Effects of non-surgical periodontal treatment on serum C-reactive protein level among coronary Artery disease patients: Meta-analysis

Ashraf Elzer[1], Emad Saleh GabAlla[2] , Khalid Omar Sassi[3] ,Mohamed Salem Jedeh[4]

1- MSc in Clinical Research, College of Medical Technology, Derna, Libya.
2-MSc in Cardiology, Faculty of Medicine, Omar Al-Mukhtar University, Derna, Libya.
3- MSc in prosthodontics, College of Medical Technology, Derna, Libya.
4-MSc in Clinical Research and conservative dentistry, Zintan College of Dentistry, Algabal Algarbi University, Zintan, Libya.
Corresponding address: Dr.Ashraf Elzer, College Of Medical Technology, Derna, Libya.
E-mail; ashrafsmelzer@yahoo.com

ABSTRACT

Objective: Periodontitis is relatively common chronic condition that has been associated with increased the risk of coronary artery disease through the elevation in the systemic inflammatory markers. . The aim of this Meta-analysis was to examine whether treatment of periodontal disease with scaling and root planning is associated with a reduction in serum level of C-reactive protein.

Design: Meta-analysis of randomized controlled trials.

Data source: PubMed/Medline database with reference lists of eligible studies that study the effect of periodontal therapy on C-reactive protein level up to February 2017 using the following keywords, C-reactive protein; Coronary artery disease; periodontal treatment; randomized controlled trail; risk factors.

Study selection: All English –language Randomized controlled trials that dealing with C-reactive protein as an inflammatory markers in patients with documented chronic sever periodontitis allocated to either treatment arm with scaling and root planning or control arm.

Data extraction: independent investigator was involved in the process of studies selection. Study design, and methodological quality were assessed according to the CONSORT 2010 statement and then included in this meta-analysis.

Results: The search in literature initially retrieved **70** related studies available in the databases. From these, and in accordance with inclusion criteria, only **11** were selected, of which only **4** fulfilled the criteria of randomized clinical trial design. According to CONSORT 2010 statement (consolidated standards of reporting trials), the studies evaluated generally presented good quality with regard to the criteria analyzed. Among the analyzed studies, treatment of periodontal disease has a positive effect with statistical significant reduction on the levels of C-reactive protein **{-0.99, p-value=0.0001}**, without evidence of heterogeneity between the studies (p=0.48);I^2=0%.

Conclusion: Treatment of periodontal disease with scaling and root planning can be considered to be an efficient way to reducing the incidence of coronary artery disease through reductions in the serum levels of C-reactive protein.

INTRODUCTION:

The relationship between oral infections diseases and systemic diseases has getting widely interesting among healthcare professionals. The main oral diseases in most studies that have linked oral diseases with systemic disease have been periodontal diseases [1,2]. Periodontitis is relatively common clinical conditions that can be define as chronic inflammatory process of multifactorial in nature that affects the tooth-supporting tissues in

response to accumulation of bacteria, which are predominantly Gram-negative. Due to it is multifactorial in nature, many of risk factors such as smoking, presence of systemic illness, use of medications and local biofilm retention, among others, may aggravate this clinical condition and presents with different degrees of progression, from the appearance of initial alterations such as gingivitis to various severity levels of periodontitis. Early diagnosis of periodontal disease can ensure that periodontal treatment will have high of success rate as the main goal of periodontal therapy is to remove the causative factors and thereby prevent the progression of disease which finally can preserving the state of health of such individuals and avoiding recurrence of periodontitis. Periodontal pathogens affect the immune system and promote local and systemic inflammatory responses[3]. Thus, periodontal therapy basically aims to control this inflammation. Long term persistent of localized infection may represent a possible contributor to influence the systemic levels of inflammatory markers. One of these inflammatory mediators is C-reactive protein, which is an acute-phase protein produced by various inflammatory stimuli such as trauma, infection and hypoxia. C-reactive protein levels guide decisions regarding diagnosis, monitoring and therapy for inflammatory processes and associated diseases[4,5]. Epidemiological studies have demonstrated an association between periodontitis and serum levels of C-reactive protein. It has been seen that its levels are generally higher in the presence of periodontal disease[6,7] and that, even if this inflammatory marker only presents a slight elevation, it may predispose individuals to coronary events. Moreover, it has been shown that C-reactive protein plays a role in the pathogenesis of atherosclerosis, and recent evidence has associated periodontal disease with a high risk of formation of atherosclerotic plaque, which is the principal cause of all cardiovascular diseases. Chronic presence of periodontal disease may lead to changes in systemic conditions via two pathological ways : (1) direct invasion of the periodontal pathogen into the arterial wall; and (2) release of inflammatory mediators in response to the inflammation, with

atherogenic effect[17]. C-reactive protein has a role in endothelial dysfunction. From this perspective, and with the recognition that more than 50% of the cases of cardiovascular diseases are not associated with classical risk factors, a chronic inflammatory process like periodontal disease may be considered to be an important complementary factor in this causal chain. The theory that sustains the hypothesis that periodontal infection may increase the serum levels of C-reactive protein is summarized in the chart below (Figure 1). In this respect, and of conflicting data regarding the topics of an association between periodontal disease and high levels of C-reactive protein and the use of periodontal therapy for reducing the levels of this protein, the aim of the present study was to conduct a meta-analysis on randomized controlled trials that have an evidence regarding the effect of periodontal treatment on the serum levels of C-reactive protein.

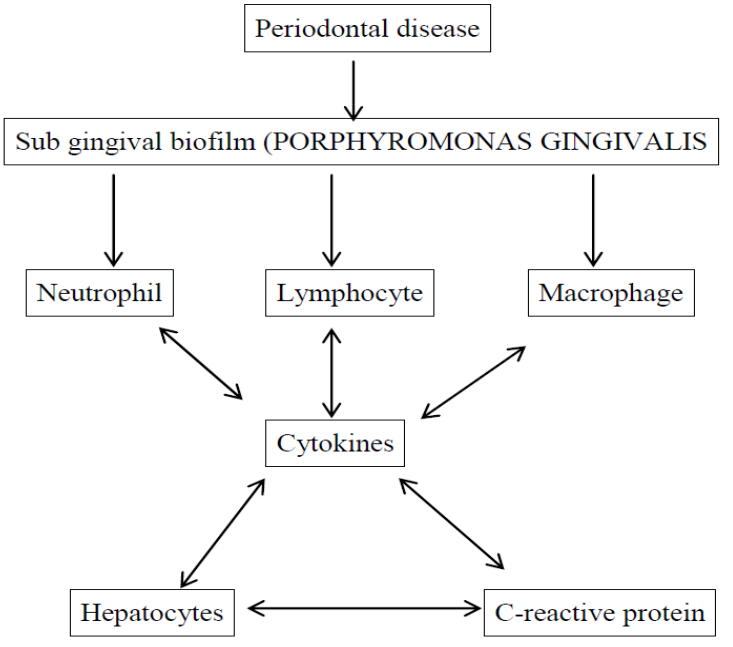

Figure 1- Biological diagram explain how the periodontal infection may increase the serum levels of C-reactive protein. [17]

Material And Methods:

Literature search:

An independent investigator searched for articles in the PUBMED/MEDLINE search site with restriction to inclusion criteria up to February 2017 by using the key words "(periodontal disease; periodontitis; c-reactive protein; cardiovascular event; periodontal treatment; periodontitis; and periodontal therapy)."All randomized controlled trials that allocated the Coronary artery disease patients to receive treatment with scaling and root planning versus no treatment or oral hygiene prophylaxis were eligible for inclusion ".

Inclusion criteria:

All articles with English-language in full-text that studied the effects of periodontal treatment on the serum levels of C-reactive protein among humans were selected. Also study designs with randomized controlled trials were selected. Because of the small number of studies found to be available, no restrictions regarding sample size or type of therapy were imposed (Fig. 2).

Procedures for obtaining data: An independed reviewer searched on PubMed /Medline search site to selected the eligible articles that fitted with the inclusion criteria. All articles that met the inclusion criteria have been obtained in full text. Subsequently, a chart compiling the following data of interest extracted from each investigation was created: author, year, study design, location where the research had been done, sample size, criteria used for determining the presence of periodontal disease, type of periodontal treatment used, C-reactive protein values before and after the therapy, method and time used for measuring C-reactive protein, and the result found, with its statistical significance value (table.1).

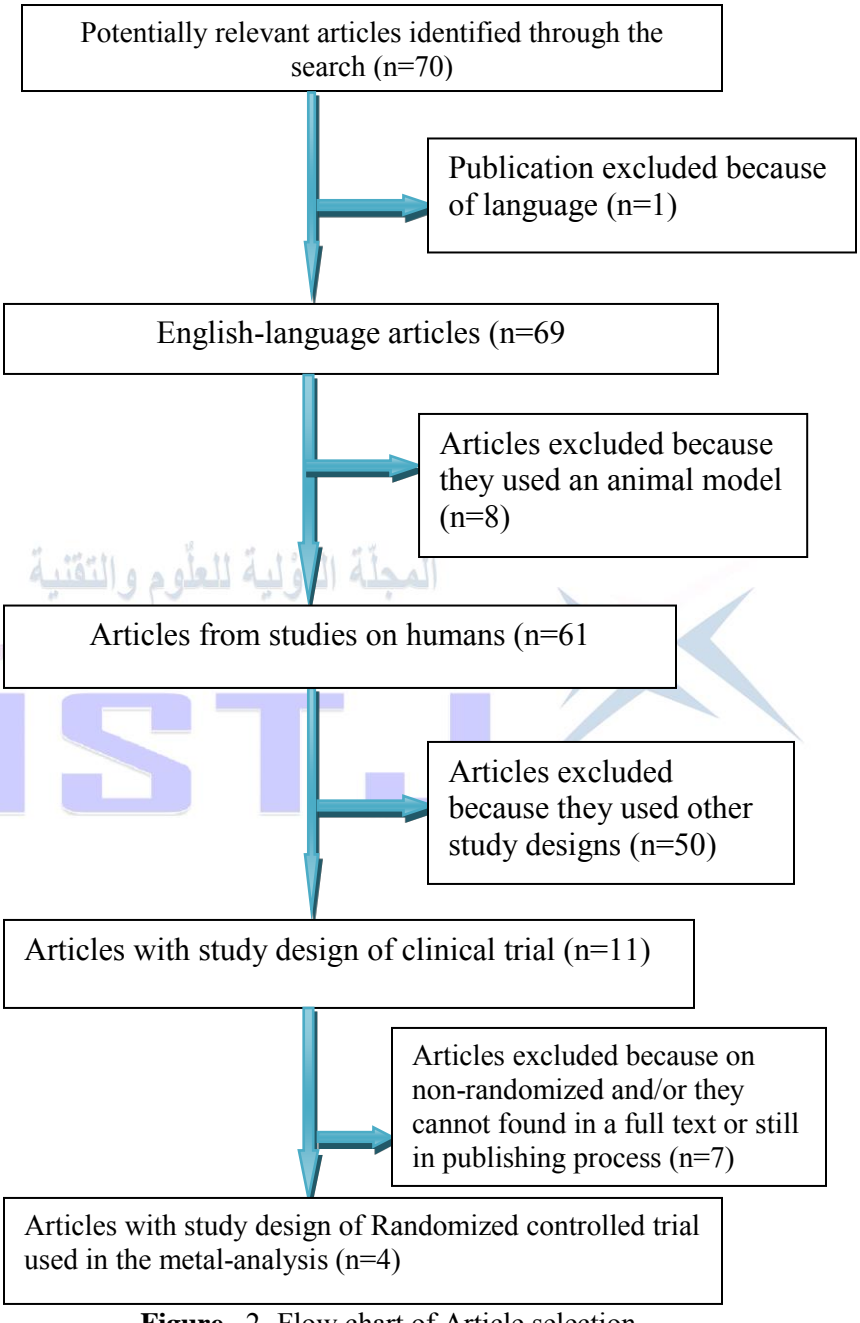

Figure. 2- Flow chart of Article selection

Table 1- Summary of characteristics included trials

Time of C-reactive measurement	**Before and after 3 months**	**Before and after 2 months**	**Before and after 2 months and 6 months**	**Before and after 1,2 months and 6 months**
Result and significance	↓ **P<0.001**	↓ **P<0.001**	↓ **P=0.09**	↓ **P= <0.05**
C-reactive values (after)	**0.07±0.26 mg/l**	**3.1±0.2 mg/l**	**2.4±2.7 mg/l**	**1.1±1.4 mg/l**
C-reactive values (before)	**1.00±0.00 mg/l**	**4.4±0.2 mg/l**	**2.5±2.7 mg/l**	**1.8±1.1 mg/l**
Type of periodontal therapy applied	**SRP**	**SRP**	**Intensive SRP+local antibiotic**	**Intensive SRP+local antibiotic**
Definition of periodontal disease	**Chronic periodontitis with PD≥4mm**	**At least 14 periodontal evaluable natural teeth present; ≥4 teeth with ≥1 sites with PD≥4mm; CAL≥3mm at same sites; BOP>20% of sites**	**Sever generalized periodontitis PD>6mm; marginal bone loss of >30% with 50% or more of their**	**Sever generalized periodontitis having at least 50% of teeth with PD > 4 mm**
Sample Size	**30**	**317**	**120**	**40**
Study design	**RCT**	**RCT**	**RCT**	**RCT**
Country	**North Sudan**	**Pakistan**	**USA**	**England**
Author, Year	**Ahmed Tawfig, et al[16].2015**	**Bokhari SAH, et al[14], 2012**	**Maurizio S.Tonetti et al[13], 2007**	**Francesco D'Aiuto, et al [11] ,2006**

RCT=Randomized Controlled Trail,
SRP=scaling and root planning, = reduced , PD= probing depth, CAL=clinical attachment loss, BOP=bleeding on probing.

Quality assessment :

The selected randomized controlled trials were assessed in accordance with CONSORT (Consolidated Standards of Reporting Trials)[9], 2010 statement. The criteria used for analyzing the quality were:

Randomization processes: assessment of whether the allocation method used was random.

Blindness procedure: assessment of whether the study participants, the sample and/or the investigators were unaware of (blind to) the details of the study method and, if so, at what level (double blind or single blind).

Intervention procedures: precise data on the intervention, such as the specific technique applied. **Statistical analysis method:** assessment of the statistical test used for analyzing the primary data, along with the additional methods for analyzing subgroups and making adjustments.

Statistical analysis method: type of statistical test used for the outcomes variables.

Attrition rate: Also we assess the participant's dropout during the study period.

Statistical analysis :

As this study designed to investigate causal relationship between periodontal therapy and C-reactive protein levels, only studies with a randomized clinical trial design were chosen for analysis via meta-analysis. The objective was to summarize the mean effect of periodontal therapy in relation to C-reactive protein levels from a series of published randomized clinical trial studies.

To perform the meta-analysis, the mean difference and standard error estimated for each of the selected studies were used. The possibility of heterogeneity between the studies was checked by means of the heterogeneity test, through adjustment of a random-effects model (Review Manager 5.3). A forest plot graphical representation for a fixed-effects model was finally generated. For all procedures, a significance level of 5%($p \leq 0.05$) was established. The statistical packed used was Review Manager (RevMan) version 5.3

RESULTS :

Literature search

From the key words used, 70 potentially relevant articles were found, among which one was excluded because it was not in the English language and 8 were excluded because it used an animal model. Among the remaining 61, 50 were excluded because they did not use a study design of clinical trial type. Only 11 fulfilled the previously established inclusion criteria. Of these, only 4 presented the characteristic of randomization of the sample. Randomization was a fundamental criterion for investigating the causality between the effect of periodontal therapy and the level of C-reactive protein, i.e., the main objective of this meta-analysis.[11, 13,14,16]

Statistical analysis :

The four randomized clinical trials were combined into a single analysis using a fixed-effected model, since no heterogeneity was observed between the studies {I^2 =0%(p=0.48)} (Figure 3). The general mean difference in C-reactive protein levels from before to after treatment was {-0.99, (95%Cl -1.05,-0.93)} mg/L, which was a statistical significant result (p=0.0001). Periodontal treatment was seen to be statistical significant protective factor for the serum levels of C-reactive protein.

Figure (3)shows a forest plot graphical representation in which the mean differences in each study can be seen individually, along with the result from grouping them (shown by the colored square point). On the left side of the figure, the studies used in the meta-analysis are listed. The horizontal lines represent 95% confidence intervals for mean differences (before and after) estimated for each study. The points on each vertical line represent the mean difference in each study, such that the size of these points is directly proportional to the respective weight. If the points crosses the thick vertical line at the center of the graph, the 95% confidence interval then contains the value zero, which gives evidence that in the respective study, the effect of the treatment on the occurrence of the event is non-significant (the interpretation is analog and usually done when the p value is greater than 5%). When the confidence intervals touch or cross line representing the

value zero, it needs to be considered that the results from the study did not, in themselves, reach statistical significance, i.e. there was no difference between before and after the experimental treatment.

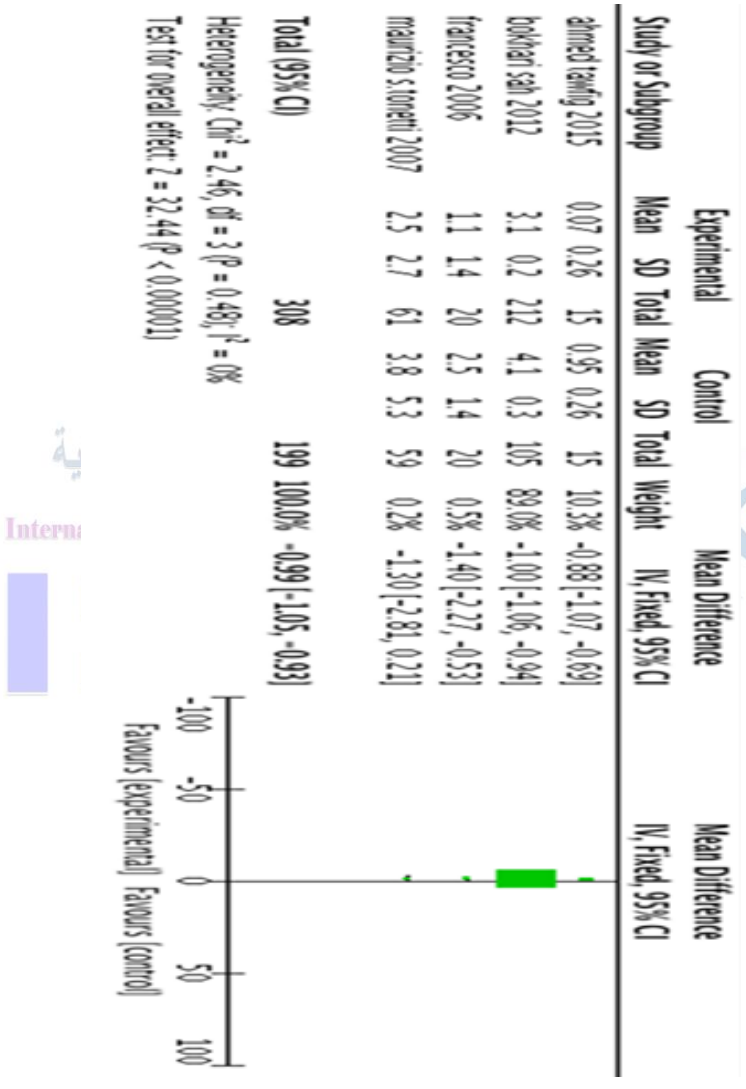

Figure 3- forest plot graphical for changing in serum levels of C-reactive protein from before to after the periodontal treatment

Table 2-Quality analysis of the randomized clinical trials included in the meta-analysis

	Bokhari,et al.[14] (2012)	Ahmed Tawfig,[16] (2015)	D'Aiuto,et al.[11] (2006)	Tonetti, et al.[13] (2007)
Attrition	**2:1 ratio**	**Unreported**	**Unreported**	**5%**
Statistical analysis method	**Positive, ITT (intent-to-treat). T –test, paired t-test and Chi-square test.**	**Positive, T-test**	**Positive (T-test, chi-squared, ANOVA,Bonferroni,Spearman)**	**positive (ANOVA, Fischer, Bonferroni**
Intervention procedures	**Positive (Mechanical non-surgical periodontal therapy included supra- and sub gingival scaling using ultrasonic instrumentation, whereas root planning was performed using Gracey curettes). Multiple appointments.**	**Positive (full-mouth instrumentation, scaling and root planning. Single session.**	**Positive (intensive full-mouth instrumentation, scaling and root planning. By means of Piezoceramic device under local anesthesia with adjunctive local delivery of minocycline microspheres in Single session.**	**Positive (intensive periodontal treatment in a single session*)**
Blindness	**Single-blind parallel-arm.**	**Not cited**	**Not cited**	**Single-blind parallel-arm.**
Randomization	**Positive (Eligible patients randomized into intervention or control group with a ratio 2:1 by computer generated simple random tables; allocation was concealed by (sealed envelopes)**	**Positive (Eligible patients randomized into intervention or control group based on periodontal examination and serum lipid level)**	**Positive (Eligible patients randomized into intervention or control group using random permuted block approach; allocation was concealed by (opaque envelopes)**	**Positive (patients were randomly assigned with the use of a computer-generated table and permuted block approach)**

Analysis of study methods

All of the **4** studies used in this meta-analysis chose non-surgical treatment consisting of scaling and root planning in a single session, except Bokhari et al (2012), use multiple sessions. Different techniques were used for evaluating the C-reactive protein levels, namely: Digital spectrophotometer[16], immunoturbidimetric test[13, 11,] and high-sensitivity assay test from commercial laboratory[13, 14,17](Table 2).

It is also emphasized that, among the **4** articles that fully fitted within the inclusion criteria, **3** presented significant reduced C-reactive protein levels after the periodontal therapy.

Analysis of clinical trial quality

All randomized clinical trial studies were evaluated for there quality . All the four studies, they presented the method used in detail, thus fulfilling the CONSORT assessment criteria[9]. In the study by Bokhari, et al. [14](2012), the masking method used was single blinding, in which the effect of the periodontal treatment at the baseline and two months after the periodontal treatment was evaluated. The periodontal therapy in this study was delay for control group and it shows significant reductions in C-reactive protein values in treatment group. In the second randomized trial by D'Aiuto, et al. (2006) [11], allocated of subjects for periodontal treatment was clear using random permuted block approach, but the evaluation period was extended and included C-reactive protein analysis at the baseline and one, two and six months after the therapy. Their results suggested that intensive periodontal treatment reduced C-reactive protein and other inflammatory markers. The third study by Tonetti, et al.[13] (2007) conducted a single-blind study on two groups of individuals, using two types of periodontal treatment to evaluate endothelial function. In addition, they analyzed inflammatory markers at the times of 1, 7, 30, 60 and 180 days after the treatment, and concluded that intensive periodontal treatment resulted in an immediate acute systemic inflammatory response of short-term nature, with alteration of endothelial function. After 7 days, the results showed that there

had been a reduction in the serum level of C-reactive protein, but this reduction did not reach the statistical significant (p=0.09)) and a large improvement in endothelial function. Finally, in the most recent randomized clinical trial on this topic by Ahmed Twafig, et al. [16](2015) conducted study in the effect of non-surgical periodontal therapy on hyperlipidemic patients to evaluated the level of c-reactive protein, in this study, the masking technique was not cited and the randomization processes was based on the result of periodontal examination and serum lipid levels and concluded that periodontal therapy resulted in improved periodontal health, with significant decrease in C-reactive proteins and others inflammatory markers.

Publication Bias:

Despite the fact that recent evidence suggests that in most meta-analyses the application of funnel plot asymmetry tests to detect publication bias is inappropriate or not meaningful, especially when few trials are included in the meta-analysis[15]. We assessed the presence of publication bias by using the funnel plots. The funnel plot seems to be symmetrical (figure 4).

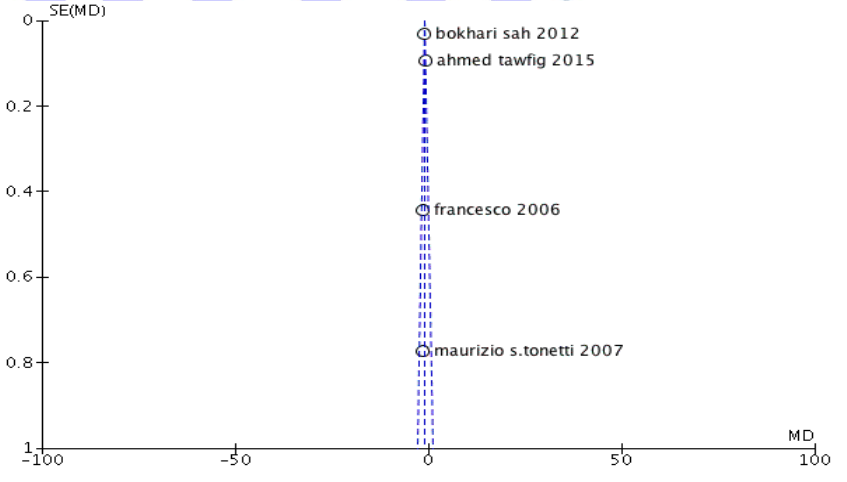

Figure 4-Funnel plot for estimation of publication bias

DISCUSSION :

Our meta-analysis finding shows that non-surgical periodontal therapy (scaling and root planning) has a positive effect in reducing the serum levels of C-reactive protein with statistically significant results. Our finding was agree with Freitas et al.[17] (2012), who conducted a systemic review and meta-analysis on the influence of periodontal therapy on C-reactive protein level and he showed statistical significant reduction on the level of serum c-reactive protein as an effect of periodontal therapy. In contrast, these findings go against those of Ioanidou, et al.[8] (2006), who conducted a systematic review and meta-analysis with the same objective and did not find statistical significance between periodontal treatment and serum levels of C-reactive protein, however, Ioanidou groups showed that treatment of periodontitis results in reduction in the level of C-reactive protein. Despite of, small number of randomized controlled trails that used in our meta-analysis, this did not seem to have a negative effect with the statistical significance. Therefore, our finding regarding the hypothesis that periodontal therapy has a reductive effect on C-reactive protein levels can be accurate and trusted. Furthermore, we included in our meta-analysis only the studies with design of randomized controlled trails that studied the effect of periodontal treatment on the reduction of level of serum C-reactive protein to support our result because of epidemiological researchers consider the well designed Randomized controlled trails as gold standard of clinical research design for establishing causal relationship.

On the other hand, many of observational epidemiological studies [6,13] had linked the periodontitis with increase level of C-reactive protein, but these studies they do not showed a causative relationship because of some confounder variables that can not be controlled due to the study design like smoking, diabetes and obesity.

In the present meta-analysis, 4 studies met the inclusion criteria and they all showed decreased in the levels of C-reactive protein after the periodontal treatment. However, after statistical analysis of the C-reactive protein values before and after the periodontal

treatment, three of these studies presented statistically statistical significant results [11,14,16], as shown in (table 1).

Heterogeneity analysis among our studies had shown no statistical difference and this homogeneity can be explained as the selected studies have the same study deigns that adhered to the inclusion criteria which raised our confidence for our result. Moreover, selection the type of periodontal treatment and the times for measuring the level of serum C-reactive protein used for analysis in this study had gave our study further unique characteristics.

Among the studies evaluated, a variety of times were used for measuring the serum level of C-reactive protein (ranging from 1 day to 6 months), as well as different lengths of periodontal treatment. For analysis purposes, we always include the values of serum C-reactive protein that measured at the end of the study, which range from 2-6 months.

Despite of the statistical significant result among the studies, it showed that the periodontal treatment used in this analysis that consist non-surgical scaling and root planning had a positive effect on reducing the serum levels of C-reactive protein and showed significant improvements in periodontal parameters. Although, it can be noted that some variability we cannot control in this analysis like genetic factors that could affect the result of our study. Finally, because of fact that the high levels of C-reactive protein have been associated with coronary artery diseases which increase the human mortality rates worldwide, further studies with double blind randomized controlled trails with large sample size are required to determine whether the treatment of sever periodontitis could contribute to the prevention of cardiovascular diseases in adults populations.

CONCLUSION:

Although, Our finding in this meta-analysis has agreement with the updated evidence that encourage the use of scaling and root planning as an efficient method of reducing the serum level of C-reactive protein in patient with chronic periodontitis and improving the cardiovascular outcome. However, another meta-analysis on larger number of well-designed Randomized controlled trails

studies that have high methodological quality with large sample size is needed to robust our result and help in reducing the risk of cardiovascular events.

REFERENCES

1. Loss BG, Craandijk J, Hoeck FJ, Wertheim-van Dillem PME, Van Der Velden U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. J Periodontol. 2000;74:1528–1534.
2. Wakai K, Kawamura T, Umemura O, Hara Y, Machida J, Anno T, et al. Associations of medical status and physical fitness with periodontal disease. J Clin Periodontol. 1999;26:664–672.
3. . Noack B, Genco RJ, Trevisan M, Grossi S, Zambon JJ, Nardin ED. Periodontal infections contribute to elevated systemic C-reactive protein level. J Periodontol. 2001;72:1221–1227.
4. Glurich I, Grossi S, Albini B, Ho A, Shah R, Zeid M, et al. Systemic inflammation in cardiovascular and periodontal disease: comparative study. Clin Diagn Lab Immunol. 2002;9:425–432.
5. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest. 2003;111:1805–1812.
6. Ebersole JL, Machen RL, Steffen MJ, Willmann DE. Systemic acute-phase reactants, C-reactive protein and haptoglobin, in adult periodontitis. Clin Exp Immunol. 1997;107:347–352.
7. Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acutephase inflammatory response to periodontal disease in the US population. J Dent Res. 2000;79:49–57.
8. Ioannidou E, Malekzadeh T, Dongari-Bagtzoglou A. Effect of periodontal treatment on serum C-reactive protein levels: a systematic review and meta-analysis. J Periodontol. 2006;77:1635–1642.
9. Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials.

Lancet. 2001;357:1191–1194.
10. D'Aiuto F, Nibali L, Parkar M, Suvan J, Tonetti MS. Short-term effects of intensive periodontal therapy on serum inflammatory markers and cholesterol. J Dent Res. 2005;84:269–273.
11. D'Aiuto F, Parkar M, Nibali L, Suvan J, Lessem J, Tonetti MS. Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. Am Heart J. 2006;151:977–984.
12. Ide M, Jagdev D, Coward PY, Crook M, Barclay GR, Wilson RF. The short-term effects of treatment of chronic periodontitis on circulating levels of endotoxin, C-reactive protein, tumor necrosis factor-alpha, and interleukin-6. J Periodontol. 2004;75:420–428.
13. Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, et al. Treatment of periodontitis and endothelial function. N Engl J Med. 2007;356:911–920.
14. Bokhari, H., et al. "Non-surgical periodontal therapy reduces coronary heart disease risk markers: a randomized controlled trial." Journal of clinical periodontology 39.11 (2012): 1065-1074.
15. loannidis JP, Trikalinos TA.The appropriateness of asymmetry tests for publication bias in meta-analyses: a large survey.CMAJ 2007;176:109-6.
16. Ahmed Twafig2015
17. Freitas, Camila Oliveira Teixeira de, et al. "Influence of periodontal therapy on C-reactive protein level: a systematic review and meta-analysis." Journal of Applied Oral Science 20.1 (2012): 1-8.