

## Gingival pigmentation in relation to anti-inflammatory salivary interleukin 10, a comparative study

Ahmed Shukur Mahmood, Ban Sahib Diab

### Abstract

**Objective:** To assess gingival pigmentation prevalence among students, and its relationship with anti-inflammatory salivary interleukin-10.

**Method:** The observational, cross-sectional, comparative study was conducted at the College of Physical Education and Sport Sciences, Diyala University, Iraq, from December 2021 to March 2022, and comprised students of either gender aged 19-22 years. Students with pigmented gingiva were in group A, while those having no gingival pigmentation were in group B. The level of interleukin-10 was tested using enzyme-linked immunosorbent assay in both groups, and values were compared using the gingival pigmentation index. Data was analysed using SPSS 22.

**Result:** Of the 1,295 students assessed, 1,044(80.62%) were males and 251(19.38%) were females. Pigmented gingiva was found in 118(9.11%), and these students represented group A, while as many subjects were enrolled in controlled group B. Pigmented gingiva was more common among males 81(68.64%) than females 37(31.36%). Salivary interleukin-10 level was significantly increased in group A compared to group B ( $p < 0.05$ ). However, there were no significant differences in mean value of salivary interleukin-10 with respect to severity in group A ( $p > 0.05$ ).

**Conclusion:** Pigmentation was beneficial in terms of gingival protection.

**Key Words:** Gingiva, Interleukin, Pigmentation, Enzyme, Immunosorbent, (JPMA 74: S191 (Supple-8); 2024) DOI: <https://doi.org/10.47391/JPMA-BAGH-16-42>

### Introduction

The gingiva is the soft tissue that surrounds the tooth necks, covers the alveolar processes, and connects with the oral mucosa at the mucogingival junction<sup>1</sup>. Healthy gum is typically coral-pink in colour, but it can range from light chestnut to dark brown, depending on keratinisation, gingiva thickness, vascularisation, and the presence of melanocytic cells<sup>2</sup>. Gingival pigmentation appears as a diffuse, deep purplish discolouration, or irregular, dark, light brown, or black spots, striae, or threads. Melanin granules are the cause. Melanin, a non-haemoglobin-derived brown pigment, is the most common endogenous pigment, and is formed by melanocytes found in the basal and suprabasal cell layers of the epithelium<sup>3</sup>.

Interleukin-10 (IL-10) is an immune-regulatory cytokine that is primarily formed by activated T-helper 2 (TH2) cells, B cells, macrophages, natural killer (NK) cells, keratinocytes and mast cells<sup>4</sup>. Substantial evidence suggests that keratinocytes are involved in immune responses and immunopathological reactions<sup>5</sup>. Many cytokines can be produced by keratinocytes, including IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18 and IL-20, tumour

Department of Preventive Dentistry, University of Baghdad, Baghdad, Iraq.

**Correspondence:** Ahmed Shukur Mahmood

**Email:** med031923@gmail.com

necrosis factor alpha (TNF- $\alpha$ ) and interferon alpha (IFN  $\alpha$ ), IFN beta (IFN- $\beta$ ) and IFN gamma (IFN- $\gamma$ )<sup>6</sup>. Cytokine imbalance appears in the skin of vitiligo patients<sup>7</sup>, such as IFN- $\gamma$ , IL-10, IL-13 and IL-17<sup>8,9</sup>. Earlier research proved that successful treatment of vitiligo with topical tacrolimus involved the unique immunosuppressive actions of IL-10, demonstrating that tacrolimus increased IL-10 expression in vitiligo lesions, and thus inhibited melanocyte destruction triggered by TH1 pathways<sup>10</sup>. IL is a powerful anti-inflammatory cytokine that plays an important and often life-saving function in the prevention of inflammatory and autoimmune diseases<sup>11</sup>. Inflammation in response to a microbial challenge can be exacerbated by IL-10 deficiency or aberrant expression. However, it can also result in the development of a number of autoimmune diseases<sup>12</sup>.

To the best of our knowledge, there has been no study done in Iraq on the relationship between gingival pigmentation and salivary IL-10. The current study was planned to fill the gap by assessing gingival pigmentation prevalence among students, and its relationship with anti-inflammatory salivary IL-10.

### Subjects and Methods

The observational, cross-sectional, comparative study was conducted at the College of Physical Education and Sport Sciences, Diyala University, Iraq, from December 2021 to March 2022. After approval from the ethics review

committee of the College of Dentistry, University of Baghdad, Iraq, and permission from the University of Diyala, the sample was raised. Written informed consent from the students was also obtained.

The sample comprised all students of either gender aged 19-22 years having pigmented gingiva. Those with medical disorders and diseases, those taking any type of medication that could cause oral pigmentation, pregnant and lactating women, those with disease in the oral cavity, and those who smoked were excluded. Those included randomly formed group A. Students without pigmented gingiva matched for age and gender were enrolled randomly as control group B.

Gingival pigmentation severity scores were determined using the gingival pigmentation index<sup>13</sup>; 0 = absence of pigmentation, 1 = spots of brown to black colour or pigments, 2 = brown to black patches but no diffuse pigmentation, and 3 = diffuse brown to black pigmentation, marginal, and attached. IL-10 levels were determined using enzyme-linked immunosorbent assay (ELISA) after saliva collection through the unstimulated method<sup>14</sup>.

Artificial light, disinfectant, gloves, masks and dental mirrors were used to measure gingival pigmentation. Disposable cups, disposable test tubes, and a saliva cooling box were used to measure IL-10.

Data was analysed using SPSS 22. The threshold of significance was set at  $p < 0.05$ .

## Results

Of the 1,295 students assessed, 1,044(80.62%) were males and 251(19.38%) were females. Pigmented gingiva was found in 118(9.11%), and these students represented group A, while as many subjects without gingival pigmentation and age and gender matched, were enrolled in control group B (Table 1).

**Table-1:** Gingival pigmentation distribution in the study groups with respect to age and gender.

Age (years)	Gender	Gingival pigmentation			
		Presence (study)		Absence (control)	
		N.	%	N	%
19-20	male	22	27.16	22	27.16
	female	15	40.54	15	40.54
	Total	37	31.36	37	31.36
21-22	male	59	72.84	59	72.84
	female	22	59.46	22	59.46
	Total	81	68.64	81	68.64
Total	male	81	68.64	81	68.64
	female	37	31.36	37	31.36
	Total	118	100.00	118	100.00

**Table-2:** Gingival pigmentation severity scores with respect to age and gender.

Gender	Gingival pigmentation scores (severity)						
	1		2		3		
	N.	%	N.	%	N.	%	
<b>Age (years)</b>							
19-20	Male	7	31.82	9	40.91	6	27.27
	Female	4	26.67	7	46.67	4	26.67
	Total	11	29.73	16	43.24	10	27.03
21-22	Male	16	27.12	26	44.07	17	28.81
	Female	9	40.91	7	31.82	6	27.27
	Total	25	30.86	33	40.74	23	28.40
Total	Male	23	28.40	35	43.21	23	28.40
	Female	13	35.14	14	37.84	10	27.03
	Total	36	30.51	49	41.53	33	27.97

Pigmented gingiva was more common among males 81(68.64%) than females 37(31.36%), and in those aged 21-22 years 81(68.64%) compared to 19-20 years 37(31.36%).

Gingival pigmentation scores within group A were compared with respect to age and gender (Table 2).

Salivary IL-10 level was significantly increased in group A compared to group B ( $p < 0.05$ ). However, there were no significant differences in mean value of IL-10 with respect to severity in group A (Table 3).

**Table-3:** Mean IL-10 in the study groups, and with respect to gingival pigmentation severity.

Gingival pigmentation scores	Mean IL-10	SD	test	P value
1	43.921	11.530		0.183
2	38.526	13.744	F test	
3	47.684	14.750	1.767	
total student with gingival pigmentation (study group)	43.377	13.641	Independent T test	0.001*
Control group	35.796	6.921	3.325*	

IL-10: Interleukin-10, SD: Standard deviation. \*Significant ( $p \leq 0.05$ ).

## Discussion

In the current study, there was no significant difference in gingival pigmentation occurrence between the genders, which was in agreement with previous studies<sup>15-17</sup>. The occurrence of gingival pigmentation was higher for the age group 21-22 compared to the 19-20 age group, indicating that gingival pigmentation may increase with age, and the finding agreed with earlier reports.<sup>18</sup> However, contrasting results have also been reported by a study in which gingival pigmentation was higher in the younger age group<sup>17</sup>. The young ones have less melanin

than adults due to smaller keratinocytes, keratinocytes and a thinner epidermis. At the same time, the stratum corneum's water content rises, reducing the need for keratinocytes to produce melanin pigments<sup>19,20</sup>.

The current study showed that IL-10 level was higher and more significant in the study group, implying that inflammation was reduced. This agrees with earlier studies<sup>21,22</sup>. Melanin, which is produced by melanocytes in the gingival epithelium's basal layer, has the ability to deactivate reactive oxygen species (ROS) produced through dento-gingival plaque-induced inflammation in the periodontal micro-environment due to its defensive role in the oral environment<sup>21</sup>. To some extent, this was found in the present study as salivary IL-10 increased among students in the severe gingival pigmentation score category.

**Limitation:** The current study has limitations as the sample size was not calculated which could have affected the power of the study.

## Conclusion

Gingival pigmentation, even when its aesthetically not pleasing, has a beneficial role in protecting the gingiva by reducing inflammation. If the pigmentation has not reached a degree of unacceptability, it is preferable to avoid intervention.

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