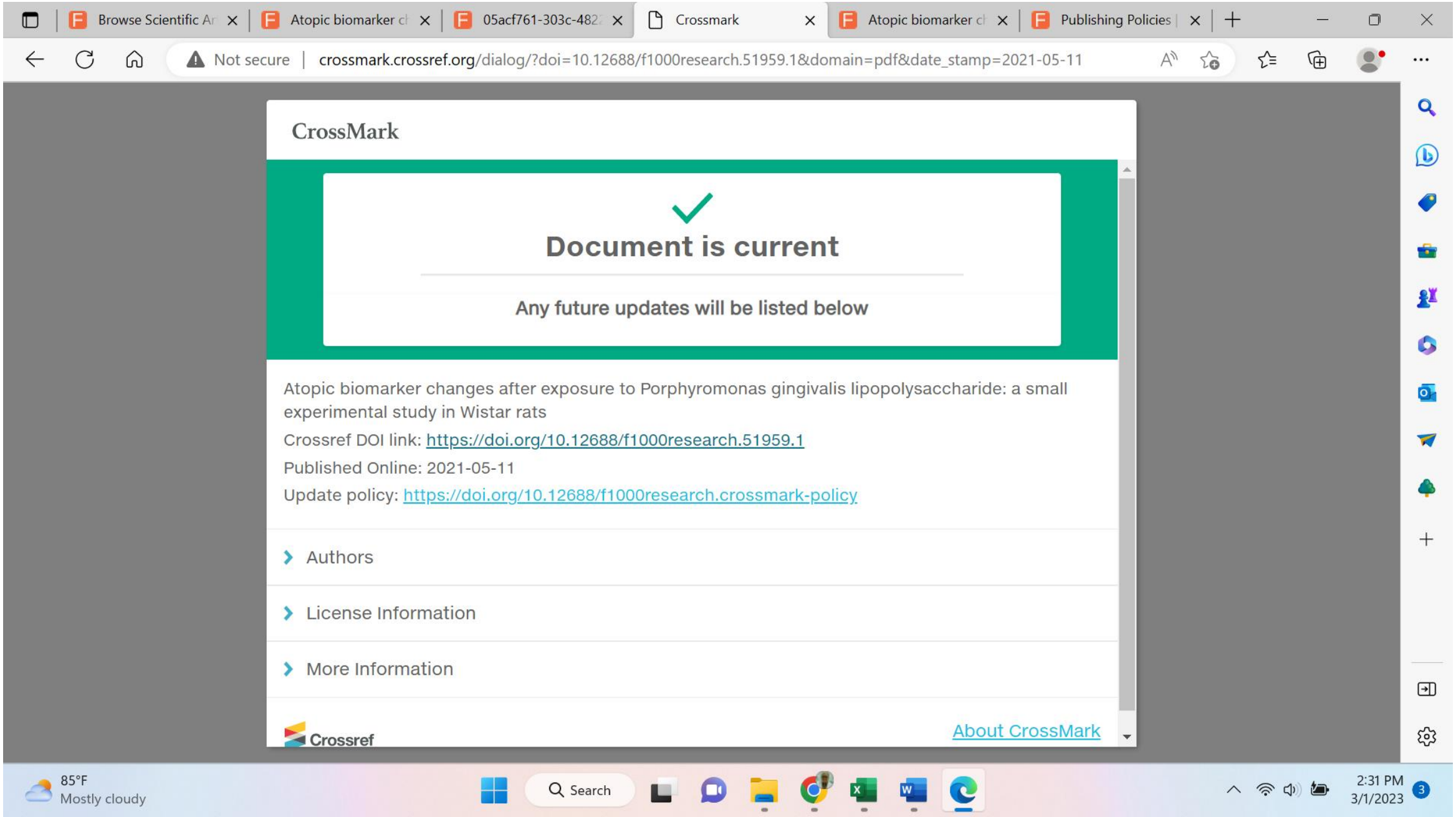
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RESEARCH ARTICLE

Atopic biomarker changes after exposure to *Porphyromonas gingivalis* lipopolysaccharide: a small experimental study in Wistar rats [version 1; peer review: 1 approved with reservations, 1 not approved]

Sindy Cornelia Nelwan[1], Ricardo Adrian Nugraha [2], Anang Endaryanto[3], Asti Meizarini[4], Udijanto Tedjosasongko[1], Seno Pradopo[1], Haryono Utomo[5], Nunthawan Nowwarote [6]

Author details

Abstract

Background: IgE and IgG_4 are implicated in atopic development and clinically utilized as major biomarkers. Atopic responses following certain pathogens, such as *Porphyromonas gingivalis* (Pg), are currently an area of interest for further research. The aim of this study is to measure the level of IgE, IgG_4, and IgG_4/IgE ratio periodically after exposure of periodontal pathogen Pg lipopolysaccharide (LPS).

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Results: Levels of IgE and IgG_4 increased significantly following exposure of LPS Pg at day-4 and day-11. Greater increase

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Results: Levels of IgE and IgG_4 increased significantly following exposure of LPS Pg at day-4 and day-11. Greater increase of IgE rather than IgG_4 contributed to rapid decline of IgG_4/IgE ratio, detected in the peripheral blood at day-4 and day-11.
Conclusion: Modulation of atopic responses following exposure to LPS Pg is reflected by a decrease in IgG_4/IgE ratio that accompanies an increase of IgE. Therefore, Pg, a keystone pathogen during periodontal disease, may have a tendency to disrupt atopic

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2. **Tomoki Maekawa**, Niigata University, Niigata, Japan

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biomarkers.

Keywords
allergic diseases, atopic inflammatory pathway, immunoglobulin, periodontal pathogen, Porphyromonas gingivalis LPS

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Introduction

The oral cavity is the habitat of numerous bacteria, including *Porphyromonas gingivalis* (Pg). Pg is a gram negative, facultative anaerobic pathogen, which is responsible in causing gingivitis or periodontitis.[1] In low-income countries, gingivitis and periodontitis can affect up to 90% of the adult population.[2] Rather than alveolar bone and ligament destruction, Pg is believed to be involved with the development of atopic responses in a susceptible host.[3] Following Pg infection, hosts' adaptive immune response (both cell-mediated and humoral-mediated) could induce a systemic inflammatory reaction, not only just local destruction of tooth-supporting tissues.[4–5] Although periodontal pathogens, such as Pg, play a major role in the initiation of local and systemic inflammatory reaction,[6] the host aberrant immune responses require further study. Since humoral immune responses are stimulated following Pg infection, there might be a link to the occurrence of atopy.

Despite long-standing research about hygiene hypothesis for several decades, there is an unequivocally accepted fact that the prevalence of atopy increases more among children who have periodontal pathogen colonization or infection.[7] While endorsing these hygiene hypothesis approaches, there is an alternative hypothesis in which exposure to some periodontal pathogens will exclusively trigger an "immunoglobulin-E skew" rather than reducing it.[8] Within the context of the hygiene hypothesis, the most essential microbial exposures needed to be studied is the biomolecular relationship between host antibody and regulatory T-cell with lipopolysaccharide (LPS), an endotoxin released by Pg to affect host immune reaction.

Hygiene hypothesis principles might not be able to answer all phenomenon of increasing incidence of atopy among children with poor oral hygiene.[9] Some studies report a positive association between the colonization/infection of Pg with the development of allergic diseases,[10–15] whereas some studies report no association.[16–21] Due to lack of conclusive evidence about the association between Pg and allergic diseases,[22] we try to measure the level of atopic biomarkers following Pg infection.

To the best of our knowledge, measuring IgG_4 and IgE antibody may have a closer association to atopic profiles, since IgG_4 and IgE are released after activation of mature B cells following the modulation of IL-4 and IL-5 released by Th-2 cells during type I hypersensitivity.[23] By looking at the alteration of IgG_4 and IgE antibodies level after exposure to these selected components of Pg in a rat model, we hope to understand more deeply the biological mechanism of B-cell production antibodies pattern and humoral immune responses before the clinical manifestation of atopy. We chose a rat model since they are inbred so they are almost identical genetically and their genetic, biological and behavior characteristics closely resemble those of humans.

Methods

Ethics approval

This article was reported in line with the ARRIVE guidelines. Animal experimental study was conducted under the approval of the Institutional Animal Research Ethics Committee of Universitas Airlangga (UNAIR), Surabaya, Indonesia (animal approval no:50/KKEPK.FKG/IV/2015) under the name of Sindy Cornelia Nelwan as the Principal Investigator. The study was carried out in strict accordance to internationally accepted standards of the Guide for the Care and Use of Laboratory Animals of the National Institute of Health. All efforts were made to ameliorate any suffering of animals through using anaesthetic to euthanize the rats at the end of the experimental procedure.

Animals

Sample size

$N = (Z_{\alpha/2})^2 \, s^2/d^2$, where s is the standard deviation obtained from previous study or pilot study, and d is the accuracy of estimate or how close to the true mean. $Z_{\alpha/2}$ is normal deviate for two- tailed alternative hypothesis at a level of significance. Suppose sample size calculated by software is 3 animals per group and researcher is expecting 10% attrition then his final sample size will be 4 animals per group or 16 animals in total.

Rats

The present study used 16 male Wistar rats (*Rattus novergicus*) between eight and ten weeks of age (average body weight 120-150 grams). The rats were housed in microisolator cages and maintained in a constant room temperature ranging from 22°C to 25°C, with a 12-h light/12-h dark cycle, under artificially controlled ventilation, with a relative humidity ranging from 50% to 60%. The rats were fed a standard balanced rodent diet (NUTRILAB CR-1®) and water were provided ad libitum.

Inclusion criteria was male Wistar rats, age 8-10 weeks, with body weight 120-150 grams. Female Wistar rats, diseased, sick, and lazy male Wistar rats were strictly excluded.

Experimental design and groups

The present study design was a pre-test post-test-controlled unblinded group design using quantitative method. The 16 male Wistar rats were randomized using randomized block sampling and classified into four groups. Each group consisted of 4 matched Wistar rats (age, weight, IgE and IgG_4 baseline characteristic). Group 1 were given placebo (0.9% normal saline solution). Group 2 were given lipopolysaccharide (LPS) of *Porphyromonas gingivalis* (Pg) (American Type Culture Collection, Rockville, Md.) at dose 0.3 µg/mL. Group 3 were given LPS Pg at dose 1 µg/mL. Group 4 were given LPS Pg at dose 3 µg/mL.

The rats received LPS by an intra-sulcular injection. Intra-sulcular injection has an advantage due to the its direct delivery of LPS to oral cavity in which the tip of needle is injected slowly at the crestal bone. Longitudinal quantitative measurement was performed; IgE level, IgG_4 level, and IgG_4/IgE ratio in both groups on day-0 (before treatment), day-4, and day-11. An average of 0.2 ml peripheral blood sera was obtained by Pasteur pipette from retro-orbital plexus, using a lateral approach on each of these days from each rat. The potential expected adverse events were anaphylactic shock, allergic reaction, bleeding and infection. However, to the best our knowledge, there were no expected nor unexpected adverse events in the experimental procedures. Following the end of the experiments, all efforts were made to ameliorate any suffering of animals through injection of sodium pentobarbital anesthetic to euthanize the rats at the end of the experimental procedure.

Level of IgG_4 and IgE

Sample of the sera were collected and stored at −70°C (−94°F) at Institute of Tropical Diseases Universitas Airlangga (UNAIR). All sera were assessed by direct-sandwich enzyme-linked immunosorbent assay (ELISA) with mouse IgE antibody (MAB9935) and IgG_4 antibody (MAB9895) under the manufacturer's (R&D System Europe Ltd, Abingdon, UK) protocol. Briefly, the sera were examined using microtiter plates using 25 ml of 3,3',5,5'-tetramethylbenzidine to 1 ml of phosphate-citrate buffer plus perborate in a mildly acidic buffer (adjust pH 5.7). Levels of IgG_4 were detected using monoclonal antibody anti-IgG_4, transferring it to microtiter plates, adding the supplied conjugate, adding blocking solution, diluting plasma sample (1:100,000), and washing between the steps. Level of IgE was detected using monoclonal antibody anti-IgE, following similar steps until diluting the plasma sample (1:200). A minimum value of 0.01 pg/mL for IgE and 0.01 ng/mL for IgG_4 were assigned for below the limit of detection. We used 3,3',5,5'-tetramethylbenzidine as chromogenic substrate, which allows direct visualization of signal development through spectrophotometer.

Statistical analysis

All measurements were performed at least three times. Results were presented as means ± standard errors (SEM). The assumption of the normality for the complete data was assessed by Shapiro-Wilk test. Test of homogeneity of variances was assessed by Levene Statistics. Statistical significance was examined by one-way ANOVA and repeated measure ANOVA using SPSS version 17.0 for Microsoft (IBM corp, Chicago, USA).

Results

General characteristic investigations

Table 1 show the baseline characteristics of the 16 Wistar rats (*Rattus norvegicus).* No significant differences were found for mean age ($p = 0.774$), body weight ($p = 0.700$), baseline IgE ($p = 0.071$), baseline IgG_4 ($p = 0.770$), and baseline IgG_4/IgE ratio ($p = 0.053$) among the four groups.

Comparison of serum IgE level between the four groups

Prior to experiments (day-0), there was no difference of serum IgE level between the four groups ($p > 0.05$). On day-4, there was a significance difference of serum IgE level between all groups ($p = 0.006$). At day-4, the highest average IgE level could be found in Group 3 treated with LPS Pg 1 µg/ml (17.00 ± 1.69 pg/ml) and the lowest average IgE level could be found in Group 1 (control) (5.31 ± 0.76 pg/ml). On day-11, there was also a significance difference of serum IgE level between both groups ($p = 0.047$). At day-11 the highest average IgE level could be found in Group 2 treated with LPS Pg 0.3 µg/ml (180.34 ± 10.42 pg/ml) and the lowest average IgE level could be found in Group 1 (5.06 ± 1.86 pg/ml) (Table 2).

Comparison of serum IgG_4 level between the four groups

Prior to experiments (day-0), there was no difference of serum IgG_4 level between the four groups ($p > 0.05$). On day-4, there was a significance difference of serum IgG_4 level between all groups ($p = 0.008$). At day-4, the highest average IgG_4 level could be found in Group 4 (LPS Pg 3 µg/ml; 23.86 ± 1.59 ng/ml) and the lowest average IgG_4 level could be found

Table 1. General characteristics of study population (n = 16; 4/group).

Variable	Group 1 (control)	Group 2 (LPS Pg 0.3 μg/ml)	Group 3 (LPS Pg 1 μg/ml)	Group 4 (LPS Pg 3 μg/ml)	*p value*
Age (weeks)	8.87 ± 0.86	9.37 ± 0.95	8.75 ± 0.96	9.12 ± 0.63	0.774
Weight (g)	135.25 ± 9.54	135.25 ± 9.55	137.25 ± 7.93	138.00 ± 10.98	0.700
Male (%)	100	100	100	100	-
IgE (pg/mL)	5.69 ± 0.28	5.36 ± 0.19	10.27 ± 0.70	6.69 ± 0.61	0.071
IgG_4 (ng/mL)	10.74 ± 1.41	11.76 ± 0.85	11.91 ± 1.66	10.14 ± 1.44	0.770
IgG_4/IgE ($x10^3$)	1.87 ± 0.18	2.20 ± 0.13	1.19 ± 0.20	1.59 ± 0.34	0.053

*Data are presented as mean ± standard error of the mean (SEM).
**One-way ANOVA for categorical variables; significant at $p < 0.05$.
***IgE, immune globulin E; IgG_4, immune globulin G_4; IgG_4/IgE, ratio between average IgG_4 levels divided by average IgE levels.

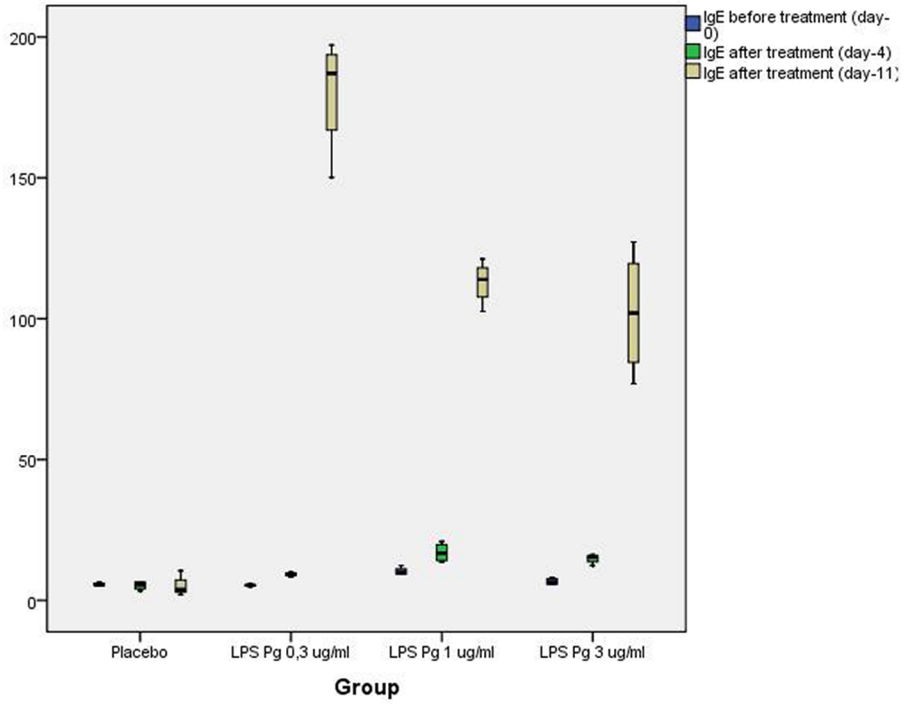

Figure 1. Longitudinal observation of serum IgE levels following exposure to LPS Pg.

in Group 1 (8.34 ± 0.58 ng/ml). On day-11, there was a greater difference of serum IgG_4 level between all groups ($p = 0.005$). At day-10, the highest average IgG_4 level could be found in Group 4 (LPS Pg 3 μg/ml; 63.74 ± 4.74 ng/ml) and the lowest average IgG_4 level could be found in Group 1 (13.91 ± 0.99 ng/ml) (Table 3).

Ratio of IgG_4/IgE antibodies between the four groups

The average IgG_4/IgE ratio for the control group at day-0, day-4, and day-11 was 1.87×10^3, 1.72×10^3, and 3.62×10^3. In Group 2 (low-dose LPS group; 0.3 μg/ml), the average IgG_4/IgE ratio was 2.20×10^3, 1.27×10^3, and 0.22×10^3, respectively. In Group 3 (medium-dose LPS group; 1 μg/ml), the average IgG_4/IgE ratio was 1.19×10^3, 0.69×10^3, and 0.56×10^3, respectively. In Group 4 (high-dose LPS group; 3 μg/ml), the average IgG_4/IgE ratio was 1.59×10^3, 1.64×10^3, and 0.65×10^3, respectively. All groups exhibited significant differences of IgG_4/IgE ratios, except at day-0. The highest IgG_4/IgE ratio at day-4 and day-11 could be found in Group 1. The lowest IgG_4/IgE ratio at day-4 could be found in Group 3, whilst the lowest ratio at day-11 could be found in Group 2 (Table 4).

Table 2. Comparisons of total serum IgE (pg/mL) between the four groups (mean ± SEM).

Group	n	IgE day 0	IgE day 4	IgE day 11
Group 1 (control)	4	5.69 ± 0.28	5.31 ± 0.76	5.06 ± 1.86
Group 2 (LPS Pg 0.3 μg/ml)	4	5.36 ± 0.19	9.26 ± 0.32	180.34 ± 10.42
Group 3 (LPS Pg 1 μg/ml)	4	10.27 ± 0.70	17.00 ± 1.69	112.90 ± 3.87
Group 4 (LPS Pg 3 μg/ml)	4	6.69 ± 0.61	14.79 ± 0.86	102.01 ± 11.04
F statistic	/	20.733	26.171	83.758
p value	/	0.071	0.006	0.047

*Measured by one-way ANOVA (df1 = 3, df2 = 12, f table 3.490; significant at $p < 0.05$).

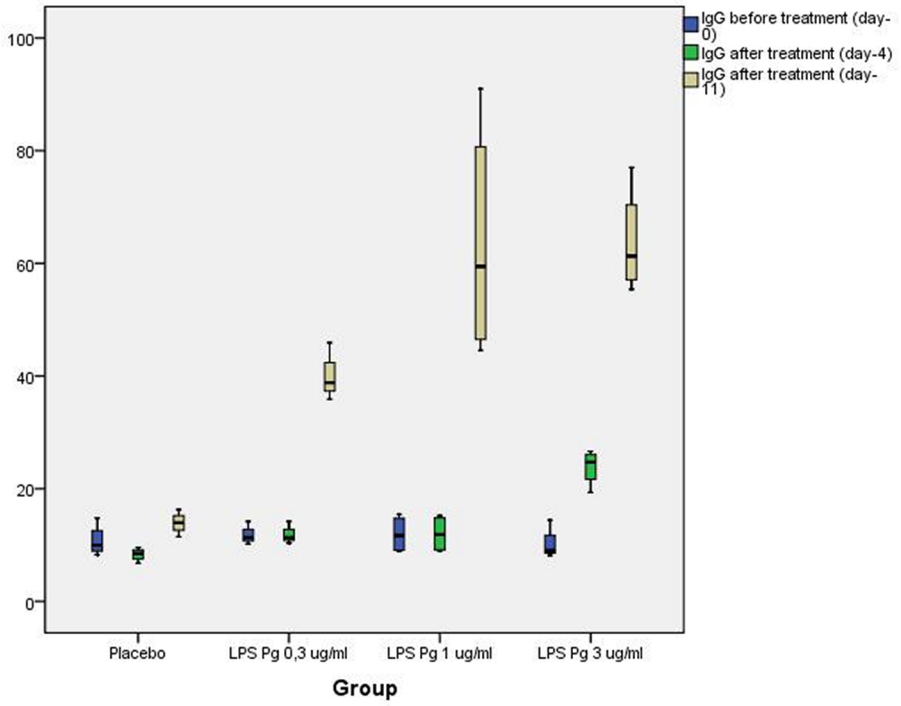

Figure 2. Longitudinal observation of serum IgG_4 levels following exposure to LPS Pg.

Subgroup analysis

Group 2 (0.3 μg/ml LPS Pg)

Level of IgE were increased dramatically from day-0 to day-11 after experiments (5.36 ± 0.19 pg/ml to 180.34 ± 10.42 pg/ml; $p = 0.011$). Level of IgG_4 also increases significantly from day-0 to day-11 after experiments (11.76 ± 0.85 ng/ml to 39.85 ± 2.14 ng/ml; $p = 0.006$). On the other hand, IgG_4/IgE ratio were decreased following experiments ($2.20 \pm 0.13 \times 10^3$ to $0.22 \pm 0.01 \times 10^3$; $p = 0.014$) (Table 5).

Group 3 (1 μg/ml LPS Pg)

Level of IgE were raised dramatically from day-0 to day-11 after experiments (10.27 ± 0.70 pg/ml to 112.90 ± 3.87 pg/ml; $p = 0.003$). Level of IgG_4 also increased significantly from day-0 to day-11 after experiments (11.91 ± 1.66 ng/ml to 63.6 ± 10.76 ng/ml; $p = 0.027$). On the other hand, IgG_4/IgE ratio declined following experiments ($1.19 \pm 0.20 \times 10^3$ to $0.56 \pm 0.09 \times 10^3$; $p = 0.362$) (Table 6).

Table 3. Comparisons of total serum IgG_4 (ng/mL) between the four groups (mean ± SEM).

Group	n	IgG_4 day 0	IgG_4 day 4	IgG_4 day 11
Group 1 (control)	4	10.74 ± 1.41	8.34 ± 0.58	13.91 ± 0.99
Group 2 (LPS Pg 0.3 μg/ml)	4	11.76 ± 0.85	11.80 ± 0.83	39.85 ± 2.14
Group 3 (LPS Pg 1 μg/ml)	4	11.91 ± 1.66	11.98 ± 1.65	63.6 ± 10.76
Group 4 (LPS Pg 3 μg/ml)	4	10.14 ± 1.44	23.86 ± 1.59	63.74 ± 4.74
F statistic	/	0.379	29.265	15.665
p value	/	0.770	0.008	0.005

*Measured by one-way ANOVA (df1 = 3, df2 = 12, f table 3.490; significant at $p < 0.05$).

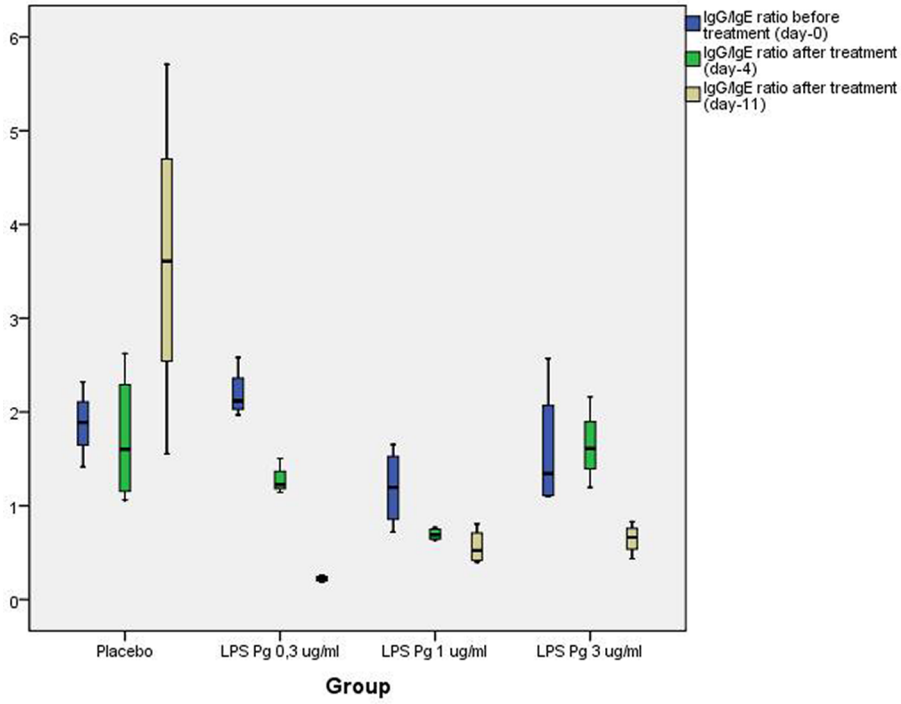

Figure 3. Comparisons of IgG_4/IgE ratio between four groups (mean ± SEM).

Table 4. Comparisons of IgG_4/IgE ratio ($x10^3$) between the four groups (mean ± SEM)

Group	n	IgG_4/IgE day 0	IgG_4/IgE day 4	IgG_4/IgE day 11
Group 1 (control)	4	1.87 ± 0.18	1.72 ± 0.36	3.62 ± 0.85
Group 2 (LPS Pg 0.3 μg/ml)	4	2.20 ± 0.13	1.27 ± 0.08	0.22 ± 0.01
Group 3 (LPS Pg 1 μg/ml)	4	1.19 ± 0.20	0.69 ± 0.03	0.56 ± 0.09
Group 4 (LPS Pg 3 μg/ml)	4	1.59 ± 0.34	1.64 ± 0.20	0.65 ± 0.08
F statistic	/	3.418	5.032	13.600
p value	/	0.053	0.017	<0.001

*Measured by one-way ANOVA (df1 = 3, df2 = 12, f table 3.490; significant at $p < 0.05$).

Table 5. Comparisons of total serum IgE, IgG_4, and IgG_4/IgE ratio before and after treatment in Group 2 (exposure of 0.3 μg/mL LPS Pg) (mean ± SEM; n = 4).

Time-point	IgE (pg/mL)	IgG_4 (ng/mL)	IgG_4/IgE (x10^3)
Day-0 before treatment	5.36 ± 0.19	11.76 ± 0.85	2.20 ± 0.13
Day-4 after treatment	9.26 ± 0.32	11.80 ± 0.83	1.27 ± 0.08
Day-11 after treatment	180.34 ± 10.42	39.85 ± 2.14	0.22 ± 0.01
F statistic	93.924	178.233	71.969
p value	0.011	0.006	0.014

*Measured by repeated measure ANOVA.
**df times = 2, df error = 14, f table 3.739; significant at $p < 0.05$.

Table 6. Comparisons of total serum IgE, IgG_4, and IgG_4/IgE ratio before and after treatment in Group 3 (exposure of 1 μg/mL LPS Pg) (mean ± SEM; n = 4).

Time-point	IgE (pg/mL)	IgG_4 (ng/mL)	IgG_4/IgE (x10^3)
Day-0 before treatment	10.27 ± 0.70	11.91 ± 1.66	1.19 ± 0.20
Day-4 after treatment	17.00 ± 1.69	11.98 ± 1.65	0.69 ± 0.03
Day-11 after treatment	112.90 ± 3.87	63.6 ± 10.76	0.56 ± 0.09
F statistic	294.526	35.437	1.760
p value	0.003	0.027	0.362

*Measured by repeated measure ANOVA.
**df times = 2, df error = 14, f table 3.739; significant at $p < 0.05$.

Table 7. Comparisons of total serum IgE, IgG_4, and IgG_4/IgE ratio before and after treatment in Group 4 (exposure of 3 μg/mL LPS Pg) (mean ± SEM; n = 4).

Time-point	IgE (pg/mL)	IgG_4 (ng/mL)	IgG_4/IgE (x10^3)
Day-0 before treatment	6.69 ± 0.61	10.14 ± 1.44	1.59 ± 0.34
Day-4 after treatment	14.79 ± 0.86	23.86 ± 1.59	1.64 ± 0.20
Day-11 after treatment	102.01 ± 11.04	63.74 ± 4.74	0.65 ± 0.08
F statistic	111.386	32.929	7.841
p value	0.009	0.029	0.113

*Measured by repeated measure ANOVA.
**df times = 2, df error = 14, f table 3.739; significant at $p < 0.05$.

Group 4 (3 μg/ml LPS Pg)

Level of IgE were raised dramatically from day-0 to day-11 after experiments (6.69 ± 0.61 pg/ml to 102.01 ± 11.04 pg/ml; $p = 0.009$). Level of IgG_4 were also increase significantly from day-0 to day-11 after experiments (10.14 ± 1.44 ng/ml to 63.74 ± 4.74 ng/ml; $p = 0.029$). On the other hand, IgG_4/IgE ratio declined following experiments ($1.59 \pm 0.34 \times 10^3$ to $0.65 \pm 0.08 \times 10^3$; $p = 0.113$) (Table 7).

Discussion

Several mechanisms have been suggested to alter atopic inflammatory responses following LPS Pg infection. One of the mechanisms proven in this study is an elevation of IgE antibody and reduction of IgG_4/IgE ratio.[24] As far as we have known, Th-1 and Th-2 cells are not two different CD4+ T-cell subsets, but it represents polarized forms of the highly heterogenous CD4+ Th cell–mediated immune response. Host genetic and microenvironmental factors could have contributed with series of modulatory factors including:[1] the ligation of T-cell receptor (TCR);[2] the activation of costimulatory molecules and its particular components;[3] the predominance of an inflammatory cytokine in the local environment; and[4] the number of postactivation cell divisions following exposure to antigens. Down-regulation of the Th-1 cell is associated with depression

of cell-mediated immune response and stimulation of humoral immune response, thus pathogens are able to evade immune clearance.[25]

Porphyromonas gingivalis possess very sophisticated defense mechanisms against host immune responses. These pathogens produce capsules containing long chain LPS which is designed effectively to counter membrane attack complex. These long chain LPS can also downgrade cell-mediated immunity by shifting Th-1 into Th-2 which less dangerous to pathogens.[26] LPS may have an essential role in switching cell-mediated to humoral-mediated immune responses.[27] LPS Pg antigen is processed and presented on its surface with MHC-II molecule. Recent studies suggest an activation of alternative complement pathway, disruption of classical complement pathway, modulation of antigen presenting cells, and downregulation of anti-inflammatory cytokines are responsible for the Th2-skewed immune response following exposure to LPS Pg. Predominance shifting from Th-1 into Th-2 occurs in several extra-lymphoid tissues; the ideal site for *Porphyromonas gingivalis* is the oral cavity.[28]

Interleukin-4 (IL-4), which is produced by naive T cells, acts as autocrine manner known to be responsible for the differentiation and activation of Th-2 phenotype.[29] Guo et al (2014) shows upon the occurrence and development of allergic diseases, there is a complex pathobiology which results in an imbalance of Th-1/Th-2.[30] In an atopic disease such as bronchial asthma or urticaria, naive T cell can differentiate into Th-2 under IL-4–induced STAT6 and GATA-3 transcription factors.[30] Th-2 predominant immune response will automatically stimulate plasma cell to release IgE and IgG_4.[31] Upon re-exposure of antigen or allergen, binding of the allergen to IgE orchestrates the adaptive immune system to initiate rapid sensitization. Frequent sensitization is a major risk factor for the development of allergic diseases such as urticaria, bronchial asthma, hay fever or atopic dermatitis/eczema.[32]

Our previous study used whole-cell body of *Porphyromonas gingivalis* to study different molecular responses in Wistar rats. Our first project studied the association between periodontal pathogen and host innate immunity. Exposure to *Porphyromonas gingivalis* had been shown to stimulate level of TLR2 and depress level of TLR4.[33] Our findings might indicate that several bacterial properties can turn-off host innate immunity and host inflammatory response. Our second project studied the association between periodontal pathogen and host adaptive immunity. We summarized that high dose CFU of Pg stimulates fold increase of Th-2 cytokines (IL-4, IL-5 and IL-13) and decrease of Th-1 cytokines (IFN-γ and IL-17).[34] These were the cornerstone to continue our project in studying LPS as the most important component of these bacteria.

At this moment, both total IgE or specific IgE antibodies have little diagnostic value in the occurrence of allergic manifestation. Even total or specific IgE is increasing, yet the manifestation of allergy doesn't usually develop, since IgG_4 level also increases as a counter-regulator.[35] It means that even human or rat become susceptible to atopic allergy due to the increasing level of IgE, body mechanism is able to provide protection, with increased IgG_4 as a counter response to prevent manifestation of allergic diseases and immediate hypersensitivity. Thus, exposure of LPS Pg will develop chance of atopic and hypersensitivity markers, but manifestation of allergic reaction is a complex pattern.[36] IgG_4/IgE ratio has closer accuracy to detect any alteration of atopic inflammatory pathway. Increase level of IgE, which isn't accompanied by IgG_4, can be seen in patients with urticaria or atopic dermatitis.[37] IgE-switched B cells are much more likely to differentiate into plasma cells, whereas IgG_4-switched B cells are less likely to differentiate.[38] This reason would explain why IgE antibody is the most dominant antibody in the development of atopic inflammatory pathway, whereas IgG_4 antibodies become prominent later during chronic non-atopic stimulation.[39] According to this reason, IgG_4/IgE ratio may predict atopic responses more accurately than total or specific IgE level.

Limitations and strengths

Several limitations should be highlighted. First, this study had limitations with regard to very small number of samples which can increase the likelihood of error and imprecision. Second, results from animal models often do not translate into replications in humans.[40] IgE antibody responses in Wistar rats are typically transient, whereas the atopic IgE response in human persists for many years.[41] Other crucial difference is IgG_4/IgE ratio, which is usually much higher in the Wistar rats than humans.[42–43] These factors may have an impact on the interpretation of our results. Thus, the findings should be interpreted within the context of this study and its limitations. The strengths of the study were its high statistical power and the homogeneity of each group to enable comparison between groups and periods.

Conclusion

Several experiments in rats indicate that exposure to LPS Pg may have a tendency to increase levels of IgE and IgG_4. On the contrary, declining IgG_4/IgE ratio following exposure to LPS Pg suggests the potential role of LPS Pg for isotype switching from IgG_4 to IgE. The results of the present study favor indirect isotype switching route for most IgE as secondary responses from LPS Pg infection that leads to systemic atopic inflammatory pathway.

Data availability

Underlying data

Figshare: Raw Data - Atopic Biomarker Changes after Exposure to Porphyromonas gingivalis Lipopolysaccharide: A Small Experimental Study in Wistar Rat, https://doi.org/10.6084/m9.figshare.14350271.v1.[44]

This project contains the following underlying data:

- Data fixed LPS grup A.sav (Group 1 results)
- Data fixed LPS grup B1.sav (Group 2 results)
- Data fixed LPS grup B2.sav (Group 3 results)
- Data fixed LPS grup B3.sav (Group 4 results)

Acknowledgments

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A previous version of this article is available on bioRXiv: https://doi.org/10.1101/2021.01.14.426656.

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Abbreviations

ANOVA	analysis of variant
BCR	B cell receptor
CD-40	Cluster Differentiation-40
CFU	colony-forming unit
CSR	class-switch recombination
df	degree of freedom
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
HDM	house dust mite
IACUC	Institutional Animal Care and Use Committee
IFN-γ	gamma-Interferon
IgE	immunoglobulin-E
IgG$_4$	immunoglobulin-G$_4$

IL	interleukin
LLPC	long-lived plasma cell
LPS	lipopolysaccharide
mAb	monoclonal antibody
MAC	membrane attack complex
MHC	major histocompatibility complex
NIH	National Institutes of Health
NGS	next-generation sequencing
NK	natural killer cells
OIT	oral immunotherapy
PAMP	pathogen-associated molecular patterns
PCR	Polymerase Chain Reaction
Pg	*Porphyromonas gingivalis*
SD	standard deviation
SEM	standard error of the mean
SPSS	Statistical Package for the Social Sciences
Th-1	type-1 helper T-cells
Th-2	type-2 helper T-cells
TNF-α	Tumor Necrosis Factor-α

Open Peer Review

Current Peer Review Status: ✗ ?

Version 1

Reviewer Report 14 September 2021

https://doi.org/10.5256/f1000research.55174.r92330

?

Tomoki Maekawa
Center for Advanced Oral Science, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan

This paper reaches its conclusions based on data from a small number of analysis samples, making it difficult to evaluate. In particular, there are few prerequisite statements and reference papers on the relevance of IgG and IgG4 in periodontitis, and the purpose of the study is not clear. No rationale was given for using Pg LPS in measuring pan-IgG4 and IgE levels that makes this manuscript a simple descriptive survey.

- Ensure all the references are appropriately selected. Some of the references in this manuscript are not presented correctly. The authors should refer to the comprehensive scientific paper in such a scientific journal.

- There are many papers that stated IgG rises when Pg (not only Pg but also the other LPS) is administered, so the authors need to be clear about what is new and what the purpose is.

- A more detailed explanation of the relationship between IgG4 and IgG and periodontal disease is needed.

- In the Introduction, Ref 2 Mazurek *et al.* paper is too specific to cite since this paper mentioned the influence of KIR gene presence/absence polymorphisms, which the description has not related to a comprehensive explanation of periodontitis. Ref 3 is also an irrelevant paper, it is a review of comorbidities. Moreover, Refs 4, 5, 7, 8 suddenly appeared immunoglobulin-E skew and is not suitable for citing. Some of the references cannot be found in NCBI (i.e. Refs 14, 24).

- Please check all references throughout the paper.

- Material and Methods are poorly written. How do the authors isolate LPS from Pg? The authors should describe the strain of Pg you selected.

- Discussion, page 8, third line from the back: wrong reference papers are cited.

- LPS Pg should be Pg LPS.

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 31 August 2021

https://doi.org/10.5256/f1000research.55174.r92329

Carla Alvarez
The Forsyth Institute, Cambridge, MA, USA

The Manuscript by Nelwan *et al.* presents a pilot study where they administer different concentrations of Pg LPS by Intra-sulcular injection to rats and then analyzed the IgE and IgG4 after 0, 4, and 11 days in the peripheral blood serum by ELISA. The results are that Pg injection increased both IgE and IgG4 at days 4 and 11 post-injection. IgE concentration was increased to a higher extend than IgG4.

Overall Comments:

- Even though the study is simple in its design and results, the data analysis results layout (plots and tables) and all sections are very confusing. The study is presented as a pilot study (small experimental study), yet they provided a sufficient sample size to achieve statistical significance, which needs to include an appropriately detailed analysis.

Specific comments:

- The introduction does not explain why the authors decided to perform this study. The sentence: 'Since humoral immune responses are stimulated following Pg infection, there might be a link to the occurrence of atopy' is extremely vague. There are no references to support that connection of ideas.

- The link between periodontitis and atopy is not explained, and the literature does not back several statements. For instance, the sentence "Rather than alveolar bone and ligament destruction, Pg is believed to be involved with the development of atopic responses in a susceptible host" has as reference Sperr *et al.*: Prevalence of Comorbidities in Periodontitis Patients Compared to the General Austrian Population. J Periodontol. 2018; 89(1): 19–27. What authors believe that Pg is involved in the development of atopic responses in a host? And why? These are critical elements missing in the introduction.

- The sentence "there is an unequivocally accepted fact that the prevalence of atopy increases more among children who have periodontal pathogen colonization or infection" is a bold statement with no literature backup. The paper cited is Dowarah R *et al.*: Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health, and antioxidant status in weaned piglets. PLoS One. 2018; 13(3): e0192978. How does that paper in piglets explain that unequivocally accepted fact in human children?

- A proper introduction must explain all the variables studied in the paper and present them in an organized and coherent way. The knowledge gap must be evident and support the hypothesis established by the authors and the experimental design. All this, using solid references that can accurately backup the statements as are presented.

- The results should be three grouped graphs: IgE and IgG4 concentrations, then the ratio of them, that's it. There is no need for most tables, and the graphs should show which group is significantly different from another group by using asterisks. The authors should use a posthoc analysis after ANOVA to show the individual comparisons in the graph.

- The discussion is very confusing; the authors often change the topic or don't explain the point they try to make. Also, several statements have no literature references, such as "Th-1 and Th-2 cells are not two different CD4+ T-cell subsets, but it represents polarized forms of the highly heterogenous CD4+ Th cell-mediated immune response", is again a bold statement with no references. Please check the relevant literature.

- I suggest including in your discussion the following elements:
  What are the significant findings of the study, their meaning, and why are they important? Relate the results to those of similar studies. Clarify alternative explanations of your results. State what is the relevance of the conclusions in the clinical scenario. Suggest future studies

and provide a conclusion with a take-home message.
Don't overinterpret the results, provide unwarranted speculation or explain tangential issues.

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Periodontology, Immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.