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The Role IgM, IgG a, Complementary Factors C3, C4 and Latex assay in C-reactive Protein Positive Assay) in Perioniditis Patients

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Abstract: Periodontal diseases include the common inflammatory disorders known as gingivitis and periodontitis, which are caused by microbes in the biofilms under the gums. The study aimed to identify the level of CRP, C3, C4, IgG and IgM in the serum of patients with periodontal diseases compared to its level in the healthy people. Sixty blood samples were collected from patients with Periodontal diseases , and 30 apparently healthy individuals who were considered as a control group .The current study showed that the prevalence of the disease was higher in females (n=33) than in males (n=27), (55.0% , 45.0%), respectively. As the highest rate of infection was within the age group from 30-39 years, at a rate of 36.7%, and the lowest was within 50-59 years at a rate of 3.3% .It was found through statistical analysis of the results that there are very high significant differences ($p<0.001$)between smoking and non-smoking patients. Chronic gingivitis had the highest prevalence rate of 51.7%, among other types. The results of the study also indicated that the CRP positivity in patients with Periodontal diseases was 68.3% and negative 31.7% . That the level of C3 and C4 in the sera of patients ,increased significantly P value (0.001,0.004) respectively, compared to its level in the sera of healthy subjects, where the mean ± standard deviation was [(208.78 ± 55.40 and 138.67 ± 39.69);(43.65 ± 10.80 and 30.03 ± 9.81)] mg/dl, respectively. It was observed from the results of the study that the level of the IgM and IgG immunoglobulin of the patients had a very high significant increase ($p <0.001$) ,compared to the healthy group, [(228.37 ± 42.83 and 147.33 ± 13.86);(169.72 ± 68.32)] mg / dl, respectively.

Keywords: Periodontal diseases, gingivitis, CRP, complement protein, immunoglobulin.

1-Introduction

Periodontal diseases include the common inflammatory conditions known as periodontitis and gingivitis, which are brought on by microbes in the biofilms under the gums (AlSharief and Alabdurubalnabi, 2023). Resulting in an immune response and inflammation, this response destroys the biofilms under the gums and surrounding and supporting tissues of the teeth, and eventually lead to tooth loss (Dubey and Mittal, 2020).Gingivitis is classified in terms of etiology into five types, plaque induced gingivitis, infectious gingivitis, nutritional gingivitis, hormonal gingivitis and drug-induced gingivitis (Abbas et al., 2023). Periodontal disease are also classified according to the severity of the disease, into several different forms, which are as, Periodontal abscesses, necrotizing periodontal diseases, aggressive periodontitis, chronic periodontitis, acute gingivitis, and chronic gingivitis (Armitage and Cullinan,2010).

Studies have shown the presence of a group of molecules in the gingival cervical fluid whose percentage increases with Periodontal disease, including interleukin-8, interleukin-1alpha, interleukin-1beta, interleukin-8, interleukin-6, Transforming Growth Factor-beta, and Monocyte Chemoattractant Protein-1 (MCP- 1) and Tumor Necrosis Factor Alpha (TNF-α), in addition to high levels of Acute-phase proteins such as C-reactive protein (CRP) (Patel, 2020). High levels of CRP were found in the serum of patients with periodontitis, compared to healthy people (Shankar, et al., 2023.). Therefore, CRP has been used for prediction and early detection of periodontal diseases (Shojaee et al., 2013). This protein increases upon activation of T, B and Natural killer cells, and upon opsonisation of pathogens and damaged cells (Luan and Yao,2018). CRP is produced, as a result of pro-inflammatory cytokines released by liver cells during infectious inflammatory processes (Abullais et al., 2023). Native pentameric CRP and monomeric CRP are the two types of CRP; they bind to distinct receptors and lipid rafts and have distinct biological activities.(Pepys and Hirschfield, 2003).It was found that there is a relationship between CRP levels and smoking, and gum disease, as it was discovered in the serum of patients with periodontitis at levels much higher than those in people without periodontitis (Xu et al., 2020);(Podzimek, et al., 2015).

Complement protein third(C3) and fourth (C4) is one of the most important components of the complement system, (Huang, et al., 2023);(Maekawa et al., 2014). C3 plays an important role in the immune and inflammatory response that is regulated during periodontal disease, as its quality in serum and cervical gingival fluid reflects the status and severity of the disease (Weinstein et al., 2021);(Sridharan and Sravani, 2019).

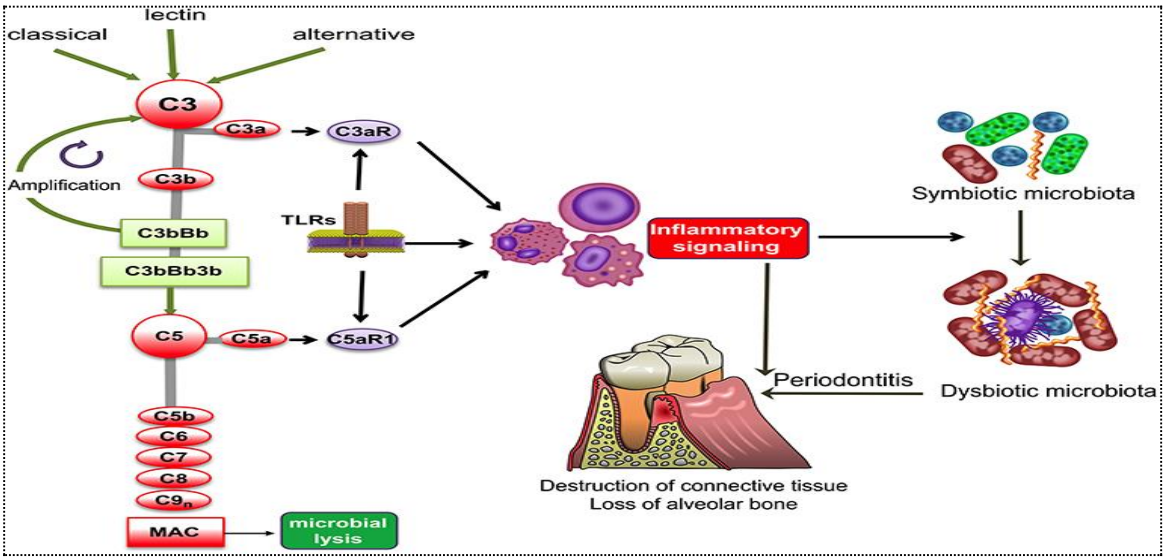

Figure 1: Activation of the complement system in periodontitis (Hajishengallis et al., 2019).

It was found that there is a relationship between periodontal disease, rheumatoid arthritis, and Immunoglobulins G (IgG) and Immunoglobulin M (IgM), to increase the concentration of these antibodies in the serum of patients suffering from periodontitis. (Gomez-Bañuelos et al.,2020);(Sakai et al., 2001).It was also note that there is an association between periodontitis and high levels of IgG and IgM and host-derived pathogens (Buhlin et al., 2015).

The objective of the current study is to determine the concentration of CRP, certain complement proteins (C3, C4), and immunoglobulins (IgG, IgM) in the serum of individuals suffering from periodontal diseases, as well as the impact of smoking, age, and gender on the spread of these conditions.

Materials and methods:

Samples collection

This study was carried out between September 2020 and January 2021, when 60 patients with gum disease who had been diagnosed by a specialist physician at the Specialized Dental Center in Diyala Governorate provided blood samples for the study. In my age range of 12 to 80 years, there were 27

males and 33 females. Thirty blood samples from individuals who appeared to be in good health were taken and utilized as a control group; in this group, there were 8 males and 22 females within the specified age range. They were (14–4) years old and free of any acute or long-term illnesses.

Methods

Five milliliters of venous blood were drawn in order to gather samples. And a centrifuge was used for five minutes to separate the serums. The serum was separated into equal portions of 250 µl in small tubes (Eppendroff) and kept at a temperature of -20°C until needed. Each section of the preserved serum was only used once to prevent the sample from being repeatedly thawed and frozen. All of this was done at a rate of 3000 rpm. .Latex agglutination test was used to examine CRP (Komoriya et al., 2010).The concentration of Complement protein (C3,C4) and immunoglobulin (IgM, IgG), was also measured by single radial immunodiffusion test.(Mancini et al., 1965).

Statistical analysis

The Statistical Package for Social Sciences, version 22, was used to perform the statistical analysis. The chi-square test was used to compare the descriptive variables, which were expressed as numbers and percentages. As for the variables of the numerical form, They were explained by means of the mean Additionally, the ANOVA test or the t-test between two groups were used to compare the totals and determine the standard deviation of the mean.

Results

1. Distribution of the two study groups according to sex percentages

The current study included 90 individuals, including 60 patients (Patients) with periodontal disease, the number (N) of males (Male) 27, or 45.0%, and the number of females (Female) 33, or 55.0%, while the healthy group (control) numbered 30 individuals., the number of males was 8, with a percentage of 26.7%, and the number of females was 22, at a rate of 73.3%, with no significant differences (P value = 0.093) between the two groups of patients and healthy subjects, as shown in (table 1).

Table 1 ; distribution of the two study groups (patients and healthy people) by sex.

			Study groups		Total	P value
			Patients	Controls		
Sex	Male	N	27	8	35	0.093 *
		%	45.0%	26.7%	38.9%	
	Female	N	33	22	55	
		%	55.0%	73.3%	61.1%	

* : Insignificant difference .

2. Distribution of the two study groups by percentage of age groups

Table (2) shows the age groups for the group of patients and the infection rates for each age group, as the highest rate of infection was within the age group from 30-39 years, at a rate of 36.7%, and the lowest was within the age group from 50-59 years at a rate of 3.3%, with the presence of non-existent differences Significant (P=0.153) .

Table 2; distribution of the two study groups (patients and healthy people) by age groups.

			Study groups		Total	P value
			Patients	Controls		
Age periods	10-19	N	12	3	15	0.153*
		%	20.0%	10.0%	16.7%	
	20-29	N	18	17	35	
		%	30.0%	56.7%	38.9%	
	30-39	N	22	8	30	
		%	36.7%	26.7%	33.3%	
	40-49	N	3	2	5	

		%	5.0%	6.7%	5.6%	
	50-59	N	2	0	2	
		%	3.3%	0.0%	2.2%	
	≥ 60	N	3	0	3	
		%	5.0%	0.0%	3.3%	

* : Insignificant difference.

3. Distribution of patient groups by smoking factor

Table (3) shows the number and proportions of the group of smoking and non-smoking patients with gum disease, as the number of smoking patients was 31, or 51.7%, while the number of non-smokers was 29, or 48.3%, and it was found through statistical analysis of the results that there are very high significant differences (p<0.001)between smoking and non-smoking patients.

Table 3 Distribution of patient groups by smoking factor

Smoking	Study groups		P value
	Count	Percent	
Yes	31	51.7%	0.001***
No	29	%48.3	

*** :very high significant differences .

4. Distribution of patient groups by type of periodontal disease

The results of the current study showed that the most prevalent type of periodontal disease is chronic gingivitis with a rate of 51.7% and the least type is acute gingivitis and acute perioditis with a rate of 8.3% and 10% respectively with the presence of Very high significant differences (**p<0.001**), as shown in (table 4).

Table 4 Distribution of patient groups by type of injury

infection types	Study groups		P value
	Count	Percent	
Chronic periodontitis	18	30%	0.001***
Acute periodontitis	6	10%	
Chronic gingivitis	31	51.7%	
Acute gingivitis	5	8.3%	

*** : very high significant differences.

5. Measurement of CRP in the serum of patients and healthy:

The concentration of CRP in the serum of all samples was measured. The results of the current study showed that 41 patients showed positive CRP with a percentage of 68.3% and 19 negative (31.7%), while in the healthy group it was (16.7% and 83.3%), respectively, and very high significant differences (p<0.001), as in (table 5).

Table 5 Comparison of CRP levels for the study group.

			Study groups		Total	P value
			Patients	Controls		
CRP	Positive	N	41	5	46	0.001***
		%	68.3%	16.7%	51.1%	
	Negative	N	19	25	44	
		%	31.7%	83.3%	48.9%	

*** :very high significant differences .

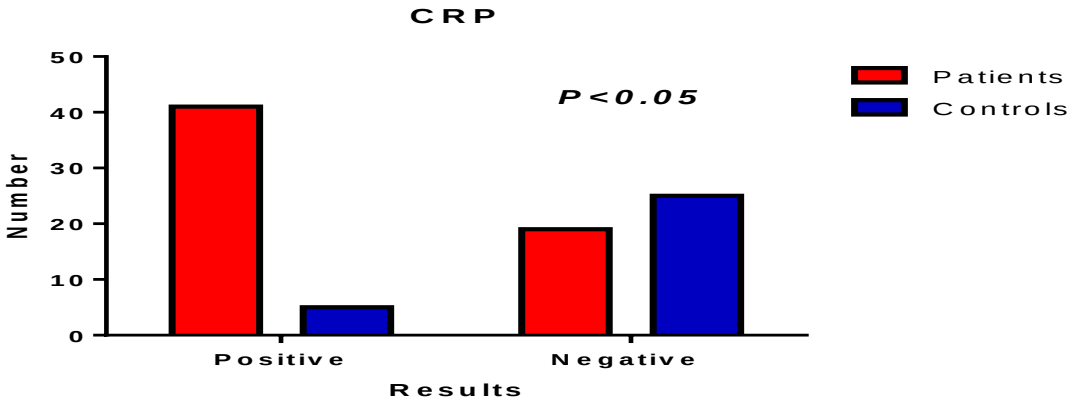

Figure-1 ; Comparison of CRP levels between the two groups of patients and healthy.

6. Measurement of the level of C4 and C3 in the serum of both groups of patients and healthy subjects:

The results of the current study showed the levels of complement components C4 and C3 estimated in (mg/dl), that the level of C3 in the sera of patients with periodontal disease, increased significantly (P = 0.001) compared to its level in the sera of healthy subjects, where the mean ± standard deviation was (208.78 ± 55.40 and 138.67 ± 39.69) mg/dl, respectively, and it was also found that the level of C4, significantly increased (P =0.004) when comparing the two groups of patients and healthy (43.65 ± 10.80 and 30.03 ± 9.81) mg/dl on the respectively, as in (table 6).

Table 6; Comparison of the levels of C4 and C3 between the two study groups.

Study groups		N	Mean	Std. Deviation	P value
C3 Mg/Dl	**Patients**	60	208.78	55.40	0.001***
	Controls	30	138.67	39.69	
C4 Mg/Dl	**Patients**	60	43.65	10.80	0.004**
	Controls	30	30.03	9.81	

*** :very high significant differences .
** : high significant differences .

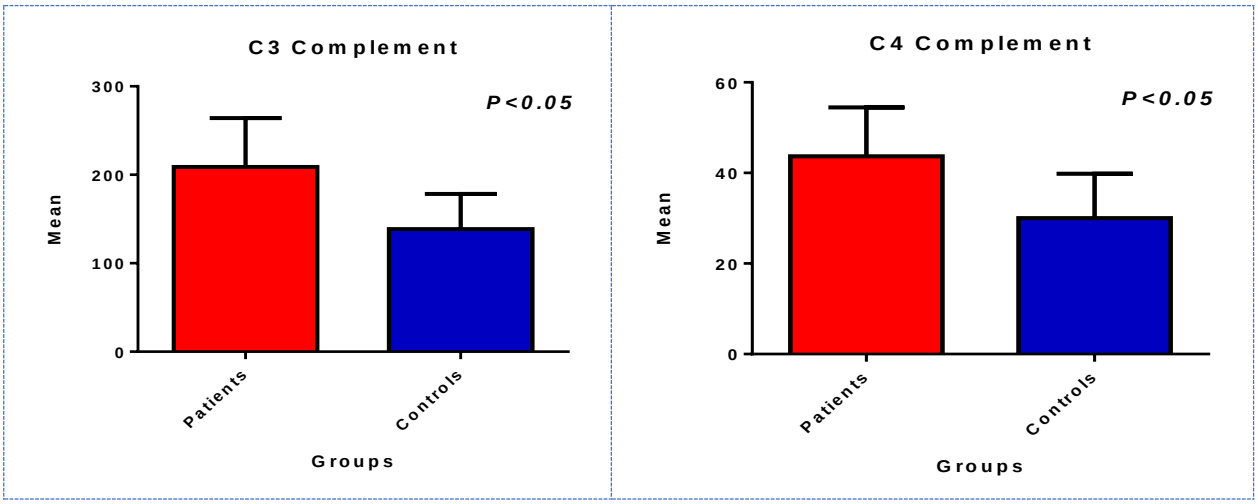

Figure-2 ; Comparison of C3 and C4 levels between the two groups of patients and healthy.

7. Measurement of the level of IgG and IgM in the sera of the two groups of patients and healthy ones

The results of the current study showed that the level of IgM and IgG in sera of patients with periodontal disease was significantly elevated (P=0.003 and 0.001), respectively, compared to its level in sera of healthy patients (228.37 ± 42.83 and 147.33 ± 13.86) (169.72 ± 68.32). and 72.57 ± 8.79) mg/dl, respectively, as in (table 7) .

Table 7; comparison of IgG and IgM levels between the two study groups.

Study groups		N	Mean	Std. Deviation	P value
IgM	Patients	60	228.37	42.83	0.003**
	Controls	30	147.33	13.86	
IgG	Patients	60	169.72	68.32	0.001***
	Controls	30	72.57	8.79	

** :high significant differences .
*** :very high significant differences .

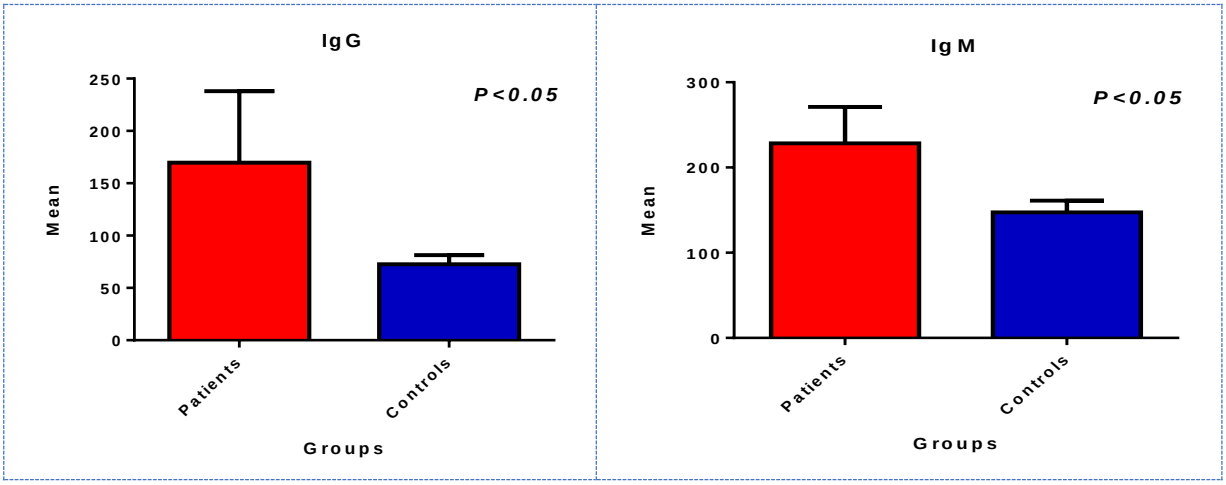

Figure-3 ; Comparison of IgM and IgG levels between the two groups of patients and healthy.

Discussion

Periodontal disease is a complex and progressive chronic inflammatory condition, It affects the gum tissue and Periodontal ligament ، If not treated, it may gradually lead to tooth loss and impaired mastication (Aizenbud and Wilensky, 2023) .Although several epidemiological Males are more likely than females to have periodontitis, according to surveys, but few studies indicated that the reasons for this difference are due to male personal habits such as smoking (Suvan et al.,2020). Furuta et al.,(2011) found that periodontitis is more common in males than in females. This does not agree with the current study's findings, which demonstrated that women are more susceptible to infection than males. The reason may be due to the hormonal changes that occur in women during life stages (Ioannidou, 2017).

Age is one of the important factors associated with the prevalence and severity of periodontal disease, as studies have confirmed that the risk of developing periodontitis increases with age. In an epidemiological study, it was found that the highest prevalence of chronic periodontal disease in the elderly (Tadjoedin et al.,2017); (Alam and Mishra,2012) .Epidemiological studies revealed a high prevalence of gum disease in the elderly compared to the younger age groups, and the reason is due to the accumulation of microbial factors over the course of life, such as dental plaques and microbial deposits (Nazir et al,.2020). As for the results of the current study, it was shown that adults are more susceptible to infection, which may be due to the increase in the sample size compared to the number of elderly people whose numbers do not exceed patients.

One of the main risk factors for periodontal disease development is smoking, and smoking facilitates early acquisition and colonization of periodontal pathogens, and as a result, the periodontal area is damaged (Jain et al,.2020). According to the current study's findings, there is a strong correlation between smokers and their vulnerability to gum disease. (Jiang et al 2020) explained that the effect of nicotine on the circulatory system is the reason for reducing the blood supply and oxygen consumption by hemoglobin, which in turn impairs the body's ability to fight infection and bone resorption. The effect of smoking habits, age and gender on periodontal treatments was statistically evaluated, as it was found that the rate of periodontitis infection among smokers group is significantly high compared to non-smokers (Grover and Bhardwaj, 2013).That tobacco consumption at an early age affects gum health and portends health risks (Ragghianti et al,.2004);(

Machuca et al,.2000).The overall The current study's results are consistent with the findings of these earlier investigations.

The chronic form of gingivitis (Chronic gingivitis) is one of the most common and prevalent types, especially in children, as its symptoms are characterized by swelling of the gum tissue and turning red and bleeding easily upon examination. Humoral and cellular immunity (Chellappa and Prabakar, 2019).This is consistent with the findings on chronic periodontitis as shown in table (4). Adults are typically affected by chronic periodontitis, although children can also develop severe cases of the disease (aggressive periodontitis). Until the afflicted tooth is extracted or the microbial biofilm is eliminated, this pathological condition will alternate between periods of activity and calm (Biofilm). therapeutically, and the swelling goes down. Periodontal disease severity is influenced by host and environmental factors such as smoking and genetic susceptibility (Shimada et al,.2013).Indications of the results of the current study, as shown in table (4), that all ages are exposed to chronic periodontitis at a rate of 30%, with a very high significant difference. Widespread periodontitis is the second most common dental disease in the world, after tooth decay, affecting 30–50% of the US population, with acute (acute periodontitis) cases accounting for only 10% of cases. (Kinane et al,.2017). This aligns with the findings of the present investigation. The current study's findings demonstrated that the rate of acute periodontitis (Acute gingivitis) was 8.3%. Caton et al (2018) stated that acute gingivitis is associated with specific infections, microorganisms or traumas. This inflammation can be easily treated in its early stages, if identified.

Shojaee et al (2013) observed that there is a significant relationship between periodontal disease and CRP concentrations, and a statistically significant result was obtained for the level of CRP when comparing the infected group with the healthy group, and it was found that there was an increase in CRP levels among the infected. This is in agreement with the results of the current study. CRP levels are higher for patients with periodontal disease compared to healthy patients and can be used as a potential biochemical marker to assess periodontal disease activity in gingivitis and periodontitis (Mann et al,.2020); (Patil and Desai, 2013). Several epidemiological studies showed that serum Patients with chronic periodontitis had elevated CRP levels, reaching hundreds of μg/ml. It was discovered that it performed better in terms of responsiveness and specificity to inflammation within hours of infection than erythrocyte sedimentation rate (ESR). Although elevated CRP in the proper clinical context indicates the presence of inflammation or infection, elevated CRP can also occur with obesity. and debilitation (Jayaprakash et al,.2014).

C3 plays an important role in the immune and inflammatory response that is regulated during periodontal disease, as its quality in serum and cervical gingival fluid reflects the status and severity of the disease (Weinstein et al., 2021). Bansal et al (2014) indicated that C3 and C4 levels in the gums or periodontal tissues are approximately 1/25 or 1/3 of the blood circulation levels. Also Kwon et al (2016) found higher levels of C3 and C4 concentrations in patients with periodontal disease compared to the healthy control group. Also Grande et al (2021); Shnawa et al (2015) patients with periodontitis had higher levels of C3 compared to healthy controls.When the results are observed in Table (6) and compared to what was mentioned above, there is a high agreement.

It was linked periodontal disease with rheumatoid arthritis and IgG and IgM antibodies and found elevated levels of these antibodies in patients with periodontitis (Gomez-Bañuelos et al,.2020) ;(Sakai et al, .2001) .There is an association between periodontitis and high levels of IgG and IgM and host-related pathogens (such as opportunistic bacteria and autoimmune diseases) (Buhlin et al., 2015). This is in agreement with the results of the current study. It does not agree with Radhi (2019); Graswinckel et al (2004), who found that IgG and IgM levels are not significantly different between healthy subjects and patients with periodontal disease.

Conclusions

The rate of periodontal disease in females compared to males increased insignificantly .Also, the age group most susceptible to infection is the age group (30-39) and the least vulnerable is the age group (50-59).An increase in the levels of CRP, C3, C4 IgG was also found in patients compared to healthy controls.

Reference

1. AlSharief, M., & Alabdurubalnabi, E. (2023). Periodontal pathogens and adverse pregnancy outcomes: *a narrative review. Life*, 13(7), 1559.
2. Dubey, P., & Mittal, N. (2020). Periodontal diseases—A brief review. *Int. J. Oral Health Dent*, 6, 177-187.
3. Abbas, Y., Elsaadany, B., & Ghallab, N. (2023). Prevalence of different stages of periodontal diseases among a sample of young adult obese Egyptian patients: *a hospital based Cross-sectional study over 1 year. BMC Oral Health*, 23(1), 573.
4. Armitage, G. C., and Cullinan, M. P. (2010). Comparison of the clinical features of chronic and aggressive periodontitis. *Periodontology 2000*, 53, 12-27.
5. Patel, A. (2020). A new approach to periodontal management. *British Dental Journal*, 228 : (4), 1-2. 304.
6. Shankar, S., Manjunath, S., Alqahtani, S. M., Ganji, K. K., Nagate, R. R., Ghokale, S. T., .. & Khader, M. A. (2023). Variations of Serum CRP Levels in Periodontal Health and Diseases: A Clinico-Biochemical Study. Diagnostics, 13(15), 2483.
7. Shojaee, M., Golpasha, M. F., Maliji, G., Bijani, A., Mir, S. M. A., and Kani, S. N. M. (2013). C-reactive protein levels in patients with periodontal disease and normal subjects. *International journal of molecular and cellular medicine*, 2:(3), 2-6.
8. Luan, Y. Y., and Yao, Y. M. (2018). The clinical significance and potential role of C-reactive protein in chronic inflammatory and neurodegenerative diseases. *Frontiers in immunology*, 9, 1-8.
9. Abullais, S. S., Wykole, Y., Khader, M. A., Shamsudeen, S. M., Alanazi, S., Khateeb, S. U., .. & Shamsuddin, S. (2023). Estimation of serum C-reactive protein activity in periodontal health and disease and response to treatment: *a clinico-biochemical study*. *PeerJ*, 11, e16495.
10. Pepys, M. B., & Hirschfield, G. M. (2003). C-reactive protein: a critical update. *The Journal of clinical investigation*, 111(12), 1805-1812.
11. Xu, C., Cw Yi, M., Nb Cheung, M., Dou, D., Dou, Y. D., and Ty Loo, W. (2020a). Cytokines and inflammatory mediators for monitoring the status of periodontitis. *J Dent Oral Biol*, 5:(1), 1157-1162.
12. Podzimek, S., Mysak, J., Janatova, T., and Duskova, J. (2015). C-reactive protein in peripheral blood of patients with chronic and aggressive periodontitis, gingivitis, and gingival recessions. *Mediators of inflammation*, 2015, 1-7.
13. Huang, R. Y., Tseng, F. Y., Cheng, C. D., Van Dyke, T. E., Sung, C. E., You, J. J., .. & Cheng, W. C. (2023). Complement components C3b and C4b as potential reliable site-specific diagnostic biomarkers for periodontitis. *Journal of Periodontal Research*, 58(5), 1020-1030.
14. Maekawa, T., Abe, T., Hajishengallis, E., Hosur, K. B., DeAngelis, R. A., Ricklin, D.,. and Hajishengallis, G. (2014). Genetic and intervention studies implicating complement C3 as a major target for the treatment of periodontitis. *The Journal of Immunology*, 192:(12), 6020-6027.
15. Weinstein, A., Alexander, R. V., and Zack, D. J. (2021). A Review of Complement Activation in SLE. *Current Rheumatology Reports*, 23:(3), 1-8.
16. Sridharan, S., and Sravani, P. M. (2019). Interrelationship between Porphyromonas gingivalis and complement C3 and factor B Levels in chronic periodontitis individuals. *Dentistry and Medical Research*, 7:(2),1-7.

17. Hajishengallis, G., Kajikawa, T., Hajishengallis, E., Maekawa, T., Reis, E. S., Mastellos, D. C., and Lambris, J. D. (2019). Complement-dependent mechanisms and interventions in periodontal disease. *Frontiers in immunology*, 10, 1-11.

18. Gomez-Bañuelos, E., Johansson, L., Konig, M. F., Lundquist, A., Paz, M., Buhlin, K., .. and Andrade, F. (2020). Exposure to Aggregatibacter Actinomycetemcomitans before Symptom Onset and the Risk of Evolving to Rheumatoid Arthritis. *Journal of clinical medicine*, 9:(6), 1-10.

19. Sakai, Y., Shimauchi, H., Ito, H. O., Kitamura, M., and Okada, H. (2001). Porphyromonas gingivalis-specific IgG subclass antibody levels as immunological risk indicators of periodontal bone loss. *Journal of clinical periodontology*, 28:(9), 853-859.

20. Buhlin, K., Holmer, J., Gustafsson, A., Hörkkö, S., Pockley, A. G., Johansson, A., .. and Pussinen, P. J. (2015). Association of periodontitis with persistent, pro-atherogenic antibody responses. *Journal of clinical periodontology*, 42:(11), 1006-1014.

21. Komoriya, T., Kikuchi, M., Terashima, Y., Okamoto, M., Ogawa, M., Moriyama, M., & Kohno, H. (2010). Development of a highly sensitive latex reagent directed against C-reactive protein (CRP) using epitope analysis with monoclonal antibodies . *Bioscience, biotechnology, and biochemistry*, 74(2), 292-297.

22. Mancini, G. J. A. J., Carbonara, A. T., and Heremans, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion . *immunochemistry*, 2:(3), 235-254.

23. Aizenbud, I., Wilensky, A., & Almoznino, G. (2023). Periodontal Disease and Its Association with Metabolic Syndrome—A Comprehensive Review. International *Journal of Molecular Sciences*, 24(16), 13011.

24. Suvan, J., Leira, Y., Moreno Sancho, F. M., Graziani, F., Derks, J., and Tomasi, C. (2020). Subgingival instrumentation for treatment of periodontitis. A systematic review . *Journal of clinical periodontology*, 47, 155-175.

25. Furuta, M., Ekuni, D., Irie, K., Azuma, T., Tomofuji, T., Ogura, T., and Morita, M. (2011). Sex differences in gingivitis relate to interaction of oral health behaviors in young people. *Journal of periodontology*, 82:(4), 558-565.

26. Ioannidou, E. (2017). The sex and gender intersection in chronic periodontitis. *Frontiers in public Health*, 5:(189),1-8.

27. Tadjoedin, F. M., Fitri, A. H., Kuswandani, S. O., Sulijaya, B., and Soeroso, Y. (2017). The correlation between age and periodontal diseases. *Journal of International Dental and Medical Research*, 10 (2): 327-332.

28. Alam, M. N., Mishra, P., and Chandrasekaran, S. C. (2012). Gender basis of periodontal diseases. Indian J Basic *Appl Med Res*, 2:(1), 128-135.

29. Nazir M., Al-Ansari A., Al-Khalifa K., Alhareky M., Gaffar B. and Almas K. (2020). Global prevalence of periodontal disease and lack of its surveillance. *The Scientific World Journal*, 2020,1-8.

30. Jain, A., Bhavsar, N. V., Baweja, A., Bhagat, A., Ohri, A., and Grover, V. (2020). Gender-Associated Oral and Periodontal Health Based on Retrospective Panoramic Radiographic Analysis of Alveolar Bone Loss. In Gender-related Differences in Health and Disease. IntechOpen.

31. Jiang, Y., Zhou, X., Cheng, L., and Li, M. (2020). The impact of smoking on subgingival microflora: From periodontal health to disease. *Frontiers in microbiology*, 11:(66),1-13.

32. Grover H. and Bhardwaj A. (2013).Smoking and Periodontal Disease. *Journal of Pharmaceutical and Scientific Innovation*, 2:(2),7-13.

33. Ragghianti, M. S., Greghi, S. L. A., Lauris, J. R. P., Sant'Ana, A. C. P., and Passanezi, E. (2004). Influence of age, sex, plaque and smoking on periodontal conditions in a population from Bauru, Brazil. *Journal of Applied Oral Science*, 12:(4), 273-279.

34. Machuca, G., Rosales, I., Lacalle, J. R., Machuca, C., and Bullón, P. (2000). Effect of cigarette smoking on periodontal status of healthy young adults. *Journal of periodontology*, 71:(1), 73-78.

35. Chellappa, L. R., and Prabakar, J. (2019). INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES . *International Journal of Research in Pharmaceutical Sciences*, 2020 : (11) 134-139 .

36. Shimada, Y., Tabeta, K., Sugita, N., and Yoshie, H. (2013). Profiling biomarkers in gingival crevicular fluid using multiplex bead immunoassay. *Archives of oral biology*, 58:(6), 724-730.

37. Kinane, D. F., Stathopoulou, P. G., and Papapanou, P. N. (2017). Authors' reply: Predictive diagnostic tests in periodontal diseases. *Nature Reviews Disease Primers*, 3:(1), 1-14.

38. Caton, J. G., Armitage, G., Berglundh, T., Chapple, I. L., Jepsen, S., Kornman, K. S., .. and Tonetti, M. S. (2018). A new classification scheme for periodontal and peri-implant diseases and conditions–Introduction and key changes from the 1999 classification. *J Clin Periodontol* ، 20, 577-568.

39. Mann, J., Bernstein, Y., and Findler, M. (2020). Periodontal disease and its prevention, by traditional and new avenues. *Experimental and therapeutic medicine*, 19:(2), 1504-1506.

40. Patil, V. A., and Desai, M. H. (2013). Effect of periodontal therapy on serum C-reactive protein levels in patients with gingivitis and chronic periodontitis: a clinicobiochemical study. *The journal of contemporary dental practice*, 14:(2), 233-237.

41. Jayaprakash, D., Aghanashini, S., Vijayendra, R. R., Chatterjee, A., Rosh, R. M., and Bharwani, A. (2014). Effect of periodontal therapy on C-reactive protein levels in gingival crevicular fluid of patients with gingivitis and chronic periodontitis: A clinical and biochemical study. *Journal of Indian Society of Periodontology*, 18:(4), 456-460.

42. Bansal, T., Pandey, A., Deepa, D., and Asthana, A. K. (2014). C-reactive protein (CRP) and its association with periodontal disease: a brief review. *Journal of clinical and diagnostic research: JCDR*, 8:(7), 21-24.

43. Kwon, E. Y., Choi, Y. K., Choi, J., Lee, J. Y., and Joo, J. Y. (2016). Effective Management of Acute Necrotizing Ulcerative Gingivitis with Proper Diagnosis and Immediate Treatment. *Journal of Korean Dental Science*, 9:(2), 81-89.

44. Grande, M. A., Belstrøm, D., Damgaard, C., Holmstrup, P., Thangaraj, S. S., Nielsen, C. H., and Palarasah, Y. (2021). Complement split product C3c in saliva as biomarker for periodontitis and response to periodontal treatment. *Journal of Periodontal Research*, 56:(1), 27-33.

45. Shnawa, I. M., Alamiedi, B. H., and Al Fatlawy, Z. M. (2015). Normo Hyper and Extreme Hypercomplementemia in Human Chronic Periodontitis. *American Journal of Biomedical and Life Sciences 2015*, 3:(4-1), 4-6.

46. Radhi, R. A. (2019). Evaluation of High Sensitivity C-reactive Protein, IgM Anticardiolipin Antibody and IgG Antiphosphoryl choline levels in Serum of Patients have Chronic Periodontitis with and without Atherosclerotic Cardiovascular Disease (Doctoral dissertation, University of Baghdad).

47. Graswinckel, J. E. M., Van Der Velden, U., Van Winkelhoff, A. J., Hoek, F. J., and Loos, B. G. (2004). Plasma antibody levels in periodontitis patients and controls . *Journal of clinical periodontology*, 31:(7), 562-568.