ORIGINAL RESEARCH

Estimation of Leptin Levels in Serum in Periodontal Health and Disease: A Clinical and Biochemical Study

[1]Dhaval Shah, [2]Deepak Dave, [3]Monali Shah, [4]Neeraj Deshpande

ABSTRACT

Introduction: Leptin is a hormone secreted by the adipocytes. As the periodontal breakdown increases, leptin concentration in serum increases with the destruction of the periodontium, but the evidence for this association is not so strong. So, the aim of our study was to evaluate the serum leptin levels in healthy, gingivitis and periodontitis group.

Materials and methods: Total 75 patients were selected and divided into three groups. Healthy (BOP < 30%, PD ≤ 4 mm), gingivitis (BOP ≥ 30%, PD ≤ 4 mm) and periodontitis (PD ≥ 4 mm). The clinical parameters that evaluated were OHI-S, PI, PD and % of BOP at baseline, 15 days and 1 month. Also, blood was collected from the anticubical fossa and was analyzed for Leptin ELISA test.

Results: There was a statistically significant reduction in all clinical parameters at the end of 15 days and 1 month. In healthy group, there was strong association between OHI-S and serum leptin levels. Whereas in gingivitis group, there was a statistically significant association between OHI-S, PD and serum leptin levels than in periodontitis group.

Conclusion: In our study, as the periodontal disease progressed, there was raise in serum leptin concentration in gingivitis ($p < 0.005$) but these results did not remain consistent for periodontitis group. From, this we can say that for leptin to be an inflammatory marker and risk indicator or risk predictor in the progression of periodontal diseases further studies are needed.

Keywords: Serum leptin levels, Health, Gingivitis, Periodontitis.

How to cite this article: Shah D, Dave D, Shah M, Deshpande N. Estimation of Leptin Levels in Serum in Periodontal Health and Disease: A Clinical and Biochemical Study. J Orofac Res 2014;4(3):138-142.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Leptin, a product of the obesity gane, is a 16-kDa non-glycosylated peptide hormone. It is synthesized mainly in adipocytes[1] and, in minor quantities by placenta,[2] T cells,[3] osteoblasts[4] and gastric epithelium,[5] which regulate weight control and modulate other physiological functions, such as regulation of neuroendocrine, reproductive and hematopoietic systems and bone remodeling. Recently, leptin has been classified as a cytokine because it shows structural similarities to the long-chain helical cytokine family, which includes interleukin-6, interleukin-11, and leukemia inhibitory factor.[6] Moreover, leptin stimulates the immune system by enhancing proinflammatory cytokine production and phagocytosis by macrophages.[7] Therefore, during infection and inflammation, leptin expression is modulated in a manner similar to the cytokine response to infection and injury. Thus, the overall increase in leptin during infection and inflammation indicates that leptin is part of the immune response and host defense mechanisms. Although, there are no adipocytes in gingiva, in a recent study by Johnson and Serio,[8] it was shown that the leptin concentration is higher in the healthy gingiva compared with the diseased gingiva and they proposed that this might be caused by entrapment of leptin within the gingiva by diffusion from the microvasculature. As leptin has a role in the inflammatory response, an increased leptin level in healthy gingiva may be a host defense mechanism similar to that which occurs during sepsis.[9] However, during gingival inflammation the concentration of leptin is decreased as a result of expansion of the vascular network caused by vascular endothelial growth factor, which may increase the net rate of leptin removal from the gingival tissues.[8]

Plasma leptin and interleukin-6 concentrations are reported to be elevated in acute critical illnesses, such as sepsis and inflammation.[10] Leptin is produced by adipocytes. It inhibits appetite and stimulates the sympathetic nervous system, reducing adipose tissue mass. IL-6 is produced by immune cells and adipocytes and participates in the inflammatory reaction. A strong negative correlation between plasma leptin and IL-6 has been reported in patients experiencing a critical illness.[11] The presence of IL-1 is essential for leptin induction, while IL-6 is not reported to be essential for this process.[12] Leptin stimulates the immune system, as it enhances cytokine production and phagocytosis by macrophages.[13] Both gingival IL-1 and IL-6 are reported to be elevated during progression of periodontal diseases,[14] while the role of leptin in this progression is unknown.

[1]Postgraduate, [2]Professor and Head, [3,4]Professor

[1-4]Department of Periodontics, KM Shah Dental College Sumandeep Vidyapeeth, Vadodara, Gujarat, India

Corresponding Author: Dhaval Shah, Postgraduate Department of Periodontics, KM Shah Dental College Sumandeep Vidyapeeth, Pipariya, Vadodara, Gujarat, India Phone: 09925105127, e-mail: drdvshah31@yahoo.in

Although, there are numerous studies of the relationship between inflammation, leptin metabolism and plasma leptin concentrations, there is no information available concerning the possible role of leptin in progression of inflammation within human tissues, in particular, gingival[11,12,15-17] diseases.

However, to date, the leptin concentration in serum in periodontal health and disease has not been explored alone. Hence, this study was designed to assess the concentration of human leptin in serum during periodontal health and disease, and, in addition, to obtain a more detailed insight into its possible role in the initiation and progression of periodontal disease.

MATERIALS AND METHODS

Study Design

1. *Place of the study*: Department of Periodontics, KM Shah Dental College and Hospital, Sumandeep Vidyapeeth, Pipariya.
2. *Source of the data*: Outpatient Department of Periodontics, KM Shah Dental Collage, Pipariya.
3. *Related approvals*: Nil.
4. *Sample description*: Was selected from the Outpatient Department of Periodontics, KM Shah Dental College. Subjects were divided into three groups as follows:

- Group 1: Healthy gingiva (BOP ≤ 30%)
- Group 2: Moderate to severe gingivitis with BOP ≥ 30%, PD ≤ 4 mm.
- Group 3: Periodontitis with BOP ≥ 30%, PD ≥ 4 mm

There were 25 patients in each group.

5. Selection criteria:
   1. Inclusion criteria
      - Age: 20 to 50 years
      - Subjects who have not received periodontal treatment in previous 6 months
      - Dentition with 20 functioning teeth
   2. Exclusion criteria
   - Patient having any systemic disease.
   - Patient taking any antibiotic/anti-inflammatory drugs for the past 6 months.
   - Patients with teeth which were partially erupted or impacted.
   - Anomalies of the immune system.
   - Pregnancy and lactation.
   - History of any influence in the inflammatory response.
   - Patients with the habit of smoking and alcoholism.
   - Patients with aggressive periodontitis
   - Patients with bleeding disorders.

Material/Equipment for the Study

- Mouth mirror
- William's graduated periodontal probe
- Surgical gloves
- Mouth mask
- Disposable syringe
- Glass tubes and stand
- Human leptin ELISA Kit
- Aliquots

Storage of samples: Collected blood samples were kept in a serum separator tube and were allowed to clot for 30 minutes before centrifugation for 15 minutes at 1000× g. after that serum was removed and Aliquoted. Storage of samples was at ≤–20°C. Repeated freeze-thaw cycles were avoided.

Methodology

Clinical Periodontal Procedure

Each patient who was enrolled in the study was examined thoroughly before commencing with scaling and root-planing procedure and oral hygiene instructions were given after the treatment. Before scaling and root planing, plaque index (PI) (Silness P and Loe H, 1964) were taken to evaluate oral hygiene maintenance of the patient, PPD the distance between the gingival margin and the bottom of the gingival pocket was measured and BOP (%) was measured as bleeding which occurred within 30 seconds of probing. All clinical recordings were performed immediately before the scaling and root-planing (baseline).

Collection of Blood Sample

With patient's consent, prior to each sample collection, disinfection of the skin at the site of venipuncture was done with surgical spirit.

After tying the tourniquet 3 to 4 inches above the antecubital fossa, blood sample of 3 ml is collected from the antecubital vein of the forearm using 20 gauge needle and 5 ml syringe and stored in tubes containing trisodium citrate anticoagulant.

Blood samples were collected before scaling and root planing.

Estimation of Serum Leptin Levels

Tubes with collected blood samples were sent to the pathology lab within 24 hours and serum leptin was estimated through ELISA test using human leptin ELISA Kit (Biovendor research and diagnostic products).

RESULTS

From Table 1, it can be interpreted that there was a significant reduction in PI from base line to 15 days and 1 month but there was no statistical significant difference between the groups.

The estimated mean PI value with standard error was 0.87 ± 0.113 (CI 0.65-1.1) at base line reduced to 0.73 ± 0.093 (CI 0.54-0.91) and 0.67 ± 0.10 (CI 0.47-0.87) in healthy group, where in gingivitis group it was 1.5 ± 0.113 (CI 1.36 -1.8) at base line reduced to 1.36 ± 0.093 (CI 1.18-1.55) and 1.17 ± 0.10 (CI 0.98-1.3) and in periodontitis group it was 1.93 ± 0.113 (CI 1.70-2.15) at base line reduced to 1.57 ± 0.93 (CI 0.1.38-1.75) and 1.05 ± 0.10 (CI 0.85-1.25) at 15 days and 1 month follow-up.

The mean probing depth in healthy, gingivitis and periodontitis groups was 3.58 ± 0.59 (mean SD), 3.95 ± 0.63 and 6.29 ± 0.72, which showed significant reduction of 3.31 ± 0.65, 3.39 ± 0.52, 5.21 ± 0.84 at 15 days and 3.16 ± 0.73, 3.21 ± 0.49 and 4.79 ± 0.67 at the end of 1 month.

The mean pocket depth reduction with standard error in healthy group was 3.58 ± 0.13 (CI 3.32-3.84) which reduced to 3.31 ± 0.13 (CI 3.05-3.57) and 3.16 ± 0.12 (CI 2.91-3.42).

In gingivitis group, it reduced from 3.95 ± 0.131 (CI 3.69-4.21) to 3.39 ± 0.13 (CI 3.13-3.65) and 3.21.12 (CI 2.95 - 3.47). In periodontitis group, reduction was from 6.29 ± 0.13 (CI 6.03-6.55) to 5.21 ± 0.13 (CI 4.95-5.47) and 4.79 ± 0.128 (CI 4.53-5.04) at the end of 15 days and 1 month.

At baseline for healthy, gingivitis and periodontitis, it was 2.16, 7.85, 4.73, 10.23 and 4.63, 9.77. At the end of 15 days, it reduced to 1.62, 6.24, 2.63, 12.13 and 3.33, 7.79. At the end of 1 month, it was 1.25, 6.57, 1.77, 10.45 and 2.32, 7.39.

The mean percentage of BOP with standard error for healthy group was 21.62, 1.87 (CI 17.89-25.34) which reduced to 16.23, 1.81 (CI 12.62-19.85) and 12.58, 1.66 (CI 9.26-15.89). For gingivitis group it was 47.37, 1.87 (CI 43.64-51.10) which reduced to 26.37, 1.8 (CI 22.75-29.99) and 17.75, 1.66 (CI 14.44-21.07) at the end of 15 days and 1 month.

In periodontitis group, it was 46.35, 1.87 (CI 42.64-50.09) which reduced to 33.30, 1.81 (CI 29.68-36.92) and 23.24, 1.66 (CI 19.92-26.55).

Table 2 is showing the inter-relationship between all the clinical parameters and the serum leptin levels in healthy group in which only OHI-S is significantly related with the serum leptin levels with p = 0.03.

Table 3 is showing the inter-relationship between all the clinical parameters and serum leptin levels in periodontitis group in which OHI-S and PD are significantly associated with serum leptin levels with the p-value of p = 0.03 with OHI-S and p = 0.01 for PD.

Table 3 is showing correlation among the all clinical parameters and serum leptin levels but, having a weak association with the serum leptin levels.

DISCUSSION

Leptin is a hormone that is secreted in the blood in varying quantities by adipocytes and controls the adipose tissue weight by stimulating lipid metabolism by the organism.[18] Adipocytes in the obese people produce a large quantity of biologically active molecules, such as leptin, and high leptin concentration in blood has been suggested as a risk factor for the cardiovascular diseases.

According to Preadeep AR et al (2007),[19] the uses of the laptin are as follows:

- It regulates weight in a central munnar by suppressing the hunger.
- Proliferation and differentiation of hematopoietic cells, angiogenesis and wound healing.
- It enhances body's immune mechanism by inducing the proliferation of human peripheral blood mononuclear cells, chemotaxis and oxidative species production by stimulated PMNs, phagocytosis by macrophages.

Table 1: Clinical parameters at baseline, 15 days and 1 month

			PI		PD		Percentage of BOP	
		N	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
At baseline	1	25	0.87	0.43	3.58	0.59	2.16	7.85
	2	25	1.58	0.63	3.95	0.63	4.73	10.23
	3	25	1.92	0.6	6.29	0.72	4.63	9.77
	Total	75	1.46	0.71	4.61	1.36	3.84	15.12
15 days	1	25	0.73	0.36	3.31	0.56	1.62	6.24
	2	25	1.36	0.57	3.39	0.52	2.63	12.13
	3	25	1.57	0.42	5.21	0.84	3.33	7.79
	Total	75	1.22	0.58	3.97	1.09	2.53	11.39
1 month	1	25	0.67	0.37	3.16	0.73	1.25	6.57
	2	25	1.17	0.57	3.21	0.49	1.77	10.45
	3	25	1.05	0.51	4.79	0.67	2.32	7.39
	Total	75	0.96	0.53	3.72	0.98	1.78	9.29

Table 2: Healthy group

		Age	*OHI-S*	*PI at baseline*	*PD at baseline*	*BOP% at baseline*	*Serum leptin levels*
Age		1	–0.4	0.002	–0.016	–0.245	–0.202
	p-value		0.042	0.991	0.941	0.237	0.332
	N	25	25	25	25	25	25
OHI-S		–0.409*	1	0.468*	0.302	0.207	0.424*
	p-value	0.042		0.018	0.143	0.321	0.035
	N	25	25	25	25	25	25
PI at baseline		0.002	0.468*	1	–0.052	–0.104	0.37
	p-value	0.991	0.018		0.805	0.621	0.069
	N	25	25	25	25	25	25
PD at baseline		–0.016	0.302	–0.052	1	0.111	0.263
	p-value	0.941	0.143	0.805		0.597	0.204
	N	25	25	25	25	25	25
BOP% at baseline		–0.245	0.207	–0.104	0.111	1	–0.038
	p-value	0.237	0.321	0.621	0.597		0.858
	N	25	25	25	25	25	25
Serum leptin levels		–0.202	0.424*	0.37	0.263	–0.038	1
	p-value	0.332	0.035	0.069	0.204	0.858	
	N	25	25	25	25	25	25

*Correlation significant at p-value ≤ 0.05

Table 3: Gingivitis group

		Age	*OHI-S*	*PI at baseline*	*PD at baseline*	*BOP% at baseline*	*Serum leptin levels*
Age		1	–0.29	0.201	–0.226	0.085	–0.323
	p-value		0.159	0.336	0.278	0.687	0.115
	N	25	25	25	25	25	25
OHI-S		–0.29	1	0.094	0.478*	0.056	0.572**
	p-value	0.159		0.655	0.016	0.791	0.003
	N	25	25	25	25	25	25
PI at baseline		0.201	0.094	1	0.12	0.097	0.028
	p-value	0.336	0.655		0.566	0.643	0.894
	N	25	25	25	25	25	25
PD at baseline		–0.226	0.478*	0.12	1	–0.274	0.607**
	p-value	0.278	0.016	0.566		0.185	0.001
	N	25	25	25	25	25	25
BOP% at baseline		0.085	0.056	0.097	–0.274	1	–0.043
	p-value	0.687	0.791	0.643	0.185		0.839
	N	25	25	25	25	25	25
Serum leptin levels		–0.323	0.572**	0.028	0.607**	–0.043	1
	p-value	0.115	0.003	0.894	0.001	0.839	
	N	25	25	25	25	25	25

*Correlation significant at p-value ≤ 0.05; **Correlation siginificant at p-value ≤0.01

- It has direct effect on osteoblast proliferation, differentiation and prolonging the life span of human primary osteoblast by inhibiting apoptosis.
- It protects the host from inflammation and infection and maintains the bone levels.

Till date, all the studies that have taken place for the estimation of the leptin were the combinations of the leptin levels either in gingival biopsies or in GCF and serum/plasma concentration of leptin.

The studies which were associating a direct significant relationship with the advancement of periodontitis and serum leptin levels were rare and hardly took place. So, in our study, we tried to establish a direct relationship between the serum concentration of leptin and periodontal disease progression.

Here, the results are showing significant reduction in all the clinical parameters at the end of 15 days and 1 month follow-up.

Zimmermann et al (2013)[20] concluded that periodontitis mainly influenced the circulating levels of resistin and adiponectin, whereas both obesity and periodontitis affected the circulating levels of leptin in favor of proinflammation. In addition, obesity upregulated the local levels of TNF-a.

When we tried to find correlation among all the clinical parameters and serum leptin levels in all the three

groups. In group 1 (healthy group), we found significant association between OHI-S and PI with $p < 0.01$ and OHI-S and serum leptin with $p < 0.03$. Where as in group 2 (gingivitis group), serum leptin levels were significantly associated with OHI-S with $p < 0.03$ and pocket depth at baseline with $p < 0.01$. But, in periodontitis group, no significant association was found between all the clinical parameters and serum leptin levels.

In our study, as the periodontal disease progressed, there was raise in serum leptin concentration in gingivitis group with the $p < 0.005$. But, these results did not remained consistent for periodontitis group. From these results, it can be hypothesized that this new inflammatory marker has the potential of being a risk indicator or risk predictor in the management of cardiovascular diseases and is having a weak association with OHI-S, PI and pocket depth with the significant increase of all these clinical parameters as the disease advances. But, it has to undergo further positive concrete evidences for being one of the same.

CONCLUSION

There is significant increase in the serum leptin levels in gingivitis group patients than from the healthy group subjects. As the disease progressed, the increase in the serum leptin levels were found in gingivitis group.

Direct positive correlation exists between raise in the serum leptin levels and periodontal disease progression in healthy and gingivitis group but a weak association is present between serum leptin levels and periodontitis group. So, it can be concluded that higher levels of leptin can be one of the helpful diagnostic parameters in occurrence and progression of periodontal diseases in future. But, further other aspects have to be checked before the acceptance of these results because of the positive association of leptin with inflammation and obesity. So, further well designed studies are still needed with better number of sample size in future to establish a concrete relationship between the serum leptin and periodontitis.

REFERENCES

1. Maffei M, Fei H, Lee GH. Increased expression in adipocyte of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus. Proc Natl Acad Sci 1995;92(15): 6957-6960.
2. Masuzaki H, Ogawa Y, Sagawa N. Nonadipose tissue production of leptin: Leptin as a novel placenta derived hormone I humans. Nat Med 1997;3(9):1029-1033.
3. Bado A, Levsseur S, Attoub S, Kemorgant S, Laigneu JP, Bortoluzzi MN, et al. The stomach is a source of leptin. Nature 1998;394(6695):790-793.
4. Sana V, Giacomo AD, Cava AL, Lechler RI, Fontana S, Zappacosta S, et al. Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathologic T cell response. J Clin Invest 2003;111(2):241-250.
5. Gordeladze JO, Drevon CA, Syversen U, Reseland JE. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis and mineralisation: impact on differentiation markers, apoptosis and osteoclastic signaling. J Cell Biochem 2002;85(4):825-836.
6. Sanchez-Margret V, Romero CM. Human leptin signaling in human peripheral blood mononuclear cells: Activation of JAK-STAT pathway. Cell Immunol 2001;211(1):30-36.
7. Ahima RS, Flier JS. Leptin. Annu Rev Physiol 2000;62(1): 413-437.
8. Jhonson RB, Serio FG. Leptin within healthy and diseased human gingival. J Periodontol 2001;72(9):1254-1257.
9. Arnalich F, Lopez J, Codoceo R, Jimnez M, Madero R, Montiel C. Relationship of plasma leptin to plasma cytokines and human survival in sepsis. J Infect Dis 1999;580(3):908-911.
10. Fantuzzi G, Faggioni R. Leptin in the regulation of immunity, inflammation and hematopoiesis. J Leukoc Biol 2000;68(4): 437-446.
11. Torpy DJ, Bornstein SR, Chrousos GP. Leptin and Interlukin-6 in sepsis. Hom Metav Res 1998;30(12):726-729.
12. Faggioni R, Fantuzzi G, Fuller J, et al. IL-1 beta mediates leptin induction during inflammation. Am J Physiol 1998;274 (Suppl 1, Part 2):204-208.
13. Gainsford T, Wilson TA, Metcalf D, et al. Leptin can induce proliferation and functional activation of hematopoietic cells. Proc Natl Acad Sci (USA) 1996;93(25):14564-14568.
14. McGee JM, Tucci MA, Edmundson TP, et al. The relationship between concentrations of proinflammatory cytokines within gingiva and the adjacent sulcular depth. J Periodontol 1998; 69(8):865-871.
15. Carlson GL, Saeed M, Little RA, et al. Serum leptin concentrations and their relation to metabolic abnormalities in human sepsis. Am J Physiol 1999;276(Suppl 4, Part 1):658-662.
16. Bornstein SR, Preas HL, Chrousos GP, et al. Circulating leptin levels during acute experimental endotoxemia and anti-inflammatory therapy in humans. J Infect Dis 1998;178(3): 887-890.
17. Hoppin AG, Kaplan LM, Zurakowski D, et al. Serum leptin in children and young adults with inflammatory bowel disease. J Pediatr Gastroenterol Nutr 1998;26(5):500-505.
18. Wang Y, Kuropatwinski KK, White DW, Hawley TS, Hawley RG, Tartaglia LA, Baumann M. Leptin receptor action in the hepatic cell. J Biol Chem 1997;277(26):16216-16223.
19. Karthikeyan BV, Preadeep AR. Gingival crevicular fluid and serum leptin: their relationship to periodontal health and disease. J Clin Periodontol 2007;32(6):467-472.
20. Zimmermann GS, Bastos MF, Eduardo dias goncxalves T, Chambrone L, Duarte MP. Local and circulating levels of adipocytokines in obese and normal weight individuals with chronic periodontitis. J Periodontol 2013;84(5):624-633.