Adherence to Mediterranean Diet, Physical Activity level and severity of periodontitis. Results from a University-based cross-sectional study.

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Abstract

Obiettivo: valutare l'associazione tra l'aderenza a dieta mediterranea (MD) e il livello di attività fisica (PA) con lo stato di salute parodontale.

Materiali e Metodi: 235 partecipanti sono stati inclusi nello studio. L'aderenza a MD e il livello di PA sono stati registrati tramite questionari validati; è stato poi eseguito un periodontogramma completo. I rapporti di probabilità (ORs) crudi e aggiustati sono stati calcolati per valutare l'associazione tra aderenza a MD, livello di PA e severità di parodontite. L'accuratezza diagnostica (Area Sotto la Curva, AUC) e la capacità predittiva per la parodontite severa/avanzata della combinazione tra MD e PA sono stati calcolati.

Risultati: Gli ORs aggiustati per la parodontite severa/avanzata sono di 1.65[0.84-3.28;*p*=.42] per la bassa PA e 5.63[3.21-9.84;*p*=.00] per la bassa MD. L'accuratezza diagnostica della loro combinazione è AUC=0.6873. Il modello di regressione è risultato in un OR=8.80[3.39-34.90;*p*=.00] di avere parodontite severa/avanzata in soggetti con bassa MD e bassa PA.

Conclusioni: MD e PA sono in grado di diagnosticare correttamente circa il 70% dei casi di parodontite severa/avanzata. Inoltre, I soggetti che seguono uno stile di vita caratterizzato da bassa aderenza a MD e scarsa PA hanno una probabilità 9 volte maggiore di avere forme severe di parodontite.

Abstract

Aim: to evaluate the association between adherence to Mediterranean Diet (MD) and Physical Activity (PA) level with the periodontal status of a University-based cohort of individuals.

Materials and Methods: 235 individuals were screened for inclusion in the study. MD adherence and PA level were registered through validated questionnaires, together with a full periodontal examination. Crude and adjusted Odds ratios (ORs) [95% Confidence Interval] were calculated to evaluate the association between MD adherence, PA level and periodontitis severity. The diagnostic accuracy (Area under the Curve, AUC) and predictive ability of the combination between low MD adherence and low PA level for severe/advanced periodontitis were then computed.

Results: The adjusted ORs for severe/advanced periodontitis were 1.65[0.84-3.28;*p*=.42] for low PA and 5.63[3.21-9.84;*p*=.00] for low MD adherence. Their combined diagnostic accuracy was AUC=0.6873. The final predictive regression model resulted in OR=8.80[3.39,34.90;*p*=.00] of having severe/advanced periodontitis in individuals with low MD adherence and low PA.

Conclusions: MD adherence and PA level were able to correctly diagnose around 70% of severe/advanced periodontitis cases. Moreover, individuals conducing a lifestyle characterized by the combination of low MD adherence and lack of regular exercise were 9 times more likely to have severe forms of periodontitis.

Introduction

Periodontitis is defined as a biofilm-mediated Non-Communicable chronic inflammatory Disease (NCD) characterized by the progressive destruction of the tooth supporting apparatus. It manifests itself through clinical attachment loss (AL), presence of periodontal pocketing, gingival recession and radiographically assessed bone loss. Periodontitis is the most common chronic inflammatory NCD, with a prevalence of its severe form ranging between 7-11%; it represents the sixth most prevalent condition worldwide (Kassebaum et al., 2017). Furthermore, periodontal infections are associated with a range of systemic diseases, including diabetes (Daudt et al., 2018), cardiovascular disease (CVD) (Kitamura et al., 2020) and adverse pregnancy outcomes (Manrique-Corredor et al., 2019). Several environmental factors, such as overweight, smoking, unhealthy diet and physical inactivity (Reynolds, 2014), are associated with disease occurrence and are shared as risk indicators with other prevalent NCDs, such as type-2 diabetes mellitus (T2DM) and CVD.

Indeed, periodontitis exerts a detrimental impact on both masticatory function and general health, thus resulting in higher dental healthcare costs (Tonetti et al., 2017). Consequently, a lot of research focused on the treatment of NCDs through a multifactorial approach targeted at improving various aspects of the patient's lifestyle, such as smoking, diet or physical activity (PA) (Iwasaki et al., 2018). In particular, the pioneer Seven Countries study in the 1950s was the first one to highlight the benefic effects of the Mediterranean Diet (MD) (Verschuren et al., 1995); since then, epidemiological evidence flourished regarding the ability of MD to significantly reduce the risk of developing NCDs such as metabolic syndrome, T2DM, CVD and cancers (Benetou et al., 2008; Sofi et al., 2008). The latest consensus on the Mediterranean pyramid (Bach-Faig et al., 2011) encompassed not only the consumption and serving size of

specific food groups, but also other lifestyle dimensions (such as regular exercise and adequate rest) which, collectively, were framed in the "Mediterranean lifestyle" (ML) (Bach-Faig et al., 2011). While on one hand a lot of studies focused on the effects of each single item of the ML (*e.g.* PA or nutrition) (Diolintzi et al., 2019; Ferreira et al., 2019; Iwasaki et al., 2021), few data are present regarding the impact of the combination of ML components or ML as a whole on oral health. In particular, while there is accumulating evidence regarding the anti-inflammatory potential of MD, on the other hand no evidence is present regarding the synergistic/antagonistic impact of adherence to both MD and PA on periodontal health. The hypothesis that we would like to figure out with this current cross-sectional design is that low MD adherence (characterized by the high consumption of saturated fatty acids and low fibers intake) combined with a sedentary behaviour would increase low-grade systemic inflammation, lipid levels and oxidative stress, as well as decrease insulin sensitivity (Beauchamp et al., 2005; Estruch et al., 2006; Tosti et al., 2018); as such, they would contribute to a more severe periodontitis phenotype. Therefore, the aim of the present cross-sectional study was to evaluate the association between MD adherence and PA level with the biometric and inflammatory periodontal parameters of a University-based cohort of individuals.

Materials and methods

Study design

The present study is reported according to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines for cross-sectional studies (von Elm et al., 2008). The research protocol was approved by the local ethics committee (protocol number: 18993/2021) and received the registration number on Clinicaltrials.gov (NCT04771949).

Setting and participants

Patients were recruited between January 2021 and April 2021 in the Unit of Periodontology at the University of Siena; the inclusion criteria were:
- age between 18 and 70 years old;
- ability and willingness to give informed consent.
The exclusion criteria were:
- pregnancy or lactation;
-periodontal therapy performed in the last 12 months
- inability to communicate effectively in Italian.
Individuals were included in the study after they read and signed the written informed consent, in accordance with the Declaration of Helsinki.

Variables

Socio-demographic characteristics

Information regarding patients' age, gender, smoking and oral hygiene habits, occupation and education level was registered. Moreover, data regarding the presence of familiarity as well as any comorbidity affecting susceptibility to periodontitis were recorded. The Body Mass Index (BMI) was computed as weight (kilograms)/height (meters^2).

Dietary assessment

A validated 15-item questionnaire to measure patients' adherence to MD (QueMD) was administered by two examiners (C.M.,J.T.) (Gnagnarella et al., 2018). The questionnaire included questions regarding the foods most frequently associated with MD (wholegrain pasta, bread or substitutes; raw or cooked vegetables; all types of fresh fruits; dairies; either red or white wine; olive oil; red meat; fish; dried fruits and pulses), as well as other commonly consumed items (white meat, carbonated beverages or soft drinks; butter, cooking cream or margarine; manufactured sweets or pastries) (Table 1). For each component, a standard portion for the Italian population was indicated (SINU, 2019) and participants could choose among 5 consumption frequencies, which differed according to food items. The alternate MD score (aMed) was drawn from the QueMD results in order to evaluate patients' adherence to MD (Gnagnarella et al., 2018); it was calculated by assigning 1 point to participants reporting food consumptions above the Italian National levels (National Research Institute for Food and Nutrition, 2003) for each of the following items typical of the MD: wholegrain products (≥1/day), vegetables (≥2/day), fresh fruits (≥2/day), olive oil (≥3/day), wine (1-2 glasses/day for males or 1 glass/day for females), red meat (≤1-3/week), fish (≥2/week), dried fruits (≥2/week), pulses (≥2/day)(Bach-Faig et al., 2011). The sum score, which ranged between 0 (minimum MD adherence) and 9 (maximum MD adherence), was dichotomized in order to define cases of low (aMed<5) and high (aMed>4) adherence to MD.

Physical activity assessment

PA was assessed through the validated short version of the International Physical Activity Questionnaire (IPAQ) (Mannocci et al., 2010). It consists of 7 items regarding the frequency and amount of time spent doing intense and moderate PA, as well as walking or doing sedentary activities during the last 7 days (Table 1). The overall PA level was classified as low, moderate or high through the IPAQ automatic report (https://sites.google.com/site/theipaq/home).

Periodontal examination

All participants received a full periodontal chart by two trained and calibrated examiners (C.M.,J.T.) (unweighted *kappa* score of 0.93). Periodontal Probing Depth (PD), gingival Recession (REC), plaque (O'Leary et al., 1972) and Bleeding on Probing (BoP) (Ainamo & Bay, 1975) were recorded with a standardized periodontal probe (UNC 15 probe, HuFriedyGroup) six sites per tooth, for a total of up to 192 sites. The presence of furcation involvement was recorded according to the classification of Hamp (Hamp et al., 1975); the classification of Miller (Miller, 1950) was used to record tooth mobility.
A periodontitis case was defined whenever interdental AL was detectable at ≥2 non-adjacent teeth, or whenever buccal or oral AL≥3mm with pocketing (PD>3mm) was detectable at ≥2 teeth (Tonetti et al., 2018). The severity, complexity, and rate of disease progression were identified through the Staging and Grading of periodontitis (Papapanou et al., 2018).

Statistical analysis

Sample size calculation

Sample size was calculated considering the prevalence of periodontitis in the reference cohort at 37.3% (Jiao et al., 2020) and its value in the study cohort as 10% higher. Considering α=0.05

and β=0.80, the computed sample size was of 185 subjects. Given a non-response rate of 20%, the inclusion of 235 participants was planned.

Descriptive and inferential statistics

Statistical analysis was performed through an *ad hoc* software (STATA BE, version 17) setting the level of significance at α=0.05. Continuous variables were reported as Mean with 95% Confidence Interval; binomial and categorical data were expressed as number of observations (proportion). After verification of data distribution, Kruskal-Wallis and Fisher's exact tests were used to compare patients' characteristics according to oral health status, MD adherence, PA level and their possible combination (low aMed and low/moderate PA, low aMed and high PA, high aMed and low/moderate PA, and high aMed and high PA).

Diagnostic accuracy

Diagnostic accuracy was tested to investigate the diagnostic ability of the combination between MD adherence and PA level for severe/advanced periodontitis (Stage III/IV). Optimal cut-off points for both tests (QueMD and IPAQ) were computed based on the Receiver operating characteristics (ROC) curve. A new binary variable, MD-PA, was created by merging data regarding MD and PA. MD-PA took the value of 1 whenever both MD and PA results were below their optimal cut-offs; it took the value of 0 whenever at least one of them was above the threshold. The ability of MD-PA to detect severe/advanced periodontitis was measured by calculating the Area Under the ROC curve (AUC), as well as its sensitivity (Se), specificity (Sp) and Positive Likelihood Ratio (LR+).

Logistic regression models

Univariate and multivariate logistic regression analyses were performed to compute the association between severe/advanced periodontitis according to PA level, aMed and each component of the aMed score; it was expressed as crude and adjusted odds ratios (ORs). A multivariate logistic regression model was then built to evaluate the predictive ability of the combination between MD adherence and PA level for severe/advanced periodontitis cases. The best model was chosen according to the highest value of AUC, and the lowest values of Akaike (AIC) and Bayesian (BIC) information criteria. The predictors included in the final model encompassed: i) age; ii) BMI; iii) smoking; iv) familiarity for periodontitis; v) presence comorbidities.

Results

Participant characteristics

A total of 235 participants were included in the present study. All individuals examined for eligibility accepted to participate, were enrolled in the study and then included in the analysis. Subjects' characteristics overall and by periodontal status are reported in Table 2. The mean age was 53.90[52.01,55.79] years, with a proportion of 57.87% females and 25.96% smokers; the mean BMI was 25.49[24.88,26.11]. Around 20% of subjects were affected by at least one comorbidity. Moreover, half the participants were affected by severe/advanced periodontitis; significant differences as to age, occupation, education and familiarity for periodontitis were

found across subgroups of periodontitis severity (Table 2). No significant differences were reported as to domiciliary oral hygiene procedures.

Outcome data

Adherence to Mediterranean Diet and periodontitis

High adherence to MD was significantly associated to a lower prevalence of severe/advanced periodontitis (29.66%) compared to those with low adherence (70.34%) (Table 3). Other biometric periodontal variables (*i.e.* %PD>4mm, %PD 5-6mm, furcation involvement, tooth mobility, number of bleeding pockets, teeth lost for periodontal causes) were reported to be significantly worse in individuals with low MD adherence (Table 3). High aMed scores as well as the frequent consumption of specific MD foods resulted to be protective against severe/advanced periodontitis, even after adjusting for age, BMI, gender, smoking and brushing frequency (Table 3).

Physical activity and periodontitis

Around 1/3 of participants reached a high PA level, while the other 2/3 were in the low/moderate PA level category (Table 3). A high PA level was significantly associated with a lower prevalence of severe/advanced periodontitis (22.03%) when compared to low/moderate PA (77.97%). Moreover, a low/moderate PA level increased the odds of having severe/advanced periodontitis, but not after adjustments (OR=1.65[0.84, 3.28], p=0.42) (Table 4).

Combining adherence to Mediterranean Diet and physical activity

Around 40% of participants belonged to the "low aMed, low/moderate PA" subgroup, while fewer subjects belonged to the other three categories (Table 5). The prevalence of severe/advanced periodontitis was significantly higher in the subgroups "low aMed, low/moderate PA" (73.68%) and "low aMed, high PA" (59.09%), when compared to the "high aMed, low/moderate PA" (30.14%) and the "high aMed, high PA" (28.89%); periodontal status was found to be significantly worse in the two former compared to the two latter categories (p=.04). The proportion of subjects who reported having lost teeth for periodontitis almost doubled when shifting from the "high aMed, high PA" (24.44%) to the "low aMed, low/moderate PA" (43.16%) subgroup (p=0.04). Subgroups "low aMed, low/moderate PA" and "low aMed, high PA" resulted in significantly positive adjusted (Table 4) ORs for severe/advanced periodontitis.

Diagnostic accuracy

The optimal cut-off points based on ROC curve for aMed (Figure 1A) and IPAQ (Figure 1B) were 5 and 2, respectively. The area under ROC curve of MD-PA for severe/advanced periodontitis was 0.6873[0.6292,0.7452], with a Se of 0.7368[0.6403,0.8149] and a Sp of 0.6525[0.5708,0.7261] (Figure 1C). The LR+ was 2.120[2.002,2.739].

Predictive multivariate logistic regression model

Results of the final model are shown in Table 6. The presence of low MD adherence, irrespective of PA level, increased the odds for severe/advanced periodontitis by almost 9 times

in both cases (p=.00). Age (OR=1.07[1.04,1.10], p=.00), BMI (OR=1.12[1.03,1.22], p=.01), the presence of smoking habit (OR=2.23[1.01, 5.24], p=.04) and familiarity for periodontitis (OR=2.19[1.09,4.40], p=.03) were significant predictors in this observation. The model resulted statistically significant (p=.00) with a pseudo R^2 of around 33%.

Discussion

Summary of findings

The present cross-sectional analysis evaluated the association between specific environmental exposure variables, *i.e.* MD adherence and PA level, with the periodontal status of a University-based cohort of individuals. The odds of severe/advanced periodontitis are double in subjects with low/moderate PA level compared to those with high PA level and are almost 6 times higher in subjects with low MD adherence compared to those with high adherence. The final predictive regression model demonstrated how the combination of poor MD adherence and PA level led to 9-time increased odds of severe/advanced periodontitis. Age, BMI, smoking and familiarity for periodontitis showed a significant mediating effect in this observation.

Dietary and physical activity assessments

This is the first study formulating the hypothesis of a significant association between PA level and MD adherence to periodontitis severity. Overall, high values of physical inactivity were detected in the present cohort (71%), maybe due to the presence of comorbidities in around 20% of the included individuals and regardless of the relatively young age of the cohort. High PA levels were associated with lower prevalence of severe forms of periodontitis, consistently to recent meta-data showing how physically-active patients are 22% less likely to have periodontitis when compared to those physically-inactive (Ferreira et al., 2019).

A linear relationship between decreasing values of aMed scores and the worsening of periodontal indices was also found, with around 70% of those with low MD adherence being affected by severe/advanced periodontitis.
Results from the present study are partially discordant from those of a previous report (Iwasaki et al., 2021) in which no differences in the biometric periodontal indices were found between subjects with high MD adherence compared to those with low adherence. This result may be due to the different target population investigated in both studies: while the former enrolled University students (mean age 20 years), participants from the current report were selected among those coming to a public University Hospital in Italy (mean age 53 years). Consequently, the reported values of periodontitis prevalence widely differ (6.6% vs 82%).

Indeed, subjects with low MD adherence and low/moderate PA presented not only worse biometric and inflammatory parameters, but also a more frequent experience of tooth mobility and tooth loss for periodontitis, irrespective of domiciliary oral hygiene measures and plaque accumulation. Therefore, a reciprocal association between such environmental factors (MD and PA) and the periodontium can be speculated. First of all, the clinical manifestations of severe periodontitis, such as advanced AL, tooth loss and tooth mobility, hamper masticatory function up to a point that they can lead to dietary changes, characterized by a decrease in fibers, fruits and vegetables intake (Zhu & Hollis, 2014). In turn from a biological standpoint, the consumption of a Western diet, rich in white flour and processed meats, as opposed to MD, induces a state of low-grade inflammation, contributing to the development of many NCDs,

including periodontitis (Christ et al., 2019). Conversely, high MD adherence was demonstrated to be protective against the most severe forms of periodontitis, supposedly due to the synergistic anti-inflammatory potential of the single MD components: wholegrain products demonstrated the strongest protective effect in the current study. In fact, a diet rich in wholegrains was associated to lower systemic inflammatory markers, such as C-reactive protein, and decreased insulin resistance (Esposito et al., 2004; Jenkins et al., 2000). The improved insulin sensitivity could positively influence periodontal health by lowering the production of glycation end-products, reducing oxidative stress and, therefore, by decreasing cytokines release. On the other hand, low MD adherence and, in particular, a low consumption of wholegrain products, increases the odds of severe periodontitis by almost 8 times plausibly by decreasing insulin sensitivity and increasing low-grade systemic inflammation (Merchant et al., 2006).

The current study also hypothesized a positive synergistic effect of regular exercise, in conjunction with high MD adherence, on periodontal health. Even though the effects of MD are prominent when compared independently to those of PA, regular physical exercise was previously demonstrated to decrease the concentration of specific pro-inflammatory markers involved in the clinical manifestations of periodontitis (Huck et al., 2019).

Diagnostic accuracy

MD adherence and PA level were able to discriminate around 70% of severe/advanced periodontitis cases, hence resulting in a good diagnostic accuracy. The ability to correctly detect severe manifestations of the disease may have relevant implications as to the epidemiological screening and prevention of those metabolic disorders, such as diabetes and metabolic syndrome, frequently associated to severe/advanced periodontitis (Daudt 2018; Genco & Borgnakke, 2020). In fact, given the peak of diabetes incidence (55 years old) (M. A. B. Khan et al., 2020), which is similar to the mean age of the present cohort (53 years), the ability of MD adherence and PA to detect severe periodontitis may increase the likelihood to also detect T2DM at its onset. Therefore, by subjecting individuals positive to such test (QueMD and IPAQ) to a blood-glucose monitoring examination we could be able to early diagnose metabolic disorders such as pre-diabetes/diabetes and metabolic syndrome, hence reducing the burden of their complications on global public healthcare.

Predictive regression model

The final regression model confirms the cooperative effect of low MD adherence and low PA to increase the odds of severe periodontitis by 9 times; nonetheless, every time MD adherence is below the selected cut-off, the OR increases irrespective of PA level. Diet and PA seem to retain a higher predictive ability than smoking for severe periodontitis. It can be speculated that such result may be inflated by the significant mediating effect exerted by BMI: in fact, low MD adherence and low PA level were correlated to higher BMI values (Shai et al., 2008), which in turn increase the odds for severe/advanced periodontitis (S. Khan et al., 2018). Such results may have critical implications not only from a diagnostic but also from a therapeutical standpoint: the Step 1 phase (Sanz et al., 2020) of periodontal therapy could be implemented by applying a holistic approach based on common risk factors (Sheiham & Watt, 2000) not only for oral health, but also for other NCDs. Therefore, risk-factor modification should encompass smoking cessation counselling and oral hygiene instructions on one side, but also

the promotion of both regular exercise and MD adherence on the other. Thus, this holistic approach based on common risk-factor modification could significantly ameliorate both the treatment efficacy and long-term management of lifestyle-related pathologies (*i.e.* periodontitis, diabetes, etc.), and also result in reduced healthcare costs.

Strengths and limitations

This is the first study testing the diagnostic accuracy and predictive ability of MD adherence and PA level for severe/advanced periodontitis. Diet and PA assessments were carried out using reliable and validated tools for the selected sample. In fact, the 15-item questionnaire used to evaluate MD adherence (QueMD) was the first one to be developed for the Italian population and validated against a Food Frequency Questionnaire (Gnagnarella et al., 2018). Moreover, PA levels were drawn from the results of the Short version of the IPAQ, whose reproducibility and validity for the Italian population were demonstrated (Mannocci et al., 2010). Altogether, these factors significantly contribute to the internal validity of the study.
The current study presents some limitations. First of all, the cross-sectional design does not allow for a longitudinal evaluation regarding the cause-effect relationship between the exposure and the outcome, and therefore it can be used only to build an hypothesis; moreover, no molecular parameters supporting the biological plausibility of such association were registered. Furthermore, all participants lived in the urban or suburban areas nearby Siena (Italy), where the vast majority of adults is Caucasian; hence, any variability in the outcome related to ethnicity may not have been detected. Overall, these factors may reduce the generalizability of the study.

Conclusion

The present study demonstrated a good diagnostic accuracy of MD adherence and PA level, resulting in the correct discrimination of around 70% of severe/advanced periodontitis cases. Moreover, individuals conducing a lifestyle characterized by the combination of low MD adherence and lack of regular exercise are 9 times more likely to have severe forms of periodontitis. Further research is needed to elucidate the periodontal response to the implementation of the Step 1 phase of periodontal treatment with nutritional and PA counselling sessions.

REFERENCES

Ainamo, J., & Bay, I. (1975). Problems and proposals for recording gingivitis and plaque. *International Dental Journal*, *25*(4), 229–235.

Bach-Faig, A., Berry, E. M., Lairon, D., Reguant, J., Trichopoulou, A., Dernini, S., Medina, F. X., Battino, M., Belahsen, R., Miranda, G., & Serra-Majem, L. (2011). Mediterranean diet pyramid today. Science and cultural updates. *Public Health Nutrition*, *14*(12A), 2274–2284. https://doi.org/10.1017/S1368980011002515

Beauchamp, G. K., Keast, R. S. J., Morel, D., Lin, J., Pika, J., Han, Q., Lee, C.-H., Smith, A. B., & Breslin, P. A. S. (2005). Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. *Nature*, *437*(7055), 45–46. https://doi.org/10.1038/437045a

Benetou, V., Trichopoulou, A., Orfanos, P., Naska, A., Lagiou, P., Boffetta, P., & Trichopoulos, D. (2008). Conformity to traditional Mediterranean diet and cancer incidence: the Greek EPIC cohort. *British Journal of Cancer*, *99*(1), 191–195. https://doi.org/10.1038/sj.bjc.6604418

Christ, A., Lauterbach, M., & Latz, E. (2019). Western Diet and the Immune System: An Inflammatory Connection. *Immunity*, *51*(5), 794–811. https://doi.org/10.1016/j.immuni.2019.09.020

Daudt LD, Musskopf ML, Mendez M, Remonti LLR, Leitão CB, Gross JL, Weidlich P, O. R. (2018). Association between metabolic syndrome and periodontitis: A systematic review and meta-analysis. *Brazilian Oral Research*, *32*, 35. https://doi.org/10.1016/j.adaj.2019.07.023

Diolintzi, A., Panagiotakos, D. B., & Sidossis, L. S. (2019). From Mediterranean diet to Mediterranean lifestyle: a narrative review. *Public Health Nutrition*, *22*(14), 2703–2713. https://doi.org/10.1017/S1368980019000612

Esposito, K., Marfella, R., Ciotola, M., Di Palo, C., Giugliano, F., Giugliano, G., D'Armiento, M., D'Andrea, F., & Giugliano, D. (2004). Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA*, *292*(12), 1440–1446. https://doi.org/10.1001/jama.292.12.1440

Estruch, R., Martínez-González, M. A., Corella, D., Salas-Salvadó, J., Ruiz-Gutiérrez, V., Covas, M. I., Fiol, M., Gómez-Gracia, E., López-Sabater, M. C., Vinyoles, E., Arós, F., Conde, M., Lahoz, C., Lapetra, J., Sáez, G., & Ros, E. (2006). Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Annals of Internal Medicine*, *145*(1), 1–11. https://doi.org/10.7326/0003-4819-145-1-200607040-00004

Ferreira, R. de O., Corrêa, M. G., Magno, M. B., Almeida, A. P. C. P. S. C., Fagundes, N. C. F., Rosing, C. K., Maia, L. C., & Lima, R. R. (2019). Physical Activity Reduces the Prevalence of Periodontal Disease: Systematic Review and Meta-Analysis. In *Frontiers in physiology* (Vol. 10, p. 234). https://doi.org/10.3389/fphys.2019.00234

Genco, R. J., & Borgnakke, W. S. (2020). Diabetes as a potential risk for periodontitis: association studies. *Periodontology 2000*, *83*(1), 40–45. https://doi.org/10.1111/prd.12270

Gnagnarella, P., Dragà, D., Misotti, A. M., Sieri, S., Spaggiari, L., Cassano, E., Baldini, F., Soldati, L., & Maisonneuve, P. (2018). Validation of a short questionnaire to record adherence to the Mediterranean diet: An Italian experience. *Nutrition, Metabolism and Cardiovascular Diseases*, *28*(11), 1140–1147. https://doi.org/10.1016/j.numecd.2018.06.006

Hamp, S. E., Nyman, S., & Lindhe, J. (1975). Periodontal treatment of multirooted teeth. Results after 5 years. *Journal of Clinical Periodontology*, *2*(3), 126–135. https://doi.org/10.1111/j.1600-051x.1975.tb01734.x

Huck, O., Buduneli, N., & Bravo, D. (2019). Inflammatory Mediators in Periodontal Pathogenesis. In *Mediators of inflammation* (Vol. 2019, p. 2610184). https://doi.org/10.1155/2019/2610184

Iwasaki, M., Borgnakke, W. S., Ogawa, H., Yamaga, T., Sato, M., Minagawa, K., Ansai, T., Yoshihara, A., & Miyazaki, H. (2018). Effect of lifestyle on 6-year periodontitis incidence or progression and tooth loss in older adults. *Journal of Clinical Periodontology*, *45*(8), 896–908. https://doi.org/10.1111/jcpe.12920

Iwasaki, M., Ennibi, O. K., Bouziane, A., Erraji, S., Lakhdar, L., Rhissassi, M., Ansai, T., Yoshida, A., & Miyazaki, H. (2021). Association between periodontitis and the Mediterranean diet in young Moroccan individuals. *Journal of Periodontal Research*, *56*(2), 408–414. https://doi.org/10.1111/jre.12833

Jenkins, D. J., Axelsen, M., Kendall, C. W., Augustin, L. S., Vuksan, V., & Smith, U. (2000). Dietary fibre, lente carbohydrates and the insulin-resistant diseases. *The British Journal of Nutrition*, *83 Suppl 1*, S157-63. https://doi.org/10.1017/s0007114500001100

Jiao, J., Jing, W., Si, Y., Feng, X., Tai, B., Hu, D., Lin, H., Wang, B., Wang, C., Zheng, S., Liu, X., Rong, W., Wang, W., Li, W., Meng, H., & Wang, X. (2020). The prevalence and severity of periodontal disease in Mainland China: Data from the Fourth National Oral Health Survey (2015-2016). *Journal of Clinical Periodontology*. https://doi.org/10.1111/jcpe.13396

Kassebaum, N. J., Smith, A. G. C., Bernabé, E., Fleming, T. D., Reynolds, A. E., Vos, T., Murray, C. J. L., &

Marcenes, W. (2017). Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990-2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors. *Journal of Dental Research*, *96*(4), 380–387. https://doi.org/10.1177/0022034517693566

Khan, M. A. B., Hashim, M. J., King, J. K., Govender, R. D., Mustafa, H., & Al Kaabi, J. (2020). Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. *Journal of Epidemiology and Global Health*, *10*(1), 107–111. https://doi.org/10.2991/jegh.k.191028.001

Khan, S., Barrington, G., Bettiol, S., Barnett, T., & Crocombe, L. (2018). Is overweight/obesity a risk factor for periodontitis in young adults and adolescents?: a systematic review. *Obesity Reviews : An Official Journal of the International Association for the Study of Obesity*, *19*(6), 852–883. https://doi.org/10.1111/obr.12668

Kitamura, M., Ikebe, K., Kamide, K., Gondo, Y., Yamashita, M., Takedachi, M., Nozaki, T., Fujihara, C., Yamada, S., Kashiwagi, Y., Miki, K., Iwayama, T., Hatta, K., Mihara, Y., Kurushima, Y., Takeshita, H., Kabayama, M., Oguro, R., Kawai, T., … Murakami, S. (2020). Association of periodontal disease with atherosclerosis in 70-year-old Japanese older adults. *Odontology*. https://doi.org/10.1007/s10266-020-00567-z

Mannocci, A., Di Thiene, D., Del Cimmuto, A., Masala, D., Boccia, A., De Vito, E., & La Torre, G. (2010). International Physical Activity Questionnaire: Validation and assessment in an Italian sample. *Italian Journal of Public Health*, *7*(4), 369–376. https://doi.org/10.2427/5694

Manrique-Corredor, E. J., Orozco-Beltran, D., Lopez-Pineda, A., Quesada, J. A., Gil-Guillen, V. F., & Carratala-Munuera, C. (2019). Maternal periodontitis and preterm birth: Systematic review and meta-analysis. *Community Dentistry and Oral Epidemiology*, *47*(3), 243–251. https://doi.org/10.1111/cdoe.12450

Merchant, A. T., Pitiphat, W., Franz, M., & Joshipura, K. J. (2006). Whole-grain and fiber intakes and periodontitis risk in men. *The American Journal of Clinical Nutrition*, *83*(6), 1395–1400. https://doi.org/10.1093/ajcn/83.6.1395

Miller, S. C. (1950). *Textbook of periodontia oral medicine.* Blakiston. http://books.google.com/books?id=MivSAAAAMAAJ

National Research Institute for Food and Nutrition. (2003). *Linee Guida Per Una Sana Alimentazione Italiana. (Guidelines for an Italian Healthy Eating).*

O'Leary, T. J., Drake, R. B., & Naylor, J. E. (1972). The plaque control record. *Journal of Periodontology*, *43*(1), 38. https://doi.org/10.1902/jop.1972.43.1.38

Papapanou, P. N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D. H., Flemmig, T. F., Garcia, R., Giannobile, W. V., Graziani, F., Greenwell, H., Herrera, D., Kao, R. T., Kebschull, M., Kinane, D. F., Kirkwood, K. L., Kocher, T., Kornman, K. S., Kumar, P. S., … Tonetti, M. S. (2018). Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Clinical Periodontology*, *45*(March), S162–S170. https://doi.org/10.1111/jcpe.12946

Reynolds, M. A. (2014). Modifiable risk factors in periodontitis: at the intersection of aging and disease. *Periodontology 2000*, *64*(1), 7–19. https://doi.org/10.1111/prd.12047

Sanz, M., Herrera, D., Kebschull, M., Chapple, I., Jepsen, S., Beglundh, T., Sculean, A., Tonetti, M. S., Merete Aass, A., Aimetti, M., Kuru, B. E., Belibasakis, G., Blanco, J., Bol-van den Hil, E., Bostanci, N., Bozic, D., Bouchard, P., Buduneli, N., Cairo, F., … Wennström, J. (2020). Treatment of stage I–III periodontitis—The EFP S3 level clinical practice guideline. *Journal of Clinical Periodontology*, *47*(S22), 4–60. https://doi.org/10.1111/jcpe.13290

Shai, I., Schwarzfuchs, D., Henkin, Y., Shahar, D. R., Witkow, S., Greenberg, I., Golan, R., Fraser, D., Bolotin, A., Vardi, H., Tangi-Rozental, O., Zuk-Ramot, R., Sarusi, B., Brickner, D., Schwartz, Z., Sheiner, E., Marko, R., Katorza, E., Thiery, J., … Stampfer, M. J. (2008). Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *The New England Journal of Medicine*, *359*(3), 229–241. https://doi.org/10.1056/NEJMoa0708681

Sheiham, A., & Watt, R. G. (2000). The common risk factor approach: a rational basis for promoting oral health. *Community Dentistry and Oral Epidemiology*, *28*(6), 399–406. https://doi.org/10.1034/j.1600-0528.2000.028006399.x

SINU. (2019). *Livelli di Assunzione di Riferimento di Nutrienti ed energia per la popolazione italiana (LARN)*. 22–23. https://sites.google.com/site/braszopykoutsrenan7/9788890685224-39niacomGEtheibu37

Sofi, F., Cesari, F., Abbate, R., Gensini, G. F., & Casini, A. (2008). Adherence to Mediterranean diet and health status: meta-analysis. *BMJ (Clinical Research Ed.)*, *337*, a1344. https://doi.org/10.1136/bmj.a1344

Tonetti, M. S., Greenwell, H., & Kornman, K. S. (2018). Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *Journal of Clinical Periodontology*, *45*(January), S149–S161. https://doi.org/10.1111/jcpe.12945

Tonetti, M. S., Jepsen, S., Jin, L., & Otomo-Corgel, J. (2017). Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. *Journal of Clinical Periodontology*,

44(5), 456–462. https://doi.org/10.1111/jcpe.12732

Tosti, V., Bertozzi, B., & Fontana, L. (2018). Health Benefits of the Mediterranean Diet: Metabolic and Molecular Mechanisms. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *73*(3), 318–326. https://doi.org/10.1093/gerona/glx227

Verschuren, W. M., Jacobs, D. R., Bloemberg, B. P., Kromhout, D., Menotti, A., Aravanis, C., Blackburn, H., Buzina, R., Dontas, A. S., & Fidanza, F. (1995). Serum total cholesterol and long-term coronary heart disease mortality in different cultures. Twenty-five-year follow-up of the seven countries study. *JAMA*, *274*(2), 131–136.

von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., & Vandenbroucke, J. P. (2008). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Journal of Clinical Epidemiology*, *61*(4), 344–349. https://doi.org/10.1016/j.jclinepi.2007.11.008

Zhu, Y., & Hollis, J. H. (2014). Tooth loss and its association with dietary intake and diet quality in American adults. *Journal of Dentistry*, *42*(11), 1428–1435. https://doi.org/10.1016/j.jdent.2014.08.012

Tables

Adherence to Mediterranean diet (QueMD)

How often do you normally consume a portion of the following foods?

Food Items	Reference portions	Daily frequency of consumption: Never or seldom	<1 per day	1 per day	2 per day	≥3 per day
1. Wholegrain pasta or rice	80 g	☐	☐	☐	☐	☐
2. Vegetables, all type (raw and cooked)	200 g 80 g (salad)	☐	☐	☐	☐	☐
3. Fruits, all types fresh and fresh juices	150 g	☐	☐	☐	☐	☐
4. Milk and yoghurt	125 g	☐	☐	☐	☐	☐

		Never or seldom	<1 per day	1-2 per day	3-4 per day	≥5 per day
5. Wholegrain bread and substitutes	50 g (1-2 slices)	☐	☐	☐	☐	☐
6. Olive oil to cook and to dress	10 ml (1 spoon)	☐	☐	☐	☐	☐
7. Butter, margarine or cooking cream	10 g (1 spoon)	☐	☐	☐	☐	☐
8. Wine (white and red)	125 ml (1 glass)	☐	☐	☐	☐	☐

		Weekly frequency of consumption: Never or seldom	<1 per week	1-3 per week	4-6 per week	≥7 per week
9. Red meat (beef, veal, pork), meat products	100 g (raw meat) 50 g (meat products)	☐	☐	☐	☐	☐
10. White meat (chicken, turkey, rabbit)	100 g	☐	☐	☐	☐	☐
11. Carbonated and/or sugar-sweetened beverages	200 ml (1 glass)	☐	☐	☐	☐	☐
12. Manufactured sweets, pastries, biscuits, creams…	100 g	☐	☐	☐	☐	☐

		Never or seldom	<1 per week	1 per week	2-3 per week	≥4 per week
13. Fish (fresh or frozen) or sea foods	150 g (fish) 50 g (fish products)	☐	☐	☐	☐	☐
14. Dried fruits (nuts, almonds, hazelnuts)	30 g (1 fist)	☐	☐	☐	☐	☐
15. Pulses (chickpeas, lentils, peas, beans)	50 g (dried) 150 g (canned/raw)	☐	☐	☐	☐	☐

International Physical Activity Questionnaire (IPAQ)

Think about all the ***vigorous activities*** that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1)	During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?	☐ Yes **…** days/week	☐ No Skip to question 3
2)	How much time did you usually spend doing vigorous physical activities on one of those days?	☐ Yes **…**minutes/day	☐ Not sure/Don't know

Think about all the ***moderate activities*** that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.			
3)	During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.	☐ Yes …days/week	☐ No Skip to question 5
4)	How much time did you usually spend doing moderate physical activities on one of those days?	☐ Yes …minutes/day	☐ Not sure/Don't know
Think about the time you spent ***walking*** in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.			
5)	During the last 7 days, on how many days did you walk for at least 10 minutes at a time?	☐ Yes …days/week	☐ No Skip to question 7
6)	How much time did you usually spend walking on one of those days?	☐ Yes …minutes/day	☐ Not sure/Don't know
The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.			
7)	During the last 7 days, how much time did you spend sitting on a week day?	☐ Yes …minutes/day	☐ Not sure/Don't know

Table 1: questionnaires regarding adherence to Mediterranean Diet (QueMD) and physical activity (International Physical Activity Questionnaire).

Variable	TOTAL	Healthy	Initial periodontitis	Moderate periodontitis	Severe/advanced periodontitis	*p*-value*
	n=235	n=40	n=26	n=51	n=118	
Socio-demographic characteristics						
Age	53.90 [52.01, 55.79]	41.16[a] [35.36, 46.96]	49.35[a,c] [43.74, 54.95]	53.14[b,c] [49.02, 57.26]	59.55[d] [57.76, 61.34]	***.00***
BMI	25.49 [24.88, 26.11]	23.32[a] [22.09, 24.56]	24.59[a] [22.96, 26.23]	24.22[a] [23.13, 25.31]	26.98[b] [26.04, 27.91]	***.00***
Gender, females	136 (57.87%)	25 (62.50%)	17 (65.38%)	33 (64.71%)	61 (51.69%)	.29
Occupation						
Unemployed	42 (17.95%)	10[a] (25.64%)	6[a,b] (23.08%)	3[b] (5.88%)	23[a] (19.49%)	
Employed	133 (56.84%)	23[a] (58.97%)	16[a,b] (61.54%)	36[b] (70.59%)	58[a] (49.15%)	***.03***
Retired	59 (25.21%)	6[a] (15.38%)	4[a,b] (15.38%)	12[b] (23.53%)	37[a] (31.36%)	
Education						
Elementary/middle school	69 (29.49%)	8[a] (20%)	1[a] (3.85%)	10[a] (20%)	50[b] (42.37%)	***.00***
High school	107 (45.73%)	21[a] (52.50%)	14[a] (53.85%)	26[a] (52%)	46[b] (38.98%)	
College or more	58 (24.79%)	11[a] (27.50%)	11[a] (42.31%)	14[a] (28%)	22[b] (18.64%)	
Smoking						
Never	107 (45.53%)	24 (60%)	14 (53.85%)	25 (49.02%)	44 (37.29%)	.13
Former	67 (28.51%)	8 (20%)	4 (15.38%)	16 (31.37%)	39 (33.05%)	
Smoker	61 (25.96%)	8 (20%)	8 (30.77%)	10 (19.61%)	35 (29.66%)	
Familiarity for periodontitis, yes	87 (37.02%)	9 (22.50%)	8 (30.77%)	18 (35.29%)	52 (44.07%)	.08
Diabetes						
No	180 (76.60%)	30 (75%)	20 (76.92%)	38 (74.51%)	91 (77.12%)	.85
Familiarity	44 (18.72%)	8 (20%)	4 (15.38%)	12 (23.53%)	20 (16.95%)	
Yes	11 (4.68%)	2 (5%)	2 (7.69%)	1 (1.96%)	7 (5.93%)	
Rheumatoid arthritis						
No	198 (84.26%)	33 (82.50%)	23 (88.46%)	44 (86.27%)	98 (83.05%)	.83
Familiarity	18 (7.66%)	4 (10%)	1 (3.85%)	5 (9.80%)	8 (6.78%)	
Yes	19 (8.09%)	3 (7.50%)	2 (7.69%)	2 (3.92%)	12 (10.17%)	
Inflammatory Bowel Diseases						
No	224 (95.32%)	37 (92.50%)	25 (96.15%)	49 (96.08%)	113 (95.76%)	.87
Familiarity	6 (2.55%)	2 (5%)	0 (0%)	1 (1.96%)	3 (2.54%)	
Yes	5 (2.13%)	1 (2.50%)	1 (3.85%)	1 (1.96%)	2 (1.69%)	
Osteoporosis						
No	192 (81.70%)	35 (87.50%)	23 (88.46%)	41 (80.39%)	93 (78.81%)	.25
Familiarity	29 (12.34%)	4 (10%)	3 (11.54%)	9 (17.65%)	13 (11.02%)	
Yes	14 (5.96%)	1 (2.50%)	0 (0%)	1 (1.96%)	12 (10.17%)	
aMed score†	4.31 [4.05, 4.56]	4.37[a] [3.74, 5.01]	6.11[b,d] [5.58, 6.64]	5.19[a,d] [4.75, 5.64]	3.5[c] [3.16, 3.84]	***.00***
High aMed	118 (50.42%)	25[a] (62.5%)	25[a] (96.15%)	38[b] (74.51%)	35[c] (29.66%)	***.00***
Low aMed	117 (49.58%)	15[a] (37.5%)	1[a] (3.85%)	13[b] (25.49%)	83[c] (70.34%)	
PA level						
High PA	67 (28.81%)	10[a] (25%)	12[a] (46.15%)	19[a] (37.25%)	26[b] (22.03%)	***.04***
Low/Moderate PA	168 (71.19%)	30[a] (75%)	14[a] (53.85%)	32[a] (62.75%)	92[b] (77.97%)	
Oral health status						
Number of teeth	24.17 [23.47, 24.86]	26.63[a] [25.37, 27.88]	25.27[a] [23.37, 27.16]	24.52[a] [22.97, 26.09]	22.94[b] [21.91, 23.97]	***.00***
CAL	2.83 [2.72, 2.94]	2[a] [1.87, 2.13]	2.22[a] [2.15, 2.30]	2.73[b] [2.56, 2.91]	3.28[c] [3.13, 3.44]	***.00***
PD	2.53 [2.45, 2.62]	1.89[a] [1.80, 1.98]	2.15[a] [2.05, 2.25]	2.47[b] [2.35, 2.59]	2.86[c] [2.73, 299]	***.00***
% PD>4mm	9.30 [7.81, 10.79]	0.10[a] [0.02, 0.22]	2.30[b,c] [1.57, 3.03]	5.29[c] [3.98, 7.07]	15.59[d] [13.22, 17.95]	***.00***
% PD 5-6mm	7.71 [6.51, 8.90]	0.10[a] [0.02, 0.22]	2.27[b,c] [1.54, 2.99]	4.61[c] [3.39, 5.82]	12.83[d] [10.93, 14.72]	***.00***
% PD>6mm	4.03 [0.19, 8.24]	0[a] [0, 0]	0.03[a,b] [0.029, 0.11]	10.63[b] [9.03, 30.29]	3.41[c] [2.28, 4.55]	***.00***
Furcation involvement, yes‡	76 (32.34%)	1[a] (2.50%)	0[a] (0%)	8[a] (15.69%)	67[b] (56.78%)	***.00***
Mobility, yes	91 (38.72%)	6[a] (15%)	0[a] (0%)	12[a] (23.53%)	73[b] (61.86%)	***.00***
Number of bleeding pockets§	7.32 [5.98, 8.65]	0.03[a] [0.025, 0.08]	1.5[b,c] [1.01, 1.98]	4.57[c] [2.85, 6.29]	12.26[d] [10.06, 14.46]	***.00***
FMPS	50.56 [47.45, 53.68]	33.95[a] [28.42, 39.48]	54.57[b,c] [45.42, 63.73]	52.27[c] [45.73, 58.82]	54.57[d,c] [50.03, 59.11]	***.02***
FMBS	28.45 [26.17, 30.74]	16.83[a] [12.02, 21.64]	21.92[a,b] [16.12, 27.73]	28.51[b,c] [23.67, 33.34]	33.81[c] [30.65, 36.97]	***.00***

Teeth lost for periodontitis, yes	76 (32.34%)	1[a] (2.50%)	1[a] (3.85%)	3[a] (5.88%)	71[b] (60.17%)	***.00***
Grade[¶]						
none	40 (17.02%)	40 (100%)	0 (0%)	0 (0%)	0 (0%)	***.00***
A	21 (8.94%)	0[a] (0%)	12[b] (46.15%)	5[c] (9.80%)	4[d] (3.39%)	
B	106 (45.11%)	0[a] (0%)	11[b] (42.31%)	38[c] (74.51%)	57[d] (48.31%)	
C	68 (28.94%)	0[a] (0%)	3[b] (11.54%)	8[c] (15.69%)	57[d] (48.31%)	
Domiciliary plaque control						
Brushing frequency						
Not performed	4 (1.70%)	1 (2.50%)	0 (0%)	0 (0%)	3 (2.54%)	.06
Occasionally	46 (19.57%)	4 (10%)	3 (11.54%)	6 (11.76%)	33 (27.97%)	
Every day	185 (78.72%)	35 (87.50%)	23 (88.46%)	45 (88.24%)	82 (69.49%)	
Toothbrush type, powered	122 (51.91%)	23 (57.50%)	13 (50%)	29 (56.86%)	63 (53.39%)	.57
Interdental cleaning (IC)						
Not performed	75 (31.91%)	17 (42.50%)	8 (30.77%)	16 (31.37%)	34 (28.81%)	.32
Interdental floss	51 (21.70%)	11 (27.50%)	7 (26.92%)	10 (19.61%)	23 (19.49%)	
Interproximal brushes	109 (45.99%)	12 (30%)	11 (42.31%)	25 (49.02%)	61 (51.69%)	
Frequency of IC						
Not performed	75 (31.91%)	16 (40%)	8 (30.77%)	16 (31.37%)	35 (29.66%)	.73
Occasionally	48 (20.43%)	7 (17.50%)	4 (15.38%)	8 (15.69%)	29 (24.58%)	
Every day	112 (47.66%)	17 (42.50%)	14 (53.85%)	27 (52.94%)	54 (45.76%)	

Note: results of continuous variables are reported as mean [95% Confidence Interval]; results of binary and categorical variables are expressed as number of observations (proportion).
Abbreviations: BMI, body mass index; aMed, alternate Mediterranean diet score; PA, physical activity; CAL, clinical attachment level; PD, probing depth; FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; IC, interdental cleaning.
[*] *p*-value of the Kruskal Wallis or Fisher's exact test for patients' characteristics across the four subgroups.
[†] High aMed if aMed>4; Low aMed if aMed<5.
[‡] Class II/III furcation involvement according to the classification of Hamp *et al.* (1975).
[§] Defined as the number of sites with probing depth≥5mm and positive to bleeding on probing.
[¶] According to the 2018 EFP/AAP classification.

Table 2: descriptive statistics overall and by oral health status. Values with different superscript letters are different at the 5% level.

Variable	aMed			PA level		
	Low (0-4)	High (5-9)	*p*-value*	Low/moderate	High	*p*-value*
	n=117	n=118		n=168	n= 67	
Age	53.63 [50.88, 56.37]	54.17 [51.54, 56.80]	.93	54.52 [52.36, 56.68]	52.39 [48.45, 56.23]	.35
BMI	25.90 [25.02, 26.78]	25.09 [24.22, 25.96]	.11	25.96 [25.19, 26.72]	24.32 [23.33, 25.31]	***.01***
Gender, females	56 (47.86%)	80 (67.80%)	***.00***	105 (62.50%)	31 (46.27%)	***.03***
Occupation						
Unemployed	26 (22.41%)	16 (13.56%)		31 (18.56%)	11 (16.42%)	
Employed	65 (56.03%)	68 (57.63%)	.15	94 (56.29%)	39 (58.21%)	.93
Retired	25 (21.55%)	34 (28.81%)		42 (25.15%)	17 (25.37%)	
Education						
Elementary/middle school	43 (37.07%)	26 (22.03%)		52 (30.95%)	17 (25.76%)	
High school	53 (45.69%)	54 (45.76%)	***.01***	76 (45.24%)	31 (46.97%)	.71
College or more	20 (17.24%)	38 (32.20%)		40 (23.81%)	18 (27.27%)	
Smoking						
Never	47 (40.17%)	60 (50.85%)		70 (41.67%)	37 (55.22%)	
Former	36 (30.77%)	31 (26.27%)	.25	51 (30.36%)	16 (23.88%)	.18
Smoker	34 (29.06%)	27 (22.88%)		47 (27.98%)	14 (20.90%)	
Familiarity for periodontitis, yes	42 (35.90%)	45 (38.14%)	0.79	65 (38.69%)	22 (32.84%)	.46
Oral health status						
Number of teeth	23.85 [22.81, 24.89]	24.48 [23.55, 25.41]	.48	24.05 [23.24, 24.88]	24.52 [23.09, 25.79]	.48
CAL	2.96 [2.79, 3.14]	2.70 [2.57, 2.83]	.06	2.88 [2.75, 3.02]	2.69 [2.52, 2.86]	.16
PD	2.62 [2.49, 2.75]	2.44 [2.34, 2.55]	.06	2.56 [2.46, 2.67]	2.45 [2.32, 2.59]	.32
% PD>4mm	11 [8.74, 13.21]	7.61 [5.69, 9.54]	***.02***	10.21 [8.32, 12.10]	7.01 [4.87, 9.15]	.12
% PD 5-6mm	9.12 [7.30, 10.95]	6.31 [4.76, 7.84]	***.02***	8.58 [7.04, 10.13]	5.51 [3.94, 7.06]	.09
% PD>6mm	5.57 [2.81, 13.96]	2.46 [1.37, 3.55]	.18	5 [0.01, 10.90]	1.56 [0.5, 2.64]	.39
Furcation involvement, yes†	51 (43.59%)	25 (21.19%)	***.00***	60 (35.71%)	16 (23.88%)	.09
Mobility, yes	55 (47.01%)	36 (30.51%)	***.01***	77 (45.83%)	14 (20.90%)	***.00***
Number of bleeding pockets‡	9.18 [6.95, 11.41]	5.46 [4.03, 6.89]	**.03**	7.91 [6.25, 9.58]	5.83 [3.69, 7.97]	.16
FMPS	51.92 [47.45, 56.39]	49.22 [44.82, 53.61]	.48	51.19 [47.27, 55.11]	48.98 [44.08, 53.88]	.61
FMBS	29.78 [26.57, 33.01]	27.14 [23.86, 30.41]	.18	28.69 [25.88, 31.51]	27.85 [23.93, 31.76]	.96
Teeth lost for periodontitis, yes	47 (40.17%)	29 (24.58%)	***.01***	59 (35.12%)	17 (25.37%)	.16
Domiciliary plaque control						
Brushing frequency						
Not performed	3 (2.56%)	1 (0.85%)		4 (2.38%)	0 (0%)	
Occasionally	29 (24.79%)	17 (14.41%)	.06	35 (20.83%)	11 (16.42%)	.39
Every day	85 (72.65%)	100 (84.75%)		129 (76.79%)	56 (83.58%)	
Toothbrush type, powered	62 (52.99%)	60 (50.85%)	.79	90 (53.57%)	32 (47.76%)	.47
Interdental cleaning (IC)						
Not performed	43 (36.75%)	32 (27.12%)	.06	58 (34.52%)	17 (25.37%)	.51
Interdental floss	29 (24.79%)	22 (18.64%)		36 (21.43%)	15 (22.39%)	

Interproximal brushes	45 (38.46%)	64 (54.24%)		74 (44.05%)	35 (42.24%)	
Frequency of IC						
Not performed	44 (37.61%)	31 (26.27%)		58 (34.52%)	17 (25.37%)	
Occasionally	28 (23.93%)	20 (16.95%)	.06	32 (19.05%)	16 (23.88%)	.37
Every day	45 (38.46%)	67 (56.78%)		78 (46.43%)	34 (50.75%)	

Note: results of continuous variables are reported as mean [95% Confidence Interval]; results of binary and categorical variables are expressed as number (proportion).
Abbreviations: BMI, body mass index; aMed, alternate Mediterranean diet score; PA, physical activity; CAL, clinical attachment level; PD, probing depth; FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; IC, interdental cleaning.
[*] *p*-value of the Mann Whitney or Fisher's exact test for patients' characteristics across the subgroups of diet and physical activity; $p<0.05$
[†] Class II/III furcation involvement according to the classification of Hamp *et al.* (1975).
[‡] Defined as the number of sites with probing depth≥5mm and positive to bleeding on probing.

Table 3: patients' characteristics by adherence to Mediterranean diet (aMed) and physical activity level.

Variable	ORs for severe/advanced periodontitis							
	Crude ORs	95% CI		*p*-value*	Adjusted† ORs	95% CI		*p*-value*
		Lower	Upper			Lower	Upper	
Low/Moderate PA level	1.83	1.04	3.26	*.04*	1.65	.84	3.28	.42
Low aMed‡	8.47	4.20	17.09	*.00*	5.63	3.21	9.84	***.00***
aMed and PA level								
Low aMed, low/moderate PA	7.69	3.03	19.47	*.01*	6.40	2.94	13.91	***.01***
Low aMed, high PA	7.66	1.98	29.95	*.00*	3.30	1.15	9.50	***.03***
High aMed‡, low/moderate PA	.96	.44	2.20	.97	.85	.32	2.23	.74
High aMed, high PA	REF.							
aMed components								
Wholegrain products	.33	.19	.56	*.00*	.25	.13	.47	***.00***
Vegetables	.47	.28	.80	*.00*	.43	.23	.80	***.01***
Fruits	.56	.33	.94	*.03*	.37	.19	.79	***.00***
Olive oil	.40	.19	.82	*.01*	.30	.15	.82	***.00***
Wine	.67	.39	1.16	.15	.62	.32	1.19	.15
Red meat and meat products	.67	.37	1.15	.07	.69	.37	1.27	.24
Fish	.36	.21	.63	*.00*	.38	.20	.71	***.00***
Dried fruits	.33	.19	.58	*.00*	.35	.15	.59	***.02***
Pulses	.54	.31	.96	*.04*	.39	.22	.73	***.00***

Abbreviations: ORs, odds ratios; CI, confidence interval; PA, physical activity; aMed, alternate Mediterranean diet score; REF., reference category.
* $p<0.05$
† Adjusted for age, body mass index, gender, smoking and brushing frequency.
‡ High aMed if aMed>4; Low aMed if aMed<5.

Table 4: Association between aMed and physical activity level with severe/advanced periodontitis.

Variable	Low aMed, low/moderate PA n=95	Low aMed, high PA n=22	High aMed, low/moderate PA n=73	High aMed, high PA n=45	*p*-value*
Socio-demographic characteristics					
Age	55.26 [52.36, 58.15]	46.58 [39.22, 53.94]	53.56 [50.26, 56.86]	55.15 [50.64, 59.66]	.26
BMI	26.06[c,d] [25.10, 27.02]	25.22[a,c,d] [22.95, 27.49]	25.81[b,c] [24.57, 27.07]	23.88[a] [22.87, 24.90]	***.04***
Gender, females	50[c] (52.63%)	6[b] (27.27%)	55[a] (75.34%)	25[a,b,c] (55.56%)	***.00***
Occupation					
Unemployed	22 (23.40%)	4 (18.18%)	9 (12.33%)	7 (15.56%)	
Employed	50 (53.19%)	15 (68.18%)	44 (60.27%)	24 (53.33%)	.48
Retired	22 (23.40%)	3 (13.64%)	20 (27.40%)	14 (31.11%)	
Education					
Elementary/middle school	35 (36.84%)	8 (38.10%)	17 (23.29%)	9 (20%)	
High school	42 (44.21%)	11 (52.38%)	34 (46.58%)	20 (44.44%)	.66
College or more	18 (18.95%)	2 (9.52%)	22 (30.14%)	16 (35.56%)	
Smoking					
Never	37 (38.95%)	10 (45.45%)	33 (45.21%)	27 (60%)	
Former	31 (32.63%)	5 (22.73%)	20 (27.40%)	11 (24.44%)	.69
Smoker	27 (28.42%)	7 (31.82%)	20 (27.40%)	7 (15.56%)	
Familiarity for periodontitis, yes	36 (37.89%)	6 (27.27%)	29 (39.73%)	16 (35.56%)	.46
Oral health status					
Periodontitis[†]					
Healthy	14 (14.74%)[a]	6 (27.27%)[a]	16 (21.92%)[b]	4 (8.89%)[b]	
Initial	1 (1.05%)[a]	0 (0%)[a]	13 (17.81%)[b]	12 (26.67%)[b]	***.04***
Moderate	10 (10.53%)[a]	3 (13.64%)[a]	22 (30.14%)[b]	16 (35.56%)[b]	
Severe/advanced	70 (73.68%)[a]	13 (59.09%)[a]	22 (30.14%)[b]	13 (28.89%)[b]	
Number of teeth	23.74 [22.60, 24.89]	24.31 [21.58, 27.04]	24.46 [23.28, 25.64]	24.51 [22.92, 26.09]	.76
CAL (mm)	3.02 [2.82, 3.22]	2.71 [2.31, 3.11]	2.70 [2.52, 2.89]	2.68 [2.50, 2.86]	.07
PD (mm)	2.64 [2.50, 2.79]	2.52 [2.20, 2.84]	2.46 [2.31, 2.61]	2.42 [2.29, 2.56]	.18
% PD>4mm	11.64 [9.07, 14.20]	8.22 [3.37, 13.07]	8.35 [5.54, 11.11]	6.42 [4.15, 8.68]	.07
% PD 5-6mm	9.92 [7,79, 12.05][a]	5.68 [2.65, 8.71][b]	6.84 [4.61, 9.07][b]	5.42 [3.54, 7.30][b]	***.04***
% PD>6mm	2.42 [1.26, 3.57]	2.63 [0.5, 5.81]	8.36 [5.26, 22]	1.04 [0.45, 1.63]	.43
Furcation involvement, yes[‡]	43 (45.26%)[a]	8 (36.36%)[a,c,d]	17 (23.29%)[b,c]	8 (17.78%)[b,d]	***.00***
Mobility, yes	50 (52.63%)[a]	5 (22.73%)[b,c]	27 (36.99%)[a,c]	9 (20%)[b,c]	***.00***
Number of bleeding pockets[§]	9.68 [7.16, 12.20]	7.05 [2.02, 12.07]	5.60 [3.68, 7.52]	5.24 [3.05, 7.43]	.07
FMPS	53.15 [48.12, 58.19]	46.59 [36.43, 56.74]	48.64 [42.34, 54.94]	50.15 [44.52, 55.78]	.59
FMBS	30.48 [26.80, 34.16]	26.77 [20.00, 33.54]	26.39 [21.97, 30.76]	28.37 [23.40, 33.35]	.35
Teeth lost for periodontitis, yes	41 (43.16%)[a]	6 (27.27%)[a,c]	18 (24.66%)[b,c]	11 (24.44%)[c]	***.04***
Domiciliary plaque control					
Brushing frequency					
Not performed	3 (3.16%)	0 (0%)	1 (1.37%)	0 (0%)	
Occasionally	25 (26.32%)	4 (18.18%)	10 (13.70%)	7 (15.56%)	.31
Every day	67 (70.53%)	18 (81.82%)	62 (84.93%)	38 (84.44%)	
Toothbrush type, powered	53 (55.79%)	9 (40.91%)	37 (50.68%)	23 (51.11%)	.63
Interdental cleaning (IC)					
Not performed	36 (37.89%)	7 (31.82%)	22 (30.14%)	10 (22.22%)	
Interdental floss	21 (22.11%)	8 (36.36%)	15 (20.55%)	7 (15.56%)	.16
Interproximal brushes	38 (40%)	7 (31.82%)	36 (49.32%)	28 (62.22%)	
Frequency of IC					
Not performed	37 (38.95%)	7 (31.82%)	21 (28.77%)	10 (22.22%)	.09
Occasionally	20 (21.05%)	8 (36.36%)	12 (16.44%)	8 (17.78%)	

Every day	38 (40%)	7 (31.82%)	40 (54.79%)	27 (60%)

Note: results of continuous variables are reported as mean [95% Confidence Interval]; results of binary and categorical variables are expressed as number (proportion).
Abbreviations: aMed, alternate Mediterranean diet score; PA, physical activity; CAL, clinical attachment level; PD, probing depth; FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; IC, interdental cleaning.
[*] *p*-value of the Kruskal Wallis or Fisher's exact test for patients' characteristics across the four subgroups; $p<0.05$
[†] According to the 2018 EFP/AAP classification.
[‡] Class II/III furcation involvement according to the classification of Hamp *et al.* (1975).
[§] Defined as the number of sites with probing depth≥5mm and positive to bleeding on probing.

Table 5: patients' characteristics summarized by the combination of adherence to Mediterranean diet (aMed score) and physical activity level. Values with different superscript letters are different at the 5% level.

Best model (AUC=0.868, AIC=232.2, BIC=266.7)						
LR chi²	**Prob>chi²**	**Pseudo R²**				
107.44	.00	.3298				
Severe/advanced periodontitis	**OR**	**SE**	**z**	***p*-value***	**95% CI**	
					Lower	**Upper**
High aMed†, high PA	REF.					
High aMed, low/moderate PA	.85	.41	-.34	.73	.33	2.18
Low aMed†, high PA	8.73	4.22	4.49	***.00***	2.22	22.50
Low aMed, low/moderate PA	8.80	6.19	3.09	***.00***	3.39	34.90
Age	1.07	.01	4.92	***.00***	1.04	1.10
BMI	1.12	.05	2.68	***.01***	1.03	1.22
Smoking						
Never	REF.					
Former	1.00	.41	.02	.99	.45	2.24
Smoker	2.23	.97	1.84	***.04***	1.01	5.24
Familiarity for periodontitis	2.19	.78	2.20	***.028***	1.09	4.40
Comorbidities‡	1.58	.71	1.02	.31	.66	3.80
_cons	.00	.00	-6.25	.00	.00	.001

Abbreviations: AUC, Area Under the Curve; AIC, Akaike information criterion; BIC, Bayesian information criterion; LR, likelihood ratio; CI, confidence interval; aMed, alternate Mediterranean diet score; PA, physical activity; REF., reference category; BMI, body mass index.
[*] $p<0.05$
[†] High aMed if aMed>4; Low aMed if aMed<5.
[‡] Presence of at least one comorbidity (*i.e.* diabetes, rheumatoid arthritis, inflammatory bowel diseases, osteoporosis).

Table 6: multivariate logistic regression analysis for the prediction of severe/advanced periodontitis by measures of the combination of adherence to Mediterranean diet (aMed score) and physical activity, and socio-demographic characteristics.

Figure

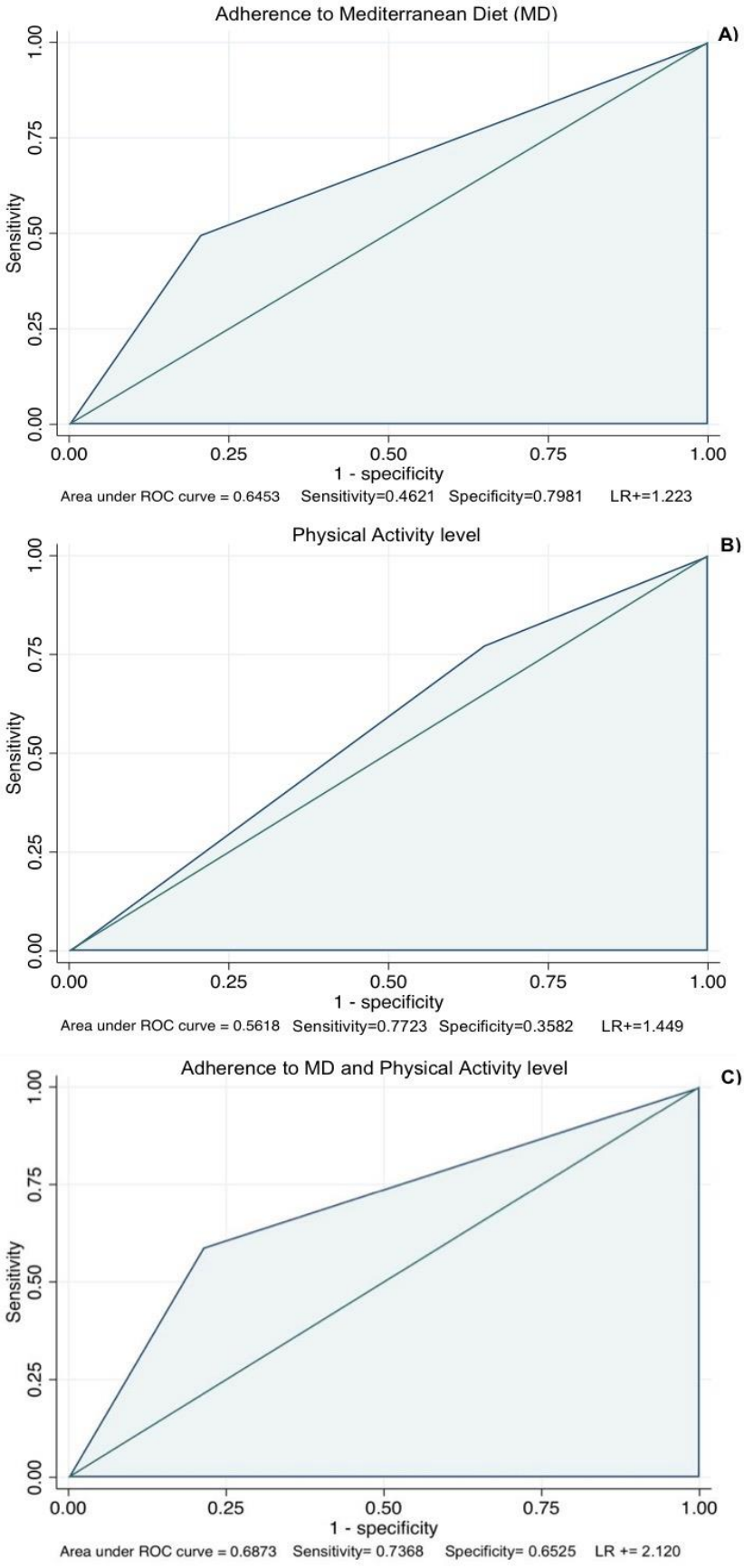
Adherence to Mediterranean Diet (MD)
A)
Sensitivity
0.00
0.25
0.50
0.75
1.00
1 - specificity
Area under ROC curve = 0.6453 Sensitivity=0.4621 Specificity=0.7981 LR+=1.223
Physical Activity level
B)
Sensitivity
0.00
0.25
0.50
0.75
1.00
1 - specificity
Area under ROC curve = 0.5618 Sensitivity=0.7723 Specificity=0.3582 LR+=1.449
Adherence to MD and Physical Activity level
C)
Sensitivity
0.00
0.25
0.50
0.75
1.00
1 - specificity
Area under ROC curve = 0.6873 Sensitivity= 0.7368 Specificity= 0.6525 LR += 2.120

Figure 1: ROC curve showing the diagnostic accuracy of adherence to Mediterranean Diet (MD) (1A), level of physical activity (PA) (1B) and the combination between low adherence to MD and low/moderate PA level (MD-PA) for severe/advanced periodontitis.