IL-12 Levels in Pregnancies with Preeclampsia and Periodontitis

Ayesha Sadiqa[1], Abdul Majeed Cheema[2]

ABSTRACT

Introduction: Periodontal pathology has become a momentous concern of public health due to its increasing prevalence in adults around the globe. Study was aimed to compare serum IL-12 p-70 levels in preeclampted and non-preeclamptic women and to observe serum IL-12 p-70 levels in preeclamptic with and without chronic periodontitis.

Material and methods: It was a Longitudinal Cohort Study. The sampled population belonged to Narowal District of Punjab, Pakistan. Duration of the study was from June 2016 to February 2018. All pregnant subjects' aged in-between 18-34 years were participated though convenience sampling and consented. Out of total 73 subjects, 33 were with healthy periodontium (6 preeclamptic and 27 normotensives) and 40 were with chronic periodontitis (6 preeclamptics and 34 normotensives). Sampling was performed in two phases; First when all subjects were in 2nd trimester and second when all were in postpartum period. Periodontal status was assessed by CPITN (Community Periodontal Index for Treatment Need) probing technique and for preeclampsia, monthly blood pressure profile of each participant was taken by her doctor. Serum estimation of IL-12 p-70 was confirmed through sandwich ELIZA technique. Microsoft Excel and Minitab were used for data analysis.

Results: Normotensives with healthy periodontium showed 17.4% high serum IL-12 p70 (pg/ml) in antepartum than postpartum. Similarly preeclamptics with healthy periodontium exhibited 13.4% high serum IL-12 p70 in antepartum than postpartum. While normotensives with periodontitis displayed 10% high serum IL-12 p70 in postpartum than antepartum. Preeclamptics with periodontitis exhibited 17.4% high serum IL-12 p70 in postpartum than antepartum.

Conclusion: Preeclampsia and chronic periodontitis both decrease serum IL-12 p-70 levels in antepartum and puerperium.

Keywords: Periodontitis, IL-12 p-70, Preeclampsia, Pregnancy, Normotensives.

INTRODUCTION

The prevalence of periodontal disease is approximately 15% in fertile women.[1,2] Chronic periodontitis is a chronic inflammatory infectious disease of periodontium of teeth caused by gram negative anaerobic bacteria. Research data advocates the relationship of periodontitis with numerous systemic disorders such as coronary heart diseases, diabetes mellitus[1], and poor gestational outcomes like preeclampsia.[3] Preeclampsia is a clinical term referred to the onset of hypertension i.e. blood pressure >140 /90 mmHg along with proteinuria >300mg/24 hours after 20th week of pregnancy. It is a prevalent source of maternal as well as perinatal expiry that affects 5–7% of expecting mothers all over the World.[4] Today the association of periodontitis with preeclampsia mainly because of the imbalance of certain immune-inflammatory mediators, has become the point of concern for researchers of present era.[2] Previously placental ischemia has been regarded as the basis of preeclampsia due to increase in oxidative stress, pro-inflammatory cytokines and anti-angiogenic factors. However now a days advanced studies suggested that enhanced inflammation along with angiogenic imbalance are the vital players that ultimately lead to preeclampsia.[4]

Many researchers found that maternal infections caused by periodontal pathogens, raise circulating mediators IL-1β, IL-6, IL-8, IL-17, and TNF-α, all are also known for preeclampsia.[5] Similarly a decrease in specific pro-inflammatory interleukins such as IL-12 p-70 has also been associated with pregnancy[6] or preeclampsia.[5] Moreover it has been revealed that IL-12 in amniotic fluid of pregnant women could not be linked with adverse pregnancy outcomes.[7] An insignificant association of high IL-12 p-70 levels in gingival crevicular fluid (GCF) in patients of chronic periodontitis has been documented.[8]

Chemically IL-12 p-70 is heterodimer of p35 (encoded via Il12a) and p40 (encoded via Il12b) chains, as a pro-inflammatory cytokine it is secreted from many antigen-presenting cells (APCs) like monocytes, macrophages and dendritic cells. IL-12 p-70 excites both innate and adaptive immune regulatory cells and also involved in antimicrobial activity.[9] The aim of current study was two-fold: To compare the maternal serum levels of IL-12 p-70 in preeclampted and non-preeclampted pregnant women, and to study the serum levels of IL-12 p-70 in preeclamptic pregnancies with and without chronic periodontitis.

MATERIAL AND METHODS

A prospective longitudinal cohort study was carried out with gestational female subjects of Narowal District of Punjab, Pakistan. Convenience sampling method was used to and the time line for the study was from June 2016 to February 2018.

[1]PhD Scholar, Department of Physiology, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan, [2]Professor Department of Physiology, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan.

Corresponding author: Ayesha Sadiqa, Assistant Professor, Physiology Department, Shalamar Medical and Dental College, Lahore, Pakistan.

How to cite this article: Ayesha Sadiqa, Abdul Majeed Cheema. IL-12 levels in pregnancies with preeclampsia and periodontitis. International Journal of Contemporary Medical Research 2019;6(12):L3-L6.

DOI: http://dx.doi.org/10.21276/ijcmr.2019.6.12.16

All the subjects in the study were in-between 18–34 years. Out of 73 total pregnant women, 33 were with healthy periodontium that were again stratified into 6 as preeclamptic and 27 as non-preeclamptics. The remaining 40 subjects were affected with chronic periodontitis that were again sub-classified into 6 as preeclamptic and 34 as non-preeclamptic. Written consent were taken from each participant after informed procedural details and use of research. In first phase sera were taken from all the participants in their second trimester, while in second phase sera were again sampled from the same participants in their postpartum period. A dentist assured the periodontal status of each participant by using CPITN (Community Periodontal Index for Treatment Need) probing technique and marked the degree of clinical attachment loss at the gingival sulcus in millimeters. To verify preeclampsia the Blood pressure record was taken by her doctor/gynecologist, after her due approval. Drawn sera were refrigerated at -80°C in labelled eppendrofs.

ELISA kits for serum IL-12 p70 (pg/ml) were used separately for antepartum and postpartum sera samples. Through interpolation on the calibrated curve, the values were obtained.

Microsoft Excel and Minitab version 18 were employed for statistical analysis of the data. Unpaired Student's t-test was used to know the level of significance between the differences of the two groups. Value of alpha was considered as 0.05. Line chart was plotted to exhibit the relative difference in IL-12 p70 (pg/ml) levels of each studied category.

RESULTS

Normotensive subjects with healthy periodontium showed 17.4% raised serum IL-12 p-70 levels in antepartum phase in comparison to postpartum phase (p=0.13), while preeclamptic subjects with healthy periodontium also revealed 13.4% high serum IL-12 p-70 in antepartum period than postpartum period (p=0.009) and the difference proved statistically highly significant.

Normotensive subjects effected with chronic periodontitis exhibited a 10% rise in their serum IL-12 p-70 levels in postpartum phase than antepartum (p=0.37), similarly preeclamptic subjects along with periodontitis also showed 17.4% more serum IL-12 p-70 levels in their postpartum phase compared to antepartum phase (p=0.07), although the difference was not statistically sound (Table 1).

Normotensive subjects effected with periodontitis showed 21.4% high serum IL-12 p-70 compared to preeclamptics with periodontitis in antepartum phase of gestation (p=0.38) and 14% raised level of IL-12 p-70 assessed in postpartum phase (0.43).

Subjects with healthy periodontium exhibited 6.4% increased serum IL-12 p-70 levels again in normotensives compared to preeclamptics in antepartum period (p=0.75) and 2.8% high

Studied Groups		Gestational phase				p-value
		Antepartum		Postpartum		
Periodontium	Preeclampsia	n	Mean±SEM	n	Mean±SEM	
Healthy	No	27	4.32±0.38	24	3.68±0.11	0.13
	Effected	6	4.06±0.1	6	3.58±0.11	0.009*
Chronic Periodontitis	No	34	3.75±0.31	28	4.13±0.28	0.37
	Effected	6	3.09±0.16	6	3.63±0.22	0.07

* Statistically significant as p<0.05.

Table-1: Gestational Comparison of IL-12 p70 (pg/ml) levels between subjects with and without Preeclampsia, in relation to Periodontitis

Studied Groups		History of preeclampsia				p-value
		No		Yes		
Gestational Phase	Periodontal Status	n	Mean±SEM	n	Mean±SEM	
Antepartum (2nd trimester)	Ch. Periodontitis	34	3.75±0.31	6	3.09±0.16	0.38
	Healthy	27	4.32±0.38	6	4.06±0.1	0.75
Postpartum (puerperium)	Ch. Periodontitis	28	4.13±0.28	6	3.63±0.22	0.43
	Healthy	24	3.68±0.11	6	3.58±0.11	0.66

Table 2: Difference between IL-12 p70 (pg/ml) in subjects with and without Preeclampsia, in relation to Periodontitis and Gestation

Comparative Groups		Periodontal status				p-value
		Healthy Periodontium		Chronic Periodontitis		
Periodontium	Preeclampsia	n	Mean±SEM	n	Mean±SEM	
Antepartum	No	27	4.32±0.38	34	3.75±0.31	0.24
	Effected	6	4.06±0.1	6	3.09±0.16	0.0004*
Postpartum	No	24	3.68±0.11	28	4.13±0.28	0.16
	Effected	6	3.58±0.11	6	3.63±0.22	0.84

* Statistically significant as p<0.05.

Table-3: Comparison of IL-12 p-70 (pg/ml) between subjects with and with periodontitis in two gestational phases, in connection to Preeclampsia

serum IL-12 p-70 levels in puerperium (p=0.66). However all differences were not found statistically significant (p>0.05) (Table 2).

Normotensives in their antepartum period showed 15.2% insignificant raised serum IL-12 p70 levels in those who were with healthy periodontium compared to those who were periodontitis (p=0.24), while preeclamptics in antepartum phase revealed up to 31.4% high serum IL-12 p70 levels in those who were with healthy periodontium than those who were effected with periodontitis (p=0.0004) also the difference proved statistically highly significant.

Normotensives in postpartum period showed 12.2% raised serum IL-12 p70 levels in those who were with chronic periodontitis compared to those who were normal periodontium (p=0.16), while preeclamptics in postpartum phase revealed only 1.4% high serum IL-12 p70 levels in those who were with periodontitis than those who were with healthy periodontium (p=0.84). Nevertheless the differences were not statistically significant as p<0.05 (Table 3).

DISCUSSION

The association of periodontitis and preeclampsia has been brought into light a few years back, that's why there has been found a scarcity in literature in this regard.[2] It has been documented that chronic periodontitis aggravates many mediators[10] such as endotoxins, exotoxins, cytokines, chemokines and interleukins[11] that damage the placental membrane by increasing oxidative stress.[12] Many researchers like Offenbacher *et al*., Lin *et al*. and Boggess *et al*. have suggested the association of periodontitis with preeclampsia by relocation of periodontal toxins along with infective bacteria to chorio-amniotic membranes.[5,13] It has also been claimed that even preeclampsia aggravates the pre-formed chronic periodontitis[14], thus this pertinent linkage of periodontitis and preeclampsia has also been announced as a two- way street.[5]

Talking about the pathogenesis of this relation, a group of researchers explained that as in gestation the immune-inflammatory responses of the body have usually been increased because of the hormonal shift that ultimately lead to enhance inflammatory pathologies like periodontitis.[15] Numerous common mediators have been reported by many studies that have been related with both pathologies i.e. chronic periodontitis and preeclampsia.[9,16] IL-12 is one of those mediators, regarding which an uncertainty has still been prevailed[17] as many researchers claimed its increasing serum levels in pregnancy[18,19], while others stated its decrease concentration in antepartum phase[20], even in those effected with periodontitis.

IL-12 is mainly released by antigen-presenting cells (APCs) like monocytes, macrophages and dendritic cells and its bioactive form i.e. IL-12 p-70 involved in regulation of immune processes and antimicrobial activity.[21] IL-12 along with TNF-α, activates the components of cellular immunity such as cytotoxic, natural killer cells, monocytes and macrophages.[22] The current study also attempted to observe the association of IL-12 p-70 with both pathologies in our local population of Narowal District. In a study it was confirmed that pregnant individuals have shown more sensitivity against lipopolysaccharides and to some specific monocyte derived cytokines such as IL-12 and TNF, so both of these mediators decreased in pregnancy as compared to non-pregnant state[22], the results of this study are in agreement with our observations.

Contrary to our findings Enninga E. A. *et al.* from USA documented the increased level of IL-12 p-70 in preeclamptic pregnant females[23], while Heikkinen J. *et al.* from Finland found no association of IL-12 p-70 with preeclampsia.[24] Consistent with our results Szarka A. *et al*. from Hungary reported a fall in serum concentration of IL-12 p-70 in pregnancy compared to non-pregnant state and with statistical significant difference.[20] Elenkov *et al.* again from USA postulated that during terminal pregnancy the raised levels of cortisol, catecholamines and 1,25-dihydroxyvitamin D3 resulted in down-regulation of IL-12, while at postpartum period, when these hormones again low down in their concentration, higher levels of IL-12 again achieved.[22]

Limitations of the study

Present study was conducted with relatively small sample size, also the daily nutritional intake of study population was ignored.

CONCLUSION

Both normotensives and preeclamptics with normal periodontium showed elevated IL-12 p70 levels during pregnancy, but with periodontitis more levels have been observed in postpartum phase. Also Normotensives with/ without periodontitis exhibited more IL-12 p70 during and post-pregnancy phase.

REFERENCES

1. Sadiqa A, Cheema A.M, Malik S. Chronic periodontitis a possible threat towards CVD. Biomedica 2016;32: 29-32.
2. Sayar F, Hoseini MS, Abbaspour S. Effect of periodontal disease on preeclampsia. Iran J Public Health 2011;40:122-7.
3. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. Int J Health Sci (Qassim) 2017;11:72-80.
4. Ramma W, Ahmed A. Is inflammation the cause of pre-eclampsia? Biochem Soc Trans. 2011;39:1619-27.
5. Desai K, Desai P, Duseja S, Kumar S, Mahendra J, Duseja S. Significance of maternal periodontal health in preeclampsia. J Int Soc Prev Community Dent 2015;5:103-7.
6. Faas MM, Kunnen A, Dekker DC, Harmsen HJ, et al. Porphyromonas Gingivalis and E-coli Induce Different Cytokine Production Patterns in Pregnant Women. PLoS One 2014;9:e86355.
7. Cift T, Uludag S, Aydin Y, Benian A. Effects of amniotic and maternal CD-146, TGF-β1, IL-12, IL-18 and IFN-γ, on adverse pregnancy outcome. J Matern Fetal Neonatal Med 2013;26:21-5.
8. Tomás I, Arias-Bujanda N, Alonso-Sampedro M, Casares-de-Cal MA, Sánchez-Sellero C. et al. Cytokine-

based Predictive Models to Estimate the Probability of Chronic Periodontitis: Development of Diagnostic Nomograms. Sci Rep 2017;7:11580.

9. Ma X, Yan W, Zheng H, Du Q. et al. (2015;) Regulation of IL-10 and IL-12 production and function in macrophages and dendritic cells. F1000Res. 4. pii: F1000 Faculty Rev-1465.
10. Sadiqa A, Cheema A.M, Malik S. Mild chronic periodontitis: a possible threat towards CVD in males with raised C-RP. Pak J Physiol 2015;11(3):23-29.
11. De Queiroz AC, Taba M Jr, O'Connell PA, da Nóbrega PB. et al. Inflammation markers in healthy and periodontitis patients: a preliminary data screening. Braz Dent J 2008;19:3-8.
12. Moura da Silva G, Coutinho SB, Piscoya MD, Ximenes RA, Jamelli SR. Periodontitis as a risk factor for preeclampsia. J Periodontol 2012;83:1388–96.
13. Pattanashetti JI, Nagathan VM, Rao SM. Evaluation of Periodontitis as a Risk for Preterm Birth among Preeclamptic and Non-Preeclamptic Pregnant Women - A Case Control Study. J Clin Diagn Res 2013;7:1776-8.
14. Srinivas SK, Sammel MD, Stamilio DM, Clothier B, Jeffcoat MK, Parry S, et al. Periodontal disease and adverse pregnancy outcomes: Is there an association? Am J Obstet Gynecol 2009:497.e1–8.
15. Jaiman G, Nayak PA, Sharma S, Nagpal K. Maternal periodontal disease and preeclampsia in Jaipur population. J Indian Soc Periodontol 2018;22:50-54.
16. Wang L, Li XH, Ning WC. Evaluation of ICAM-1 and VCAM-1 Gene Polymorphisms in Patients with Periodontal Disease. Med Sci Monit 2016;22:2386-91.
17. Yaghini J, Mostajeran F, Afshari E, and Naghsh N. Is periodontal disease related to preeclampsia? Dent Res J (Isfahan) 2012;9:770–73.
18. Shetty M, Shetty PK, Ramesh A, Thomas B, Prabhu S, Rao A. Periodontal disease in pregnancy is a risk factor for preeclampsia. Acta Obstet Gynecol Scand 2010;89:718–21.
19. Boggess KA, Edelstein BL. Oral health in women during preconception and pregnancy: Implications for birth outcomes and infant oral health. Matern Child Health J. 2006;10(Suppl):S169–74.
20. Szarka A, Rigó J, Jr, Lázár L, Bekő G. and Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunol 2010;11: 59.
21. Jianguo Liu, Shanjin Cao, Sunjung Kim. (2015) Interleukin-12: an update on its immunological activities, signaling and regulation of gene expression. Curr Immunol Rev 1(2): 119–37.
22. Elenkov IJ, Wilder RL, Bakalov VK. et al. IL-12, TNF-alpha, and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. J Clin Endocrinol Metab 2001;86:4933-8.
23. Enninga EA, Nevala WK, Creedon DJ, Markovic SN, Holtan SG. Fetal sex-based differences in maternal hormones, angiogenic factors, and immune mediators during pregnancy and the postpartum period. Am J Reprod Immunol 2015;73:251-62.
24. Heikkinen J, Möttönen M, Pulkki K, Lassila O, Alanen A. Cytokine levels in midtrimester amniotic fluid in normal pregnancy and in the prediction of pre-eclampsia. Scand J Immunol 2001;53:310-4.

Source of Support: Nil; **Conflict of Interest:** None

Submitted: 04-11-2019; **Accepted:** 30-11-2019; **Published:** 26-12-2019