Article
JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)
Vol.33 ; No. 4 | 2025

Immunological Insights into White Spot Lesions in Fixed Orthodontic Patients: Focus on Salivary Inflammatory Markers

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رؤى مناعية حول آفات البقع البيضاء لدى مرضى تقويم الاسنان الثابت: التركيز على علامات الالتهاب اللعابية

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Accepted: 7/9/2025 **Published:** 31/12/2025

ABSTRACT

Background: Orthodontics is a dental specialty focused on the diagnosis, prevention, and management of malaligned teeth and craniofacial discrepancies, and prompting facial esthetics. The use of fixed orthodontic appliances, creates numerous retentive niches that predispose individuals to increased plaque accumulation and food debris retention. This, in turn complicates effective oral hygiene practices.

Objective: The study's objective is immunological evaluation of immunoglobulin A (IgA) and interleukin 26(IL-26) in white spot lesions in fixed orthodontic treatment.

Material and method: A total of 100 individuals, including 50(50%) experimental and 50 (50%) controls, 36 males and 64 females, aged 15-35, underwent fixed orthodontic treatment for those measuring salivary biomarkers by ELISA for at least one month after appliance activation. **Results**: The findings showed that the case group had higher significant differences as opposed to the control group with *p* value = 0.002 regarding IgA, while IL-26 showed no significant difference between the two groups with p value 0.557. **Conclusion**: Compared to the control group, which exhibited no sign of white spot lesions, the case group demonstrated elevated levels of salivary IgA, while IL-26 levels remained relatively unchanged.

.**Keyword: White spot lesions (WSLs), Immunity, Fixed orthodontic appliances, Cytokines, IgA**

INTRODUCTION

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Orthodontics is a specialized discipline in dentistry concerned with management and treatment of malocclusions, including misalignment of teeth and jaws. Treatment modalities frequently involve orthodontic devices such as braces, designed to reposition teeth and harmonize occlusal relationships. With growing public awareness and demand for aesthetic dental improvements, the prevalence of orthodontic interventions has notably increased. Despite these advancements, a prominent aesthetic complication often encountered post-treatment is the development WSLs, demineralized enamel regions that manifest as opaque, chalky spots and may compromise overall treatment outcome [1]. The demineralization underlying WSLs is primarily driven by the acidogenic bacteria, resulting in subsurface porosities, particularly noticeable on smooth surfaces[2]. Orthodontic appliances, including brackets, bands, molar tubes, and wires, are niches for plaque retention, ultimately leading to enamel breakdown [3], [4]. Dental caries arises from acid-producing bacteria within the biofilm, specific microbial species lower PH, driving enamel demineralization, however timely diagnosis and targeted strategies are key to halting progression with effective care preserving tooth integrity [5] [6]. Early enamel mineral loss begins with acid-induced breakdown of enamel rods accordingly, this process leads to structural changes, including expansion of the surrounding enamel sheath. Enamel hypoplasia alters surface morphology through pits, grooves, and irregularities, where the affected areas may exhibit reduced enamel thickness or complete surface deficiency of enamel [7], [8]. Under dry conditions, initial carious lesions are observable but virtually disappear when the enamel is wet; on the other hand, hypocalcification remains noticeable regardless of hydration status[9]. Fixed orthodontic appliances contribute to plaque retention, altering the oral ecosystem and promoting the growth of acidogenic bacteria, predominantly *Streptococcus mutans*, thus this microbial shift plays a central role in the development of WSLs, as the elevated acid production progressively lowers the local PH [10]. Immunoglobulin A (IgA) is the most prevalent antibody in the human body, playing a crucial role in mucosal immunity and demonstrating involvement in the regulation of oral microbiota. Variations in IgA levels after orthodontic treatment may indicate modifications in the immune response to the oral environment, perhaps contributing to the development of white spot lesions[11]. The immune system is essential for sustaining dental health, and increasing data suggests that interluekine-26, a cytokine synthesized by T cells, may be altered due to the formation of white spot lesions during orthodontic treatment[12] . IL-26 is produced by activated Th17 and NK cells and is overexpressed in chronically inflamed tissues. It promotes the production of proinflammatory cytokines (e.g., IL-1β, IL-6, IL-8) and enhances the recruitment of immune cells to mucosal surfaces [13].

MATERIALS AND METHODS

Study design and patients

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A Total 100 study samples, (expeimental; 50 control), with age ranging from 15-35 years, unstimulated saliva was obtained from both groups after at least 4 weeks of fixed orthodontic treatment start. All specimens were obtained from Babil specialized dental center during the time span of the study Between November 2024 and March 2025.

Unstimulated saliva collection

The participant were asked not to brush their teeth a night preceding the time of sample collection, and they were requested not to consume any food or beverages for at least one hour (before the sample was taken).Every 60 seconds, saliva was spited into specimen tube after allowing it to accumulate in the floor of mouth for at minimum for 2 mL of unstimulated saliva [14]. Saliva samples took for IgA and IL-26 assay been centrifuged at 10000 rpm for 5 minutes, the supernatant was used for immunological analysis frozen at -20˚C [14]

Estimation of Immunological Biomarkers (IL-26 and IgA)

Test Principle

A system known as Sandwich-ELISA was added to the ELISA package, The micro-ELISA plate of kit has only been pre-coated with an antibody specific to IL-26 and IgA. According to object protein. The ELISA microplate wells were filled with standards or samples and bound with a particular antibody. Next, a biotinylated detection antibody for IgA or IL-26 and an avidin-HRP peroxidase (HRP) conjugate were added one at a time to each microplate well. After that, they were incubated for one hour at least in the incubation or overnight in the refrigerator to get the reaction between the antigen and two types of antibodies, free components which did not link to the antigen-antibody complex were washed. And then, the substrate solution was put to every well of the micro plate. Only those wells which have IgA or IL-26 biotinylated detection antibody, which reacted with A-HRP conjugate, would be visible in different degrees of blue based on reaction intensity, with a particular antibody. Next, a biotinylated detection antibody for IgA or IL-26 and an avidin-HRP peroxidase (HRP) conjugate were added one at a time to each microplate well. After that, they were incubated for one hour at least in the incubation or overnight in the refrigerator to get the reaction between the antigen and two types of antibodies, free components which did not link to the antigen-antibody complex were washed. And then, the substrate solution was put to every well of the micro plate. Only those wells which have IgA or IL-26 biotinylated detection antibody, which reacted with A-HRP conjugate, would be visible in different degrees of blue based on reaction intensity, with the particular antibody. Next, a biotinylated detection antibody for IgA or IL-26 and an avidin-HRP peroxidase (HRP) conjugate were added one at a time to each microplate well. After that, they were incubated for one hour at least in the incubation or overnight in the refrigerator to get the reaction between the antigen and two types of antibodies, free components which did not link to the antigen-antibody complex were washed. And then, the substrate solution was put to every well of the micro plate. Only those wells which have IgA or IL-26 biotinylated detection antibody, which reacted with A-HRP conjugate, would be visible in

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different degrees of blue based on reaction intensity. The enzyme - substrate solution reaction was finished by the addition of strong acids or strong base(stop solution) , and the color turned yellow. After that the (OD) was measured with an ELISA reader or whatever device works spectrophotometrically at a wavelength of 450 nm ± 2 nm. Concentration value of the IgA or IL-26 is directly proportional to the optical density, the concentration increased as the OD increased. So, the results can be calculated through the straight-line equation and represented on the curve point to point through a unique software program linked to the computer (Gan & Patel, 2013).

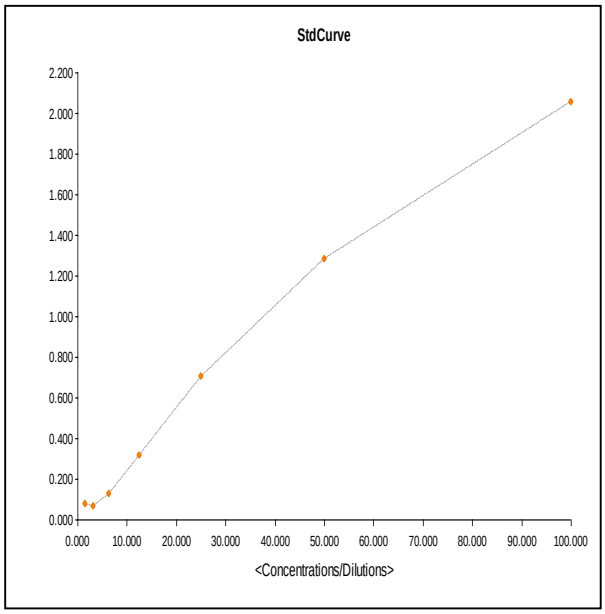

Figure (1) standard curve of IgA

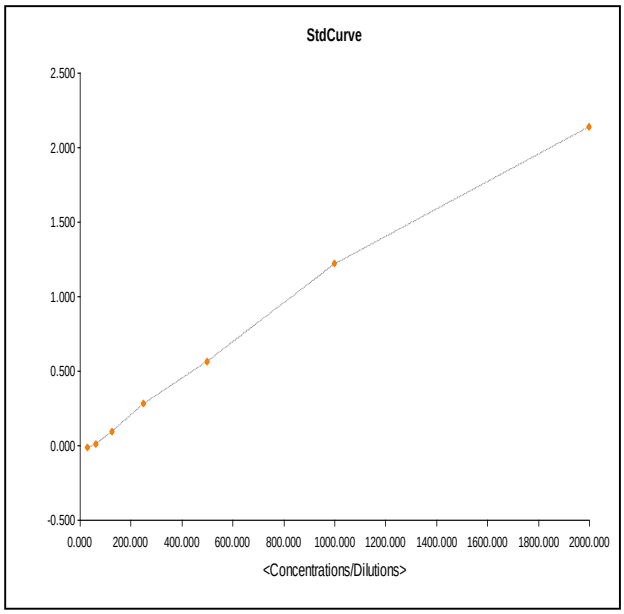

Figure (2) standard curve of IL-26

Statistical analysis

All statistical analyses were conducted using Microsoft Excel 2016 and IBM SPSS Statistics version 26. The Chi-square test of independence was applied to evaluate associations between categorical variables like IgA and IL-26. A p-value of < 0.05 was considered statistically significant throughout all analyses.

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RESULTS

This study includes 100 individuals with fixed orthodontic appliances, 50 experimental and 50 controls comprising 36 males,64 females *p* value = 0.298 . The control group samples were collected through unstimulated saliva from individuals free of WSLs with fixed orthodontic appliances, whereas the case group with WSLs. The samples have been diagnosed by ELISA. In this study the results revealed that *P* value 0.002 which indicates there is significant variation between experimental group and control group as displayed in the Table (1) and Figure (3) and found there was no significant difference between case a control group regarding to sex as illustrated in Figure (4). The results of our study, weak negative correlation between age and IgA (r = –0.113, *p* = 0.26) as shown in Figure (7). The results of the study illustrated that there was no statistical association between the experimental group and control group , *p* value 0.557 in IL-26 as presented in Table (2) and Figure (5), and also no statistical difference in sex in the experimental group with *p* = 0.37 as shown in Figure (6). The finding of our study found that IL-26 levels showed no correlation with age (r = 0.024, *p* = 0.81) as presented in Figure (7).

Table (1) IgA concentration between experimental and control group

status	mean	Std	Sem	P-value
experimental	10.07	6.53	0.92	0.002
control	6.56	4.83	0.68	

*p value of ≤0.05 is significant.

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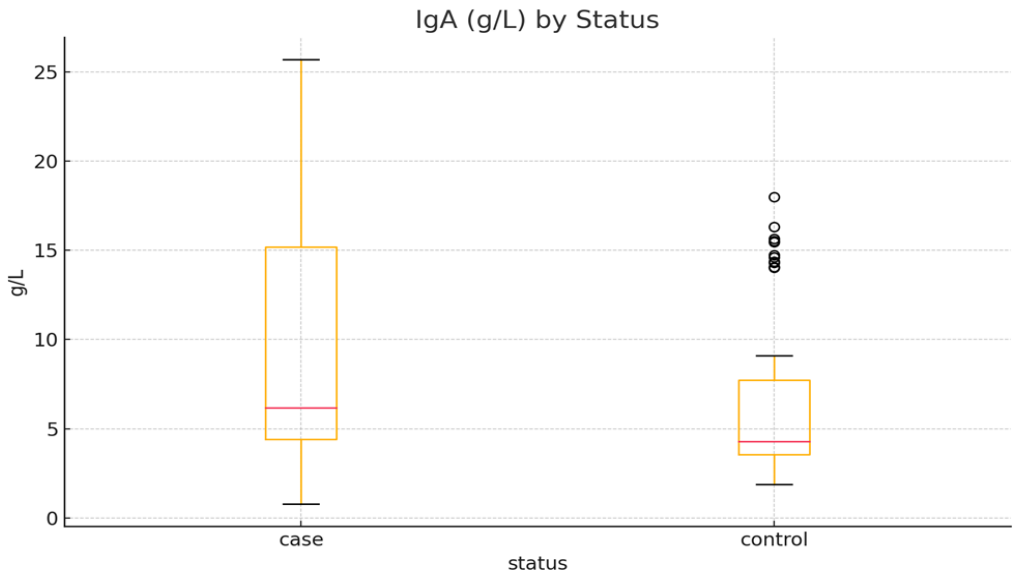

Figure(3)This box plot shows the distribution of IgA levels in both the experimental and control groups. The case group has a wider range, and higher median values compared to the control group, indicating a higher presence of IgA.

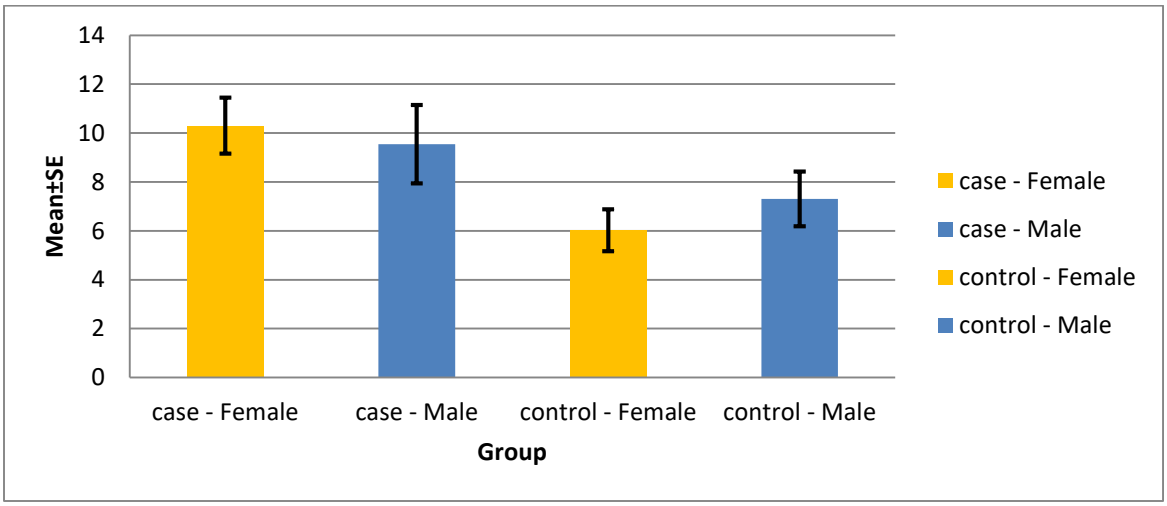

Figure (4) IgA and Sex

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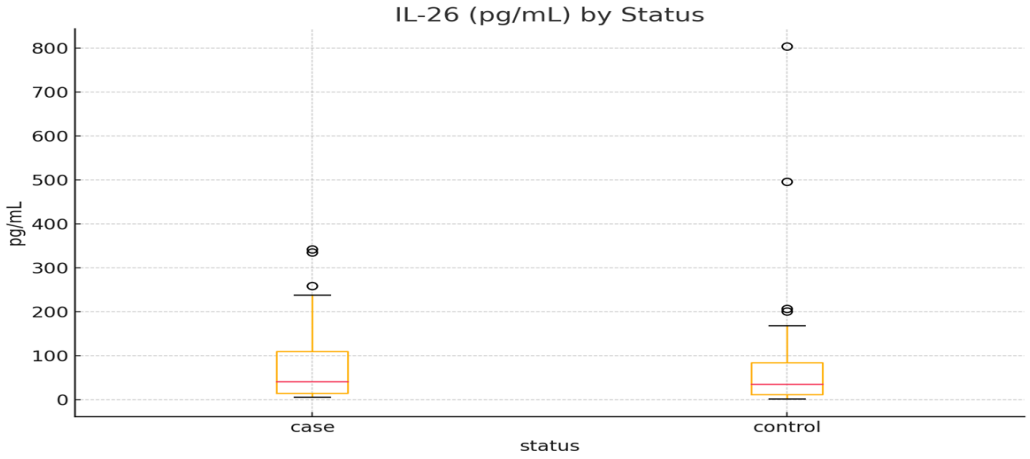
Table (2) display distribution of IL-26 between experimental and control group

status	mean	Std	sem	P-value
experimental	75.58	86.78	12.27	0.55
control	75.42	132.50	18.73	



Figure(5) box plot compares the IL-26 levels. The experimental group exhibits higher values and a wider range of IL-26 concentrations compared to the control group

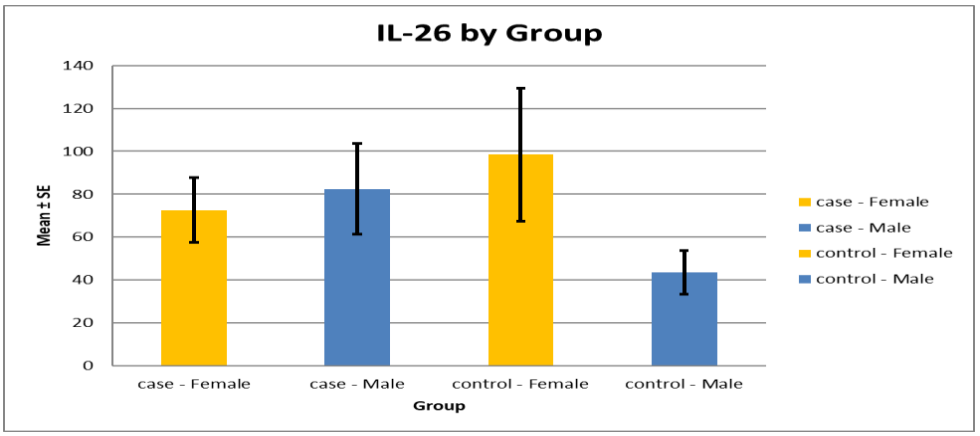


Figure (6) IL-26 level and Sex

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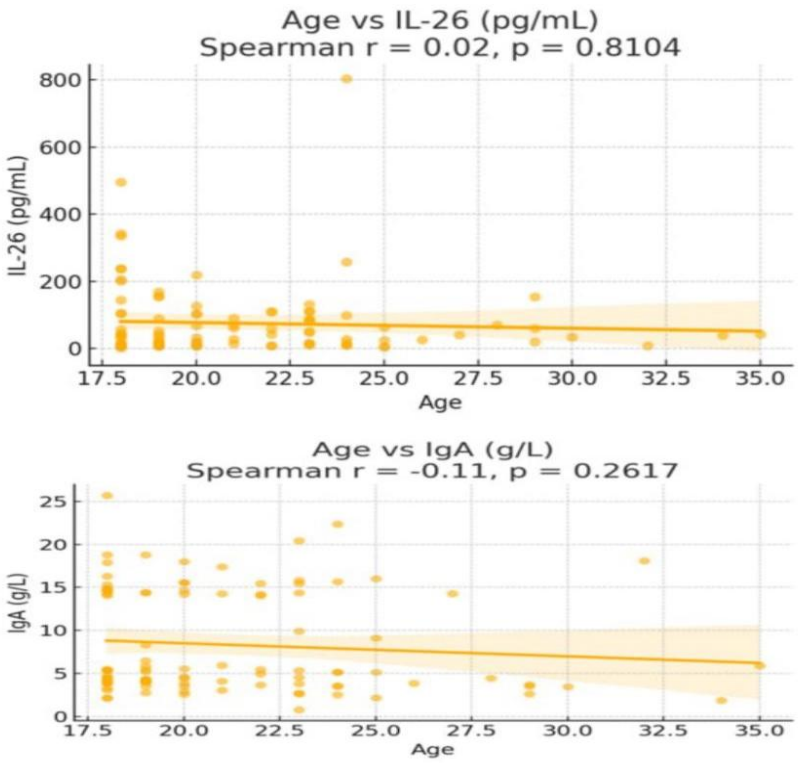

Figure (7) Age correlation with IgA and IL-26

DISCUSSION

Immunoglobulin A(IgA) is the chief isotype of antibody in humans and is highly concentrated on mucosal surfaces, which includes the mouth. IgA exist as monomers and dimers in the saliva, with the dimeric form being important for mucosal immunity. It participates in saliva, with the dimeric form being important for mucosal immunity. It participates in immune response through antigen binding, pathogen neutralization, and inflammatory response regulation, meanwhile escape complement activation.[16].Fixed appliances in orthodontic practice may offer compartments for plaque accumulation, creating a conductive atmosphere for colonies of S.mutans to grow [17]. This risk counterbalanced by IgA, which blocks bacterial adhesion and neutralizes virulence factors, such as glucosyltransferase.[18]. In The absence of IgA, these protective mechanisms are weakened, rendering the individual more susceptible to caries during fixed orthodontic therapy [19]. This cumulative risk reinforces the value of maintaining immune integrity by the patient and compliance of the oral appliance wearer. In this study the outcomes exposed that there was a significant difference between experimental group and control group with *p* value 0.002 as shown in the Table (1) and Figure (3) and that suggest there is a strong association between WSLs and

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level IgA and that agrees with (Parisotto et al., 2011; Nogueira et al., 2005). Our findings in this study also reached that there was no significant association between sex and IgA level as in demonstrated in Figure (4) in the interim that comply with [22] who found that there was no significant association between sex and changes IgA level. The results of our study showed as demonstrated in Figure (7) weak negative correlation between age and IgA ($r = –0.113$, $p = 0.26$) aligns with previous reports suggesting that while IgA levels may increase during early childhood, they tend to plateau or show minimal variation in adulthood[23]. The results of this study as in Table (2) there was no statical difference between experimental group and control group in the level of IL-26 and since there is a gap in researches regarding to IL-26 and WSLs, we are going to compare the results of this study with interleukin 6 since its production promoted by IL-26 [24], then in comparison to our result, Zawawi & Almosa (2025) reviewed that WSLs form rapidly—sometimes within a month of appliance placement—and are associated with increased plaque retention and acidic microenvironments, both of which can stimulate IL-6 production [25] which disagreed with our findings. Govula, Anumula concluded that IL-6 could be used as potential salivary biomarker to assess dental caries severity and the effectiveness of the dental treatment and that away from our results regarding IL-26 [26].The results of this study also revealed that there was no association between IL-26 and sex as shown in Figure (6), these findings corrospond with [27] found no notable differences were observed in the levels of IL-6 levels between males and females undergoing orthodontic treatment, meanwhile Bernardi found that IL-6 significantly associated with sex [28]. The finding of our study illustrated that IL-26 levels showed weak negative correlation with age ($r = 0.024$, $p = 0.81$) as evidinced by Figure (7) , which is consistent with limited literature indicating that IL-26 expression is more closely linked to inflammatory states than to chronological aging [29], [30].

CONCLUSION

There was an over-representation of females in this study sample. Patients aged less than 20 years were predominant compared to other age groups. IgA and IL-26 levels were measured using ELISA, IgA showed a positive significant association with WSLs, but IL-26 did not show association with WSLs.

ACKNOWLEDGMENTS

The authors extend their sincere appreciation to the specialized dental center for providing essential samples throughout the study. This research was made possible through the generous cooperation of orthodontic patients; whose participation was instrumental in successful completion. The authors are deeply grateful for their commitment and invaluable contribution to this work.

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Ethical approval

The study was performed in line with the ethical principles of the Helsinki declaration. Written and verbal consents were obtained from the patients before sampling. The study protocol and the subject information and consent documents were approved by a local ethics committee (Ref NO.80 on July 7,2025).

Financial support and sponsorship

Nil.

Conflict of interest.

There are non-conflicts of interest.

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مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية
ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

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الخلاصة

تقويم الأسنان تخصصٌ في طب الأسنان يُركز على تشخيص سوء اصطفاف الأسنان والاختلافات بين الوجه والجمجمة والوقاية منها وإدارتها، بالإضافة إلى تحسين جمال الوجه. يُؤدي استخدام أجهزة تقويم الأسنان الثابتة إلى خلق العديد من الفجوات الاحتجازية التي تُهيئ الأفراد لزيادة تراكم البلاك وبقايا الطعام. وهذا بدوره يُعقّد ممارسات نظافة الفم الفعالة. الهدف: هدف الدراسة هو التقييم المناعي للغلوبولين المناعي (IgA) والإنترلوكين 26 (IL-26) في آفات البقع البيضاء في علاج تقويم الأسنان الثابت. المواد والطريقة: خضع ما مجموعه 100 فرد، بما في ذلك 50 (50%) مريضًا و50 (50%) من الضوابط، 36 ذكرًا و64 أنثى، تتراوح أعمارهم بين 15 و35 عامًا، لعلاج تقويم الأسنان الثابت لأولئك الذين يقيسون المؤشرات الحيوية اللعابية بواسطة ELISA لمدة شهر واحد على الأقل بعد تنشيط الجهاز. النتائج: أظهرت النتائج أن مجموعة الدراسة أظهرت فروقًا معنوية أعلى مقارنةً بمجموعة الضبط، حيث بلغت قيمة الاحتمالية 0.002 فيما يتعلق بمستوىIgA ، بينما لم يُظهر مستوى IL-26 أي فرق معنوي بين المجموعتين، حيث بلغت قيمة الاحتمالية 0.557. الاستنتاج: مقارنةً بمجموعة الضبط، التي لم تظهر عليها أي علامات لآفات البقع البيضاء، أظهرت مجموعة الدراسة مستويات مرتفعة . IgAاللعابي، بينما ظلت مستويات IL-26 ثابتة نسبيًا