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"QUANTIFICATION OF YKL-40 IN GINGIVAL TISSUES OF CHRONIC AND AGGRESSIVE PERIODONTITIS"

Dental Science

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ABSTRACT

Background and objectives: YKL-40 is an acute phase protein chiefly regulated by IL-6 and is expressed during the course of inflammation. Multiple cytokines have been identified in gingival tissues that causes tissue destruction associated with progressing periodontitis . In this context the present study was undertaken to quantify YKL-40 levels in chronic and aggressive periodontitis patients. Clinical parameters were compared and correlated.

Material & Methods: Gingival tissue samples were obtained from three groups:- Healthy (n=30), chronic periodontitis (n=30) and aggressive periodontitis subjects (n=30) .The samples were quantitative analysed by ELISA.

Results : YKL-40 levels significantly increased in chronic periodontitis as compared to aggressive periodontitis and healthy subjects.

Conclusion: YKL-40 protein has a role in the immunopathogenesis of periodontitis and can be used as an useful inflammatory biomarker in periodontal diseases.

KEYWORDS:

YKL-40 , Periodontitis, Cytokines, ELISA, Inflammation

Introduction

Periodontitis is a polymicrobial infectious disease of multifactorial origin resulting in inflammatory breakdown of tooth- supporting osseous and soft tissue components.[1,2] It activates an array of events which involves inflammatory responses in the host. This response causes infiltration of multiple inflammatory cells which include polymorphonuclear neutrophils, macrophages, lymphocytes & plasma cells within the periodontal tissue.[3] With the advent of advanced technologies it has become progressively clear in the recent years that periodontal disease can cause remarkable increase as well as decrease in the levels of markers of systemic inflammation. These markers include primarily the plasma proteins such as acute phase proteins.[4]

These acute phase proteins(APP) are mainly produced in the liver as a response to infection and inflammation. They are generated in the host by a process called as acute phase reaction which is mediated by cytokines like IL-1, IL-6, IL-8 and tumor necrosis factor- α. The rise in the plasma concentration of APPs thereby helps the host defense to detect the invading microbes, mobilizes the leukocytes into the circulation, and increases the blood flow to injured or infected sites.[5]

In search of new bone proteins, YKL-40 was first discovered in 1989 as a protein to be secreted *in vitro* in large amounts by the MG63 human osteosarcoma cell line.[6] YKL-40 is a glycoprotein with a molecular weight of approximately 40 kDa and its name is an abbreviation of three NH2-terminal amino acids Tyrosine (Y), Lysine (K) and Leucine (L).[7] It is a member of the "mammalian chitinase –like proteins" family but has no chitinase/enzymatic activity.[8] The protein has several names like human cartilage glycoprotein 39(HC-gp39), Chitinase -3 like-1 protein (CHI3L1), 38-kDa heparin-binding glycopr otein(gp38k), chondrex.[9] YKL-40 has been expressed by a variety of cell species including neutrophils, macrophages, chondrocytes, synovial cells, osteoblastic cells, endothelial cells, and cancer cells [10] and is not a disease specific inflammatory biomarker. Although the exact role of YKL-40 are still unknown, it may probably participates in cell proliferation, adhesion, migration and differentiation, inflam mation, protection from apoptosis.[11,12] They also trigger the AKT and phosphoinositide 3-kinase signaling pathway (PI3K)[13] and may play a role in innate immune responses.[14]

Periodontal disease is an inflammatory disease with complex interaction between periodontal microorganism and host immune response, in which as a part of the host response the mediators of tissue breakdown like acute phase protein, cytokines and prostaglandins are produced to combat the bacterial endotoxins.[2] Cytokines play a major role in tissue destruction associated with progressing periodontitis. They originate from activated macrophages and act locally and distally to increase its production in local tissues and commences the systemic acute phase reactants (APRs). Since multiple cytokines have been detected in both gingival tissues and GCF, changes in local acute-phase reactants might be expected.[15] YKL-40 is an acute phase protein chiefly regulated by IL-6 so it becomes highly important to study YKL-40 and correlate its association with periodontal disease.[16,17]

To the best of our knowledge till date no study has been carried out to quantify the levels of YKL-40 protein expression in gingival tissues of chronic and aggressive periodontitis. So the present study was carried out with the purpose to quantify YKL-40 protein levels in gingival tissue of subjects with healthy periodontium, chronic periodontitis and aggressive periodontitis and also to compare and correlate YKL-40 levels with the clinical parameters.

MATERIALS AND METHODS

A total of 90 subjects, age ranging from 18 to 65 years, were selected from the Outpatient Department of Periodontics, P.M.N.M. Dental College and Hospital at Bagalkot, Karnataka.

All the participants were explained about the need and design of the study. Written informed consent was obtained from the study participants. Ethical approval for the study was obtained from the Institutional Ethical Committee of P.M.N.M. Dental College and Hospital at Bagalkot

Subjects were selected randomly and categorized into 3 groups as follows:

Group I: 30 subjects with healthy periodontium
Group II: 30 patients with chronic periodontitis
Group III: 30 patients with aggressive periodontitis

Periodontally healthy patients included patients who had no clinical signs of any form of periodontal disease. Subjects who had at least 20 teeth and were diagnosed as either generalized chronic or generalized aggressive periodontitis according to 1999 consensus classification of periodontal diseases.[18] Diagnosis of generalized aggressive periodontitis was based only on the clinical findings, which included periodontal breakdown without evidence of any systemic disease, rapid attachment loss and bone destruction, history of familial aggregation and radiographic diagnosis. Diagnosis of generalized chronic periodontitis was based on periodontal destruction, which was consistent with amount of local factors slow progression of the disease with absence of any systemic disease .Clinical measurements were taken at 6 sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual/ mesiopalatal, lingual/palatal and distolingual / distopalatal) of every tooth present, except third molars, with a William's periodontal probe. The clinical parameters measured as Gingival index(GI) ,probing pocket depth (PPD), clinical attachment level (CAL). Full-mouth orthopantamograph were recorded to evaluate the bone condition.

Subjects were excluded if they had any systemic condition,which would influence the course of periodontal disease or treatment and medical conditions, which would require antibiotic prophylaxis for routine dental procedures. Individuals who had taken antibiotics in the previous 6 months or pregnant or lactating women were also excluded. Care was taken to exclude patients who were smokers or those who had other abnormal habits.

Procedure

Under local anaesthesia, rectangular biopsies of 2 to 3 mm were obtained by surgical excision from labial / buccal surface of gingiva with no.15 BP blade The surgical blade point was directed to the portion of the alveolar bone crest in order to obtain a well oriented and representative biopsy. The gingival tissue samples were transported in phosphate buffer solution and stored at -80°C until further analysed using commercially available YKL-40 Elisa kit [Figure 1,2,3]

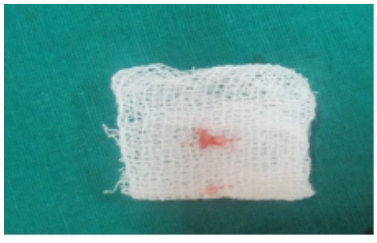

Figure 1: HARVESTED TISSUE



Figure 2: HUMAN YKL-40 ELISA KIT

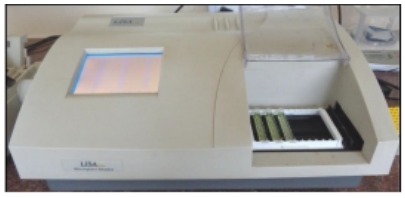

Figure 3: OPTICAL DENSITY DETERMINED AT 450 nm USING ELISA READER

Statistical Analysis

Kruskal Wallis test was used for the analysis and comparison of results between the study groups. $P < 0.05$ if obtained would be considered as statistically significant. Spearman's rank correlation used for the correlation of clinical parameters with YKL-40.

Results:

The results of present study showed significantly increased YKL-40 levels in chronic periodontitis group (162.27±114.95) as compared to aggressive periodontitis group (36.73± 26.56) and healthy (23.88±50.41) .Table 1

Compared the age groups with YKL-40 levels and they found that YKL-40 levels significantly increased in chronic periodontitis group as compared to aggressive periodontits and healthy group. Table 2

Compared the three groups with clinical parameters such as gingival index (GI), probing pocket depth(PPD) and clinical attachment level(CAL) were found to be statistically significant in all three groups. Table 3,4,5

Correlation of clinical parameters with YKL-40 levels revealed a positive correlation between YKL-40 levels with GI in chronic periodontitis and aggressive periodontitis groups .A non-significant positive correlation was found between YKL40 levels with PPD in chronic periodontitis and aggressive periodontitis groups. Table 6

Table 1:- comparison of YKL-40 levels between the study groups

Group	N	Mean (SD)	Median (Q1, Q3)	Min	Max	Kruskal Wallis test		Mann Whitney U test (p-value)		
						Chi square value	P-value	H vs CP	H vs AP	CP vs AP
Healthy	30	23.88 (50. 41)	18.87 (-1.22, 33.08)	-29.41	268.70	49.52	<0.001*	<0.001*	0.002*	<0.001*
CP	30	162.27 (114.95)	100.45 (57.62, 265.29)	32.84	384.86					
AP	30	36.73 (26. 56)	34.46 (23.00, 45.48)	-4.77	148.39					

*p<0.05 statistically significant

Table 2:- Comparison of age between the study groups

Group	N	Mean (SD)	Median (Q1, Q3)	Min	Kruskal Wallis test		Mann Whitney U test (p-value)		
					Chi square value	P-value	H vs CP	H vs AP	CP vs AP
Healthy	30	25.83 (8.55)	23(19, 30.5)	18 - 48	39.21	<0.001*	<0.001*	0.04*	<0.001*
CP	30	41.27 (8.92)	38.5(34.5, 47.25)	28 - 59					
AP	30	28.17 (5.71)	27.5(24, 33)	18 - 37					

*p<0.05 statistically significant

Table 3:- Comparison of GI scores between the study groups

Group	N	Mean (SD)	Median (Q1, Q3)	Min	Max	Kruskal Wallis test		Mann Whitney U test (p-value)		
						Chi square value	P-value	H vs CP	H vs AP	CP vs AP
Healthy	30	0.79 (0.08)	0.8 (0.7, 0.83)	0.7	0.9	75.654	<0.001*	<0.001*	<0.001*	<0.001*
CP	30	1.93 (0.52)	2 (2, 2)	1	3					
AP	30	1.10 (0.31)	1 (1, 1)	1	2					

*p<0.05 statistically significant

Table 4:- Comparison of PPD between the study groups

Group	N	Mean (SD)	Median (Q1, Q3)	Min	Max	Kruskal Wallis test		Mann Whitney U test (p-value)		
						Chi square value	P-value	H vs CP	H vs AP	CP vs AP
Healthy	30	1.10 (0.31)	1 (1, 1)	1	2	81.96	<0.001*	<0.001*	<0.001*	<0.001*
CP	30	5.53 (0.63)	5 (5, 6)	5	7					
AP	30	7.73 (0.58)	8(7, 8)	7	9					

*p<0.05 statistically significant

Table 5:- Comparison of CAL between the study groups

Group	N	Mean (SD)	Median (Q1, Q3)	Min	Max	Kruskal Wallis test		Mann Whitney U test (p-value)		
						Chi square value	P-value	H vs CP	H vs AP	CP vs AP
Healthy	30	0.00 (0.00)	0 (0, 0)	0	0	75.43	<0.001*	<0.001*	<0.001*	<0.001*
CP	30	6.53 (0.97)	7 (6, 7)	5	8					
AP	30	8.00 (0.64)	8(8, 8)	7	9					

*p<0.05 statistically significant

Table 6:- Correlation between the YKL -40 study parameters among three groups

Group			PPD	CAL	YKL-40 levels (pg/ml)
Healthy	GI	Correlation Coefficient	0.2		-0.43
		p-value	0.29(NS)		0.02*
	PPD	Correlation Coefficient			-0.006
		p-value			0.97(NS)
CP	GI	Correlation Coefficient	0.12	0.15	0.52
		p-value	0.54(NS)	0.42(NS)	0.003*
	PPD	Correlation Coefficient		0.53	0.16
		p-value		0.003*	0.39(NS)
	CAL	Correlation Coefficient			0.26
		p-value		.	0.16(NS)
AP	GI	Correlation Coefficient	0.33	0.18	0.15
		p-value	0.08(NS)	0.35(NS)	0.44(NS)
	PPD	Correlation Coefficient		0.07	0.11
		p-value		0.71(NS)	0.57(NS)
	CAL	Correlation Coefficient			-0.07
		p-value			0.70(NS)

Spearmans correlation test

*p<0.05 statistically significant, p>0.05 Non significant, NS

Discussion

The acute-phase response (APR) is a major systemic reaction of the organism to local or systemic disturbances caused by infection, tissue injury, trauma or surgery, or immunological disorders. The principle cause of these responses is to re-establish homeostasis and to eliminate the source of disturbance. Through direct stimulation and secretion of various cell communicating factors these APR is initiated by the tissue macrophages. The APR is activated by the release of cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-α) from macrophages and monocytes at the site of inflammatory lesions or infections.[19] So the current case control study was intended to compare and correlate its levels with clinical parameters among the chronic and aggressive periodontitis patients so as to obtain an insight into the probable role of YKL-40 in immunopathogenesis of periodontal diseases.

The present study quantified the levels of YKL-40 in gingival tissues of healthy ,chronic periodontitis and aggressive periodontitis groups and results showed that levels of YKL-40 was increased in Chronic periodontitis group (162.27±114.95) as compared to Aggressive periodontitis group (36.73±26.56) and least expressed in Healthy group(23.88±50.41) (Table 1) and these levels were compared with their mean age in healthy subjects (25.83 ±8.55), less than that of aggressive periodontitis (28.17±5.71) and chronic periodontitis (41.27±8.92) (Table 2).So the increase in YKL-40 levels in chronic periodontitis could be attributed to the fact that this glycoprotein increases with age; and differs from population to population. These results were in accordance with study done by Bojesen SE et al in 3,130 Danish population of age group 20–80 years which showed mean YKL-40 level was 40 µg/l and Gungen G et al where in Turkish population serum YKL-40 levels was 114.16 ± 43.73 ng/ml with age groups ranging between 44 and 65 years.[20,21]

The high expression of YKL-40 levels in chronic periodontitis group could be probably due to more release of acute-phase reactants that might be related to the inflammatory status of periodontal tissues and that YKL-40 is released by a variety of cells, mainly neutrophils and macrophages that are found excessive during periodontal inflamm ation. The results were similar to the results of a study conducted by Keles ZP et al wherein YKL-40 levels in serum and GCF were increased in chronic periodontitis group as compared gingivitis group and healthy controls.[2]

The leukotoxin of *A. actinomycetemcomitans* has the ability to selectively kill human leukocytes, either by lysis or apoptosis .The cytotoxicity elicited by the leukotoxin may impair local defence mechanisms in the periodontium [22] and therefore might be the probable reason for lower levels of YKL-40 in aggressive periodontitis group in the present study. Also lipopolysaccharide induced expression of YKL-40 mRNA in human monocytes have also been studied by Suzuki et al.[23] *A. actinomycetemcomitans* LPS appears to be a weaker stimulator of cytokine synthesis compared to *A. actinomycete mcomitans* surface associated proteins, or to LPS from other species such as *E. coli.*[24] The above explanation could be another probable reason for lower expression of these proteins in aggressive periodontitis.

Clinical parameters like gingival index(GI), probing pocket depth (PPD) and clinical attachment level (CAL) were compared with YKL-40 levels. In the present study GI scores were significantly lower in healthy(0.79±0.08) as compared to chronic periodontitis group (1.93±0.52) and aggressive periodontitis group (1.10±0.31) (Table 3) YKL-40 levels and GI scores showed significant negative correlation in healthy group, significant positive correlation in chronic periodontitis group and non-significant positive correlation in aggressive periodontitis group (Table 6). So this could be accredited to the fact that there is lack of clinical inflammation seen in aggressive periodontitis than chronic periodontitis.[25] The negative correlation in

healthy group could be due to predominance of gram positive organisms in plaque of healthy group that have influenced the negative outcome.[26]

PPD scores were significantly higher in aggressive periodontitis (7.73±0.58) as compared to chronic periodontitis (5.53±0.63) and healthy group (1.10±0.31) (Table 4). When YKL-40 levels were correlated with PPD of each of the three groups there was a non-significant negative correlation in healthy group and non-significant positive correlation in both chronic and aggressive periodontitis groups (Table 6). But contrasting results were obtained in studies conducted by Keles ZP et al [2] and Kido J et al,[27] thereby showing that YKL-40 levels were significantly correlated with PD and GI suggesting role of YKL-40 in GCF as a useful marker for inflammation in periodontal disease. This is the first research of its kind so further elaborative studies are needed to justify role of YKL-40 in the severity of periodontal disease progression.

CAL score was significantly lower in Healthy (0.00±0.00) when compared to Chronic periodontitis (6.53±0.97) and Aggressive periodontitis group (8.00±0.64) (Table 5). When CAL was correlated with YKL-40 levels in aggressive periodontitis group, it showed a non-significant negative correlation whereas in chronic periodontitis it showed a non-significant positive correlation (Table 6). This could be attributed to the fact that there is variation in disease severity between the two study population and TLR-4 mutation Asp299Gly has been associated against aggressive periodontitis than chronic periodontitis. This mutation modulates the TLR mediated responses, thereby damping down the inflammatory responses initiated by bacterial products in the periodontal tissues.[28,29]

To the best of our knowledge and available literature till date there is no published evidence supporting the expression of YKL-40 in gingival tissues of healthy, chronic periodontitis and aggressive periodontitis . More elaborative studies with larger sample size are required to ascertain the role of YKL-40 in pathogenesis of periodontal disease is required. So the present study is a footmark study to represent YKL-40 as an inflammatory biomarker in periodontal diseases.

Conclusion

This being the first study of its kind, within limitations it can be concluded that the expression of YKL-40 protein may have a potential role in the immunopathogenesis of chronic and aggressive periodontitis and can be used as an inflammatory marker in periodontal diseases. It is also suggested that this protein could be used in the preparation of therapeutic agents to limit excessive matrix degradation characteristic of various inflammatory conditions including periodontal diseases.

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