Association between an anti-inflammatory low-carbohydrate diet and gingivitis in women with obesity

Khadiga O. Ali[1], Mohammad M. Hammad[1], Rola A. Al-Habashneh[1], Jowan M. Al-Nusair[3], Mustafa M. Ababneh[2], Mohammad B. Al-Zghoul[2]

[1]Department of Preventive Dentistry, Faculty of Dentistry, Jordan University of Science & Technology, Irbid, Jordan.

[2]Department of Basic Veterinary Medical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, Jordan.

[3]Department of Internal Medicine, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV, USA

Corresponding author: Prof. Rola Al-Habashneh
Jordan-Irbid 0000-0003-1298-0635
Email: raalhabashneh@just.edu.jo

Author Contributions

KA: Study concept and design, and wrote the manuscript. MH and RH: Study supervisions, reviewed the manuscript, and approved the submitted version. JA interpretation of data, edit and revised manuscript, MA and MZ supervised the laboratory work and analyzed the data.

ABSTRACT

Background: The Western diet contributes to the onset and progression of gingivitis. In contrast, an anti-inflammatory low-carbohydrate diet (AILCD) has been proposed as a dietary recommendation to improve gingival health. Few studies have investigated the influence of an anti-inflammatory diet on gingivitis in individuals of normal weight. This study aimed to evaluate the association between AILCD and gingivitis in women with obesity. **Methods:** Twenty-three women with obesity and gingivitis participated in a two-month study of AILCD. Clinical parameters, including the Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD), and Bleeding on Probing (BOP), were recorded at baseline, one month, and two months. Mouthwash samples were collected for real-time PCR detection of oral microbiota, including *Streptococcus mutans, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis, and Fusobacterium nucleatum*. Salivary inflammatory cytokines (IL-6, TNF-α, and IL-10) were measured using ELISA. Anthropometric parameters, including weight, BMI, skeletal muscle mass (SMM), and percent body fat (PBF), were assessed using body composition analysis. **Results:** All clinical parameters were significantly reduced. The baseline PI was 1.14 ±.48 reduced to .54 ± .38, GI was .77 ± .32 reduced to .38 ± .17, PPD was 1.83mm ± .22mm reduced to 1.35mm ± .30mm, and BOP% reduced from 37% to 19%. Baseline weight, BMI, SMM, and PBF were significantly reduced by 3.8 kg, 1.3 kg/m^2, 0.5 kg, and 1.6%, respectively. However, no significant differences were observed in the periodontal pathogens and salivary inflammatory cytokines except for *Tannerella forsythia*, which showed a significant increase in its counts with a p-value of 0.01. **Conclusion:** The AILCD used in this study was associated with significant improvements in clinical and anthropometric parameters. However, *Tannerella forsythia* showed a significant increase in women with obesity.

CLINICAL RELEVANCE

1. **Scientific Rationale:** Evidence on the association between AILCD and gingivitis was limited. The current study evaluated this relationship among women with obesity.

2. **Principal findings:** The AILCD was associated with improved clinical and anthropometric parameters. However, *Tannerella forsythia* showed a significant increase in women with obesity during this study duration.
3. **Practical implications:** The AILCD might lead to weight loss and improve gingival health; further research is indicated.

Keywords: anti-inflammatory low-carbohydrate diet; body mass index; gingivitis; obesity; periodontal pathogens

CDHA Research Agenda category: risk assessment and management

INTRODUCTION

Biofilm-induced gingivitis (BIG) is a site-specific inflammatory condition caused by dental biofilm deposition, characterized by gingival inflammation without attachment loss or alveolar bone loss [1, 2]. Epidemiological studies indicate that BIG is more common and severe in individuals who neglect oral hygiene [3]. The severity of periodontal diseases ranges from reversible gingivitis to more severe irreversible periodontitis, which is considered a risk factor for long-term tooth loss.[4] Moreover, periodontitis is associated with more serious systemic conditions such as diabetes [5] , and Cardiovascular disease. [6]

The literature provides substantial evidence that the accumulation of dental biofilm is the primary cause of gingivitis; however, its role in promoting gingival inflammation is mainly observed in Western diet environments. [1, 7] Dental biofilm composed of a complex community of bacteria that accumulate supragingival along the gingival margin and involves the initial colonization by gram-positive bacteria, such as *Streptococcus mutans* [8], a member of the "oral streptococci" group. *S. mutans* produces sticky, branched glucose polymers known as glucans, which play a critical role in the formation and adhesion of dental plaque by providing a matrix for bacterial attachment and biofilm development. In addition to glucans, other components such as proteins, lipids, and extracellular polysaccharides also contribute to plaque formation. When dysbiosis exceeds the immune system's capacity to control it, inflammation extends subgingivally, increasing the proportion of pathogenic gram-negative anaerobic bacteria and driving the progression of periodontitis. [9, 10]

The relationships between different bacterial species in dental plaque are complex. The presence, absence, and relative proportions of these species are influenced by various factors, including the host's diet [11]. Therefore, dietary recommendations can help manage gingival and periodontal inflammation [12-14], as well as systemic chronic diseases such as diabetes and cardiovascular disease (CVD)[13]. Omega-3 fatty acids in fatty fish reduce inflammation in both the gingiva and cardiovascular system[15]. Antioxidants, such as vitamins C and E from fruits and vegetables, improve gingival health by neutralizing free radicals[16]. Additionally, limiting processed sugars and refined carbohydrates helps regulate blood glucose and inflammation, benefiting both periodontal and systemic health [17].

The role of a highly refined carbohydrate diet in supragingival plaque accumulation and dental caries is well known [18]. Additionally, it has been reported that a reduction in carbohydrate intake reduces gingivitis despite constant plaque values [7, 19]. In a study lasting four weeks, an anti-inflammatory diet (AID) reduced the load of the oral Streptococci group, *Granulicatella adiacens, Actinomyces* spp., and *Fusobacterium* spp. in the supragingival plaque [20].

El Makaky et al. [21] studied the effects of a four-week AID, that was low in processed carbohydrates and high in vitamin C, antioxidants, omega-3 fatty acids, plant nitrates, vitamin D , and fibers on clinical and serological inflammatory markers in children with gingivitis. They concluded that an AID can significantly reduce serological markers and gingival inflammation in children. Woelber et al. [22] studied the influence of an AID on gingivitis in a randomized controlled trial. They observed that this diet could significantly reduce gingivitis in normal-weight individuals, although serological inflammatory markers and the subgingival microbiota appeared to be unaltered during the four-week course of the study. Moreover, Rajaram et al. [12] studied the effect of AID on gingival inflammatory parameters and found that this type of diet significantly reduces gingival inflammation in individuals with chronic gingivitis.

Furthermore, studies have revealed a correlation between obesity and the severity of periodontal disease, the majority of the meta-analyses performed revealed that individuals with obesity had significantly greater clinical parameters of gingival inflammation [23, 24]. Furthermore, an association has been identified between obesity and periodontal disease in women, with the connection becoming more pronounced as obesity levels rise [25, 26]. Interestingly, this pattern is not observed in men with elevated BMI. This difference may be attributed to the fact that, in men, a higher BMI often reflects greater muscle mass or bone density rather than body fat, additionally, the distribution of body fat varies between genders, so a higher BMI in men does not necessarily indicate obesity [23].

To the best of our knowledge, no studies in the literature have investigated the association between AILCD and gingivitis in women with obesity. Therefore, this study aimed to evaluate the association between AILCD and clinical parameters, periodontal pathogens, and salivary inflammatory cytokines in women with obesity and gingivitis using real-time polymerase chain reaction (RT-PCR) over a two-month duration, to investigate a dietary intervention as an adjunctive method for the resolution of gingivitis.

Study hypothesis

This study hypothesized that AILCD would reduce gingival inflammation, improve clinical and anthropometric parameters, and lead to positive changes in salivary inflammatory cytokines and periodontal pathogens in women with obesity and gingivitis after two months, compared to baseline.

MATERIALS AND METHODS

Study design

The Institutional Review Board (IRB) of Jordan University of Science and Technology (JUST) approved this non-controlled repeated measures clinical trial (Reference number: 196/2022). Additionally, the study was registered for funding under Grant number 20220426. It was conducted at the Preventive Dentistry Department (Periodontics Clinics) of Jordan University of Science and Technology, Irbid, Jordan, between October 2022 and July 2023. Participants were informed about the study's objectives and methodology, and written informed consent was obtained.

Sample selection

The selected women fulfilled the following criteria:

Inclusion criteria:

1. Generalized gingivitis (BOP ≥10%)(27).
2. Western diet conditions with a processed carbohydrate intake >45%(28).
3. Women with BMI ≥30kg/m^2.
4. Age 18-45 years.

Exclusion criteria:

1. Smoking.
2. Severe or life-threatening illnesses.
3. Intake of antibiotics within 3 months before the start of or during the study period.
4. Drugs influencing gingival inflammation or bleeding (e.g., anticoagulants, cortisone).

5. Carbohydrate- or insulin-related diseases (e.g., diabetes)
6. Pregnancy or breastfeeding.

Recruitment was based on a Google questionnaire, which included specific questions to determine whether potential participants met the inclusion criteria, along with advertisements at Jordan University of Science and Technology, the JUST Dental Center, and a private nutrition center.

Dietary Intervention

All selected participants who met the inclusion criteria underwent testing for vitamin D and C levels, and then they were instructed to follow an AILCD under the supervision of the same dietitian for two months, with in-person follow-up visits every ten days. During these visits, participants engaged in a one-hour discussion with the dietitian to evaluate their progress, adherence, and any challenges faced during the previous period. Compliance was monitored using written dietary checklists. Frequent follow-up visits were designed to ensure participant's adherence to the diet and provide periodic motivation. Participants were instructed to maintain their routine oral hygiene methods and avoid changes to their physical activity during the observation period.

The dietary recommendations were largely based on those of a study by Woelber et al. [19], with some adjustments that omega 3 was not taken as a supplement, it was obtained through the diet. The diet was a 1500-calorie program with 20% carbohydrates (75g/d), 25% protein (94g/d), and 55% fat (92g/d). Participants had three main meals per day without snacks.
The following components were included in the dietary intervention:

Macronutrients

• Omission of processed carbohydrates like sugar, honey, white flour, white rice, or fruit juices. Reduction of the intake of starches as far as possible to 75g/day [28].
There were no restrictions regarding fruits and vegetables (polysaccharides).
• Intake of omega-3 fatty acids (salmon fish, walnuts, and beans), a restriction in the amount of trans-fatty acids as far as possible (such as fried meals, crisps, donuts, croissants, etc.) and a

reduction in omega-6 fatty acids as far as possible (such as safflower oil, grape seed oil, sunflower oil, margarine, sesame oil, and corn oil).

• Reduction of processed animal proteins (like sausages and cold cuts) as far as possible and favoring of plant proteins (like legumes, nuts, etc.).

Micronutrients

• Daily intake of a source of vitamin C (like two kiwis, one orange, one bell pepper, etc.)

• Daily intake of a source of vitamin D either by exposing the body to 15 min unprotected in the sun or supplementation with 1,000 international units (25µg). In the case of lower baseline serological values, (≤30 ng/ml) individually higher values were administered.

• Daily intake of antioxidants (such as a handful of berries, a cup of green tea, coffee without milk, one pinch of Curcuma and/or ginger).

• Daily intake of fiber (vegetables, fruits, legumes, and whole grains).

• Daily intake of nitrate-containing plants (such as a portion of spinach, beetroot, or rocket).

Sample collection

Samples were collected at baseline, after one month, and after two months follow-up visits.

***For immunological assessment*:** 4ml of unstimulated whole saliva (UWS) was collected before mouthwash samples and periodontal examination by using the resting drooling method. Participants were instructed to sit comfortably in an upright position, with their heads slightly down to pool saliva in their mouths, and to allow saliva to fall into 5.0ml sterile cryotubes [(29)].

The investigation of inflammatory cytokines IL6, IL10, and TNF-α was done using commercially available Enzyme-linked Immunosorbent Assay (ELISA) kits.

***For microbiological assessment*:** mouthwash samples were collected after plaque index recording and before the assessment of probing depth and bleeding on probing. The samples were collected by rinsing with 10 ml sterile saline (0.9% w/v Sodium Chloride, KSA) for 30 seconds and spitting the same amount in 15ml sterilized conical tubes (VACUETTE, Austria)[(30)].

Real-time PCR was used to quantify the following bacteria (*S. mutans, A. actinomycetemcomitans, T. forsythia, P. gingivalis,* and *F. nucleatum*).

After collection, both samples were immediately kept in an icebox and then stored in a -80 ℃ (Electrolux ML 402, Sweden freezer) until analysis.

Follow-up visits

After the baseline clinical examination, sample collection, and anthropometric assessment (T_0), patients underwent the same procedures at the one-month (T_1) and two-month (T_2) follow-up visits, as shown in (Figure 2).

Methods of Assessment

***Anthropometric assessment*:** Participants underwent anthropometric analysis using the InBody-770 scale (InBody Co., Ltd., South Korea). Body composition analysis determined weight, body mass index (BMI), skeletal muscle mass (SMM), and percent body fat (PBF).

Clinical examination: The first clinical periodontal parameter recorded was the plaque index, according to Silness and Löe[31]. Next, the gingival index was measured following the method of Löe and Silness. [32] Bleeding on probing (BOP) was then assessed according to Ainamo and Bay[33], with all six surfaces of each tooth evaluated to determine whether probing elicited bleeding (+) or not (−) within ten seconds, the severity of BOP was expressed as a percentage. Finally, probing pocket depth (PPD) was measured using standardized periodontal probes (UNC 15, MEDENSY, Italy) at six sites per tooth across the entire dentition (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual). One dentist who was calibrated by an expert periodontist conducted the clinical examination for all study participants.

***Microbiological assessment*:** after receiving the samples in the Research Labs at JUST, they were transferred and stored in a deep freezer at -70 °C before they were analyzed using a real-time polymerase chain reaction (RT-PCR) machine. Immediately before analysis, samples were removed from the deep freezer and DNA in each mouthwash sample was extracted using the DNeasy® PowerSoil® Pro Kit (Qiagen, Hilden, Germany). The concentration and purity of DNA

were evaluated using a BiotekPowerWave XS2 Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA).

***DNA extraction*:** The DNeasy kit was used according to the manufacturer's instructions (DNeasy Kit Handbook), allowing rapid and efficient purification of total cellular DNA.

***DNA quantification using RT-PCR technique*:** for positive control, the plasmid cloning vector (pUC 19) of known DNA concentration was used and the PCR target sequence was inserted into it. The plasmid was diluted in a 7-time serial dilution. Each DNA sample was tested in triplicate, one negative control and two positive controls of bacterial dilution 10^{-5} ng/µl and 10^{-6} ng/µl were used for the RT-PCR reaction [34]. The copy number of a plasmid DNA template was determined by using the equation of the following website http://cels.uri.edu/gsc/cndna.html, for DNA of *Streptococcus mutans, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis, Fusobacterium nucleatum*.

The machine used for RT-PCR was the Rotor-Gene Q MDx 5 plex system (Qiagen, Hilden, Germany), and the BlasTaq™ 2X qPCR MasterMix Kit (Applied Biological Materials Inc. abm, Canada, Cat. No. G891) was used to detect amplified DNA according to manufacturer instructions.
For the RT- PCR reaction, a mixture of the following components was prepared: 10 µl of BlasTaq™2XqPCR MasterMix, 0.5 µl of Forward Primer (10 µM), 0.5 µl of Reverse Primer (10 µM), and 7 µl of Nuclease-free H2O. An 18µl of this mixture was placed in a 1.5ml strip tube and a 2µl of the extracted DNA was added, finally 20µl of the reaction mix was obtained. The SYBR Green dye source wavelength was 470 nm and the detection wavelength was 510 nm. Each run in the RT-PCR machine took approximately one hundred minutes and was composed of one cycle for enzyme activation under 95°C for 3min, 40 cycles for denaturation, and annealing/ extension under 95°C, 60°C for 15 sec, 1min, respectively. Melting curve obtained under 55- 59°C to confirm the specificity of primers to the amplified target DNA sequence.

The forward and reverse primers were used for all bacterial species [35-39] as shown in (Table 1).

***Immunological assessment*:** Unstimulated whole saliva (UWS) collected at baseline, after one month, and at the end of the study after two months was examined using Enzyme-linked Immunosorbent Assay (ELISA) to detect the levels of inflammatory cytokines (IL6, TNF-α, and IL10). The kits used by ELK Biotechnology CO., Ltd, China were (Human IL6 ELISA Kit Cat: ELK1156, Human TNF-α ELISA Kit Cat: ELK1190, and Human IL10 ELISA Kit Cat: ELK1142). ELISA analysis was performed according to manufacturer instructions for each inflammatory cytokine. The wavelength for ELISA was 450 nm. Samples were analyzed on the Biotek PowerWave XS2 Spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA).

Statistical analysis

Data were described using means and standard deviations for normally distributed variables. Changes in parameters over time were compared across the three timelines using repeated measures ANOVA. Data analysis was performed using SPSS version 25 (IBM Corporation, USA), and a p-value ≤ 0.05 was considered statistically significant.

RESULTS

Seventy potential participants were assessed for eligibility. Thirteen refused to participate, and twenty-three did not meet the inclusion criteria. Thirty-four participants were initially included in the study; however, eleven were excluded: two took antibiotics, seven did not comply with sample collection, and two missed follow-up visits with the dietitian (Figure 1). Twenty-three participants completed the study and were included in the analysis, with a follow-up period of two months. The mean age of the participants was 25.7 ± 6.8 years, with 82.6% holding a bachelor's degree and 17.4% a master's degree. Demographic data are shown in Table 2.

Anthropometric outcomes

The anthropometric parameters results were available for 23 patients at three timelines. After two months, significant reductions were observed in the mean differences of all anthropometric parameters, including weight, BMI, SMM, and PBF. The reductions were -3.8 kg, -1.3 kg/m², -0.5 kg, and -1.6 %, respectively, as shown in Table 3.

Clinical outcomes

After two months, significant reductions were observed in the mean differences of all clinical periodontal parameters, including BOP, GI, PI, and PPD, with a p-value of 0.01, as shown in Table 4.

Microbiological outcomes

The microbiological results were available for all samples at three timelines for the five bacterial species. According to the mean bacterial counts at the three timelines, the highest count was for *A. actinomycetemcomitans*, while the lowest was for *P. gingivalis*. No significant mean differences for all bacteria were observed after two months except for *T. forsythia,* which showed a significant increase in the mean differences with a p-value of 0.01, as shown in Table 5.

Immunological outcomes

The results of the ELISA analysis were available for 23 patients at three timelines. No significant differences were detected in the mean values of all salivary inflammatory cytokines IL-6, IL-10, and TNFα after two months, as shown in Table 6.

DISCUSSION

The influence of diet on the oral microbial ecosystem and its correlation with oral diseases, such as caries and periodontal disease, has been under focus in the last two decades. Few studies have investigated the influence of AID on gingivitis in individuals with normal weight. [12, 19-22]. To the best of our knowledge, there is no research published about the association between AILCD and gingivitis in women with obesity. The key findings of this study were the significant reductions in clinical and anthropometric parameters; however, there was a significant increase in *T. forsythia* counts after two months.

In terms of demographics, this study included twenty-three female participants, as an association between obesity and periodontal disease has been identified in females, with the link becoming more pronounced as obesity levels increase[25, 40]. Interestingly, this pattern does not appear in males with higher BMI. This discrepancy may be because, in men, a higher BMI often reflects

increased muscle mass or bone density rather than excess body fat. Additionally, fat distribution differs between genders, so a higher BMI in men does not necessarily indicate obesity[23].

Regarding anthropometric parameters, the consistent reduction in anthropometric parameters emphasized the beneficial effect of AILCD in weight loss and obesity management. In agreement with the current study findings, Woelber et al. [22] observed significant weight loss in the test group, attributed to a lower total energy intake from the AID. A recent study showed that a low-carbohydrate diet affects body composition by reducing weight and body fat, and when coupled with physical activity, it helps preserve muscle mass[41]. The significant loss of SMM in this study can be attributed to the 1500-calorie diet with 20% carbohydrates (75g/day) and 25% protein (94g/day), which induces caloric restriction. In this case, the body breaks down muscle for energy, leading to further muscle protein breakdown. However, incorporating resistance exercise with the diet helps preserve muscle mass while promoting fat loss.

Regarding the clinical findings of the current study, the key assumption why the participants showed a reduction in the severity of gingival inflammatory parameters is likely the restriction of sugar intake and the consumption of an anti-inflammatory dietary regimen. The baseline findings support the hypothesis that a modern Western diet, high in refined carbohydrates and with an elevated Omega-6 to Omega-3 fatty acid ratio, contributes to increased inflammatory processes[42].

The reduction in PI can also be attributed to the Hawthorne effect because all participants were aware that they would be examined after brushing their teeth; they may have brushed their teeth more meticulously than usual [43], there is also a hypothesis that a reduction in inflammation leads to a decrease in plaque formation[3]. The findings of the current study are in agreement with clinical trial findings where there was a significant reduction in gingival inflammation parameters (PI, GI, BOP, and PPD) even in the absence of interdental hygiene. [22] On the other hand, Baumgartner et al. [11] found that the association of plaque and gingival inflammation might only be valid under Western diet conditions. As PI increased, BOP and PD decreased significantly in this study. Similar results were observed by El Makaky et al. [21] in which gingival inflammation and BOP were significantly reduced, but there was an increase in PI in the test and control groups. In a controlled clinical trial, Woelber et al. [19] observed that a diet low in carbohydrates, high in Omega-3 fatty acids, high in vitamins C and D, and high in fibers could significantly reduce

gingival and periodontal inflammation. Despite constant plaque values in both groups, GI, BOP, and PPD were significantly reduced to roughly half of the baseline values in the experimental group. In a study by Rajaram et al. [12] significant reductions were observed in the test group compared with the control group in GI and BOP, However, no significant differences were observed in the PI in the test group compared with the control group.

The findings of the previously mentioned studies align with the current study on clinical periodontal parameters (GI, BOP, and PPD); however, there were differences in the PI findings, which supports the crucial debate about the role of plaque as an etiological factor for gingivitis under various dietary conditions, emphasizes its contribution to gingival inflammation in Western diet conditions, and highlights the importance of host modulation. [7, 11, 43, 44]

Regarding the microbial counts of *S. mutans* and *A. actinomycetemcomitans*, the current study's findings contrast with those of Baumgartner et al. [11] in which *Streptococcus* species and *A. actinomycetemcomitans* counts in tongue samples had significant reduction after four weeks of the Stone Age diet compared to the baseline. Regarding *F. nucleatum,* the findings of the current study align with those of Woelber et al. [22] where there were no significant changes in the counts of *F. nucleatum* after four weeks of AID. In contrast, Tennert et al. [20] observed a significant reduction in the total count of *Fusobacterium* spp. in plaque samples from a healthy diet group. Conversely, Baumgartner et al. [11] found a significant increase in the total count of *F.nucleatum* from subgingival samples after four weeks of Stone Age diet.

Regarding *T. forsythia* counts in the current study, there was a significant increase in its counts after two months. This may be related to the BMI (31.9 kg/m2 at baseline) of the subjects; these women with obesity may have experienced an overgrowth of *T. forsythia*, due to obesity-related dysbiosis in periodontal pathogens. Therefore, preventive measures of longer dietary interventions, physical activity, and periodontal care are recommended to alleviate the complications of obesity-related periodontal dysbiosis. Woelber et al. [22] had contradictory results, finding no significant differences in the total counts of subgingival *T. forsythia* after four weeks of AID. On the other hand, Baumgartner et al. [11] observed a significant reduction in the total counts of *T. forsythia* from tongue samples in all subjects after four weeks of the Stone Age diet.

In the current study, there were no significant differences in the *P. gingivalis* counts after two months. Likewise, Woelber et al. [22] found no significant differences in the total count of *P. gingivalis* in subjects with gingivitis after four weeks of AID.

The difference in results regarding periodontal pathogens count may be due to heterogeneity between studies in terms of the subject's BMI, adherence to the diet, oral hygiene measures, gingival immunological reactions, and physical activity.

Despite the clinical reduction in the periodontal parameters, no significant changes in the periodontal pathogens were observed, except for *T. forsythia*. These findings require further discussion as they relate to the etiological significance of the dental biofilm. First, it is possible that the clinical outcomes were generated by an altered immunological reaction in the periodontal tissues rather than an altered microbial composition of the dental biofilm. Second, there could have been direct effects on the periodontal microbiota that DNA sequencing did not detect. The intervention may not have affected the counts of bacteria but rather their metabolic activity [45].

Regarding the immunological findings of the salivary cytokines IL-6, IL-10, and TNFα, there were no significant differences in all cytokines after two months. A possible explanation for this is that obese individuals have elevated levels of salivary IL-6 and TNFα, which are used as an alternative method to serum TNFα in diagnosing obesity. Additionally, many of the participants were young adults (25.7±6.8 years) and the salivary cytokine concentration declined with age. This correlation may be due to different age-related changes, such as a decrease in total salivary protein concentration and structural and histological variations in salivary glands, such as acinar cell atrophy and replacement of normal parenchyma with fibrosis. Third, the sampling technique by collecting unstimulated whole saliva (UWS) may affect the concentration of salivary cytokines. The UWS has a lower salivary flow rate and a higher possibility of sample blood contamination compared with stimulated whole saliva. These factors could alter the salivary cytokine concentration in UWS samples.

The current study's findings align with those of Woelber et al.[22], Who showed no significant changes in serum IL-6 and TNFα after four weeks of AID. Moreover, Ruth et al. [46] observed that serum TNFα level did not differ after twelve weeks of a dietary intervention with a high-fat, low-carbohydrate diet.

In contrast, El Makaky et al. [21] found a significant reduction in salivary TNFα and IL-6 levels within a test group after four weeks of AID in children with gingivitis. These contradictory results may be due to differences in the sampling techniques and the kits used for the assessments in the studies.

Although clinical periodontal parameters were reduced during the study, periodontal pathogens and inflammatory cytokines did not change significantly in these women with obesity. More time may be required to achieve greater weight loss and, subsequently, a reduction in periodontal pathogens.

The main limitation of the current study is the absence of a control group, a relatively small sample size, and a short follow-up period.

Future studies are recommended to conduct long-term follow-up RCTs with larger sample sizes, non-obese periodontitis patients, and a broader range of bacterial species. Furthermore, developing a protocol to ensure participant adherence to the diet, such as a food delivery service for diet standardization, is advisable.

CONCLUSION

The AILCD used in this study was associated with significant improvements in clinical periodontal and anthropometric parameters. However, *Tannerella forsythia* showed a significant increase in women with obesity.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest

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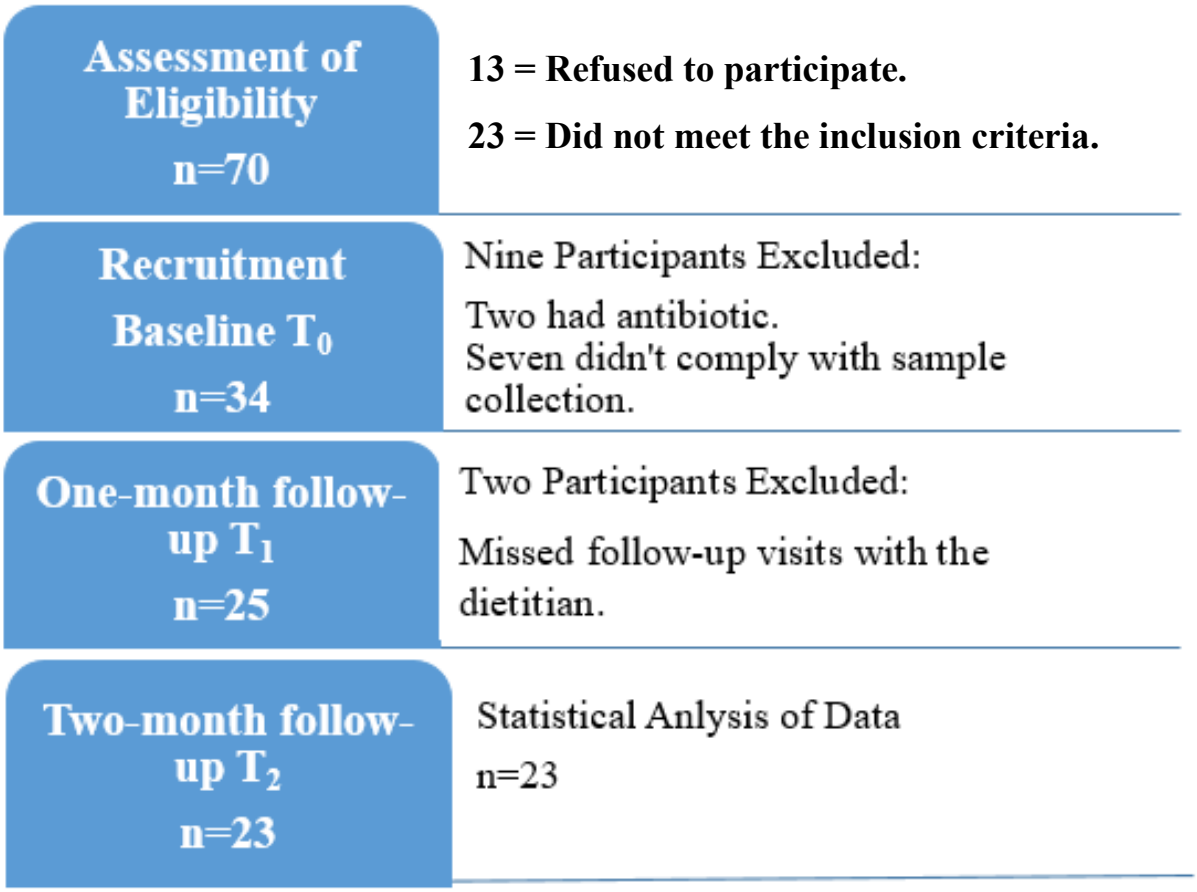
Figure 1: Flowchart of participants



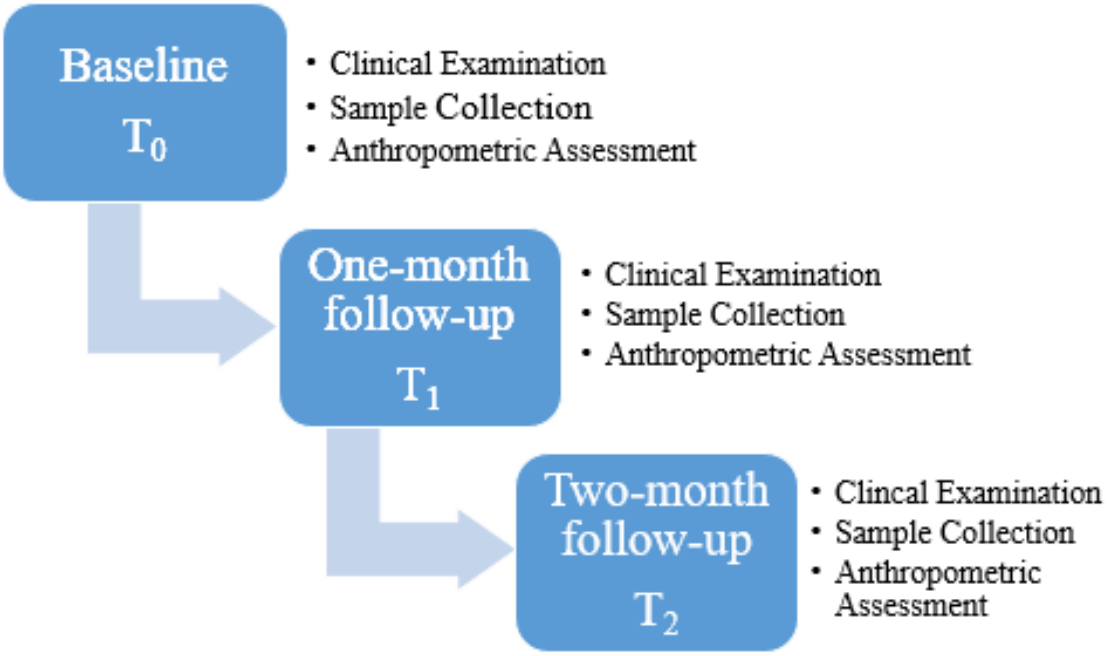


Figure 2: Follow-up visits

Table 1. Forward and reverse RT-PCR primer sequences for the examined bacterial species.

Bacteria	Forward Sequence	Reverse Sequence
S. mutans [27]	GCCTACAGCTCAGAGATGCTATTCT	GCCATACACCACTCATGAATTGA
A. actinomycetemcomitans [28]	CTTACCTACTCTTGACATCCGAA	ATGCAGCACCTGTCTCAAAGC
T. forsythia [29]	GGGTGAGTAACGCGTATGTAACCT	ACCCATCCGCAACCAATAAA
P. gingivalis [30]	TGGTTTCATGCAGCTTCTTT	TCGGCACCTTCGTAATTCTT
F. nucleatum [31]	AAGCGCGTCTAGGTGGTTATGT	TGTAGTTCCGCTTACCTCTCCAG

Table 2. Demographic data

Total number	Age (year)		Educational level %	
	Mean± SD	Range	Bachelor's degree	Master's degree
23	25.7± 6.8	18-45	82.6%	17.4%

Values are expressed as means and standard deviations.

Table 3. Anthropometric parameters at different timelines.

	T_0 (N=23)		T_1 (N= 23)		T_2 (N=23)		Net	P-
Variables	Mean	SD	Mean	SD	Mean	SD	Difference	value
Weight (Kg)	84.6	19.4	81.8	17.7	80.8	17.3	-3.8	0.01
BMI (kg/m^2)	31.9	6.8	30.9	6.3	30.6	6.1	-1.3	0.01
SMM (Kg)	25.6	5.5	25.4	5.2	25.1	5.1	-0.5	0.02
PBF (%)	43.9	7.1	42.6	7.3	42.3	7.4	-1.60	0.01

Values are expressed as means and standard deviations. A p-value ≤ 0.05 is considered statistically significant. BMI: Body mass index, SMM: Skeletal muscle mass, PBF: Percent body fat.

Table 4. Clinical parameters at different timelines.

Clinical Parameters	T_0 (N=23)		T_1 (N=23)		T_2 (N=23)		
	Mean	SD	Mean	SD	Mean	SD	P-value
BOP%	37	0.16	24	0.12	19	0.08	0.01
GI	0.77	0.32	0.47	0.24	0.38	0.17	0.01
PI	1.14	0.48	0.69	0.57	0.54	0.38	0.01
PPD	1.83	0.22	1.53	0.29	1.35	0.3	0.01

Values are expressed as means and standard deviations. A p-value ≤ 0.05 is considered statistically significant. BOP: Bleeding on probing, GI: Gingival index, PI: Plaque index, PPD: Periodontal probing depth.

Table 5. Average copy number of bacteria: copy number /µl at different timelines.

Bacteria	T_0 (N=23)		T_1 (N= 23)		T_2 (N=23)		
	Mean	SD	Mean	SD	Mean	SD	P-value
S. mutans	10.526	10.292	10.449	10.249	10.484	10.415	0.42
A.actinomycetemcomitans	12.747	12.273	12.719	12.181	12.744	12.208	0.36
F. nucleatum	12.456	11.811	12.479	11.774	12.479	11.786	0.34
T. forsythia	9.566	9.147	9.717	9.456	9.622	9.246	0.01
P. gingivalis	9.274	9.235	9.279	9.184	9.365	9.358	0.42

Values are expressed as means and standard deviations. A p-value ≤ 0.05 is considered statistically significant.

Table 6. Inflammatory cytokines at different timelines.

Cytokines	T_0 (N=23)		T_1 (N= 23)		T_2 (N=23)		
	Mean pg/ml	SD	Mean pg/ml	SD	Mean pg/ml	SD	P- value
IL-6	110.6	36.2	101	34.4	102.5	18.8	0.54
IL-10	63.7	15	78.9	38	62.5	22.8	0.13
TNFα	125.1	27.5	136.7	32.8	125.7	29	0.33

Values are expressed as means and standard deviations. IL-6: Interleukin-6, IL-10: Interleukin-10, TNFα: Tumor necrosis factor α.