobacillus*.

Table (1) shows that, all cases with yellow colored caries had an ICDAS score of 3. However, 83.3% of cases with light brown caries had a score of 3, while 8.3% had a score of 5, and the same percentage had a score of 6. In addition, 65.9% of cases with dark brown colored caries had a score of 3, 22.7% had a score of 4, 6.8% had a score of 5, and 4.5% had a score of 6. On the other hand, 44.4% of cases with black colored caries had a score of 3, 22.2% had a score of 4, and the same for score 6, while 11.2% had a score of 5. As for the relation between caries color and ICDAS scores, the results were statistically non-significant (p value > 0.05). While the median for the ICDAS score was compared regarding caries color, the results were statistically non-significant (p value > 0.05).

ICDAS	Caries color				P value
	Yellow (n=3)	Light brown (n=24)	Dark brown (n=44)	Black (n=9)	
Score 3	3(100%)	20(83.3%)	29(65.9%)	4(44.4%)	0.20
Score 4	0(0%)	0(0%)	10(22.7%)	2(22.2%)	
Score 5	0(0%)	2(8.3%)	3(6.8%)	1(11.2%)	
Score 6	0(0%)	2(8.3%)	2(4.5%)	2(22.2%)	
Median (Min-Max)	3 3-3	3 3-6	3 3-6	4 3-6	0.12

Table 1: Relation between caries color and ICDAS

Table (2) shows, the total CFU/ml for *lactobacilli* in anaerobic media in cases with different caries colors were examined and showed that the highest CFU was in cases with yellow caries (median 10 $x10^3$) compared to 5.5 x 10^3 in cases with light brown caries, 2 $x10^3$ in cases with dark brown caries, and 3 $x10^3$ in cases with black caries with a non-significant p value among studied groups.

The total CFU/ml for *S. mutans* in anaerobic media in the cases with different caries colors were examined and showed that the highest CFU was in cases with black caries (median 50 $x10^3$), compared to 1 x 10^3 in cases with yellow caries, 5 $x10^3$ for cases with light brown caries, and 2 $x10^3$ in cases with dark brown caries, with a non-significant p value among studied groups.

Microbial load	Caries color				P value
	Yellow (n=3)	Light brown (n=24)	Dark brown (n=44)	Black (n=9)	
Total CFU/ml for anaerobic bacteria (lactobacilli) Median	10 $x10^3$	5.5 $x10^3$	2 $x10^3$	$3x10^3$	0.59
Total CFU/ml for anaerobic bacteria (S. mutans) Median	1x 10^3	$5x10^3$	2 $x10^3$	50 $x10^3$	0.65

Table 2: Relation between caries color and microbial load

Table (3) shows the correlation among cases with black colored caries, and there is a strong positive correlation between the ICDAS score and total CFU/ml for anaerobic media (*lactobacilli and S. mutans*) (r = 0.76 and 0.94, respectively) and a significant correlation for (*S. mutans*) (p value <0.05). In addition, among cases with light brown- colored caries and dark brown-colored caries, there is a non-significant correlation between the ICDAS score and microbial load in cases with light brown and dark brown colored caries (p value > 0.05). Regarding cases with yellow-colored caries, the correlation was not applicable as the microbial load was constant.

Table 3: Correlation between ICDAS score and microbial load according to caries color

		ICDAS score			
		Yellow caries	Light brown caries	Dark brown Caries	Black caries
	r	NA	0.30	0.36	0.76

Total CFU/ml for anerobic media (lactobacilli)	**p value**	__	0.38	0.18	0.15
Total CFU/ml for anerobic media (S. mutans)	**r**	NA	0.16	-0.30	0.94
	p value	__	0.54	0.18	0.05*

Discussion

Caries were noticeable clinically when discoloration appeared in the enamel and dentin. However, initial caries were evident as a white opaque lesion on the enamel surface after drying. [(14)] In clinical practice, visual and tactile assessments were the most frequently utilized procedures for diagnosing dental caries. [(6)] Modern diagnostic approaches included a visual assessment centered around the color of the carious lesion and hardness evaluation with an excavator. Nevertheless, the caries diagnosis procedures in clinical settings were considered subjective. [(15)]

The purpose of the present investigation was to assess the relationship between the caries color to ICDAS score and its microbial load.

Numerous studied affirmed that the color of carious lesions is a clinical variable applied for identifying the extent of carious progression and carious lesion activity [(16,17)]. (Usha & Sathyanarayanan 2009) [(18)] mentioned that the demineralized tooth structure and the occurrence of dental caries are linked to acid generation caused by bacteria sugar metabolism.

The present study assessed the teeth distribution based on caries color and exhibited that primary molars with dark brown caries had the highest percentage 55% followed by 30% with light brown caries, then 11.3% with black color, and only 3.7% with yellow caries. These findings are inconsistent with those of Ghaderi et al. (2020) [(19)] who investigated 80 teeth from 66 children aged between 5 and 12 years old discovered that 41,25% had black color, the same for dark brown color 41,25%, and 17,5% had light brown color, while there were no instances of yellow dental caries among the teeth.

Concerning the relation between caries color and ICDAS score, no statistically significant difference was recognized in the current research. The present study demonstrated

that 100% of yellow caries cases had an ICDAS score 3 compared to 83.3% of light brown caries, 65.9% of dark brown caries, and 44.4% of black caries. Also, when median total ICDAS score was compared regarding caries color, the results were statistically non-significant.These results concurred with those of Dikmen et al. (2015) [(20)], who studied coronal primary caries and found that the enamel's surface was whitish/yellowish opaque with loss of luster; feels rough when the tip of the probe is moved (active lesion) in the ICDAS score 1, 2, and 3, compared to whitish, brownish or black enamel's surface in the inactive lesion. Moreover, Honkala et al. (2011) [(21)] clarified that ICDAS criteria might offer suitable data about the existence of caries lesion.

In the current research, the total colony-forming units per milliliter for *lactobacilli* in anaerobic media showed that the highest CFU was in cases with yellow caries compared to light brown caries, dark brown caries and black caries with non-significant p-value among the studied groups. The total colony-forming units per milliliter for *S. mutans* in anaerobic media showed that the highest CFU was in black caries compared to yellow caries, light brown caries and dark brown caries with non-significant p-value among studied groups.

These results are consistent with those of Orhan et al. (2008) [(22)], who affirmed that in general, lesions classified on the basis of their color did not harbor significantly different levels of bacteria. Furthermore, Maltz et al. (2007) [(23)] assumed that dentin color is not a reliable predictor of the existence of microorganisms following partial caries treatment.

However, based on my knowledge, there were no previous studies that examined the correlations between the ICDAS score and microbial load according to caries colors.

Conclusion

Caries color is not related to the ICDAS score nor is it related to its microbial load (*Streptococcus mutans and Lactobacilli in blood agar culture media*).

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