Reconnoitering the Association of Interleukin-6 with Chronic Periodontitis and Covid-19: An Observational Study.

Abstract:

Aim: The aim of the present study was to investigate comparatively the systemic levels of interleukin-6 in COVID-19 positive patients with/without chronic periodontitis and recovered COVID-19 patient with/without chronic periodontitis respectively. Methods: Total 200 consecutive confirmed cases were included and categorized equally into four groups- Group1A & Group 1B (COVID-19 positive), Group 2A & Group 2B (COVID-19 recovered),based on periodontal status along with Interleukin-6 levels. Peripheral blood samples were taken and Serum Interleukin-6 concentrations were tested using a highly sensitive (Chemiluminescence immunoassay) CLIA method on admission during hospital stay. Results: There were 50 subjects taken in all study groups. The mean age was 40.80, 38.02, 41.06, 38.98 in Group (1A,1B,2A&2B) respectively and the difference in mean age group was not significant (P>0.05). The mean interleukin-6 level was 26.0752, 1.5306, 4.1290, 0.7514 pg/ml in Group1A, 1B, 2A, 2B respectively and the difference was statistically significant (P-0.0001*). The mean difference in inter-group comparison was seen significant (P<0.05) among group1A vs(1B,2A,2B) & non-significant (P>0.05) among group1Bvs(2A,2B) & group2A vs 2B respectively. Conclusion: Chronic periodontitis must be included in diagnostic work-up to stratify SARS- CoV-2 severity.

Key Words: COVID 19, Periodontitis, IL6.

Introduction:

Periodontitis is one of the most prevalent chronic inflammatory diseases of human being which is characterized by inflammatory-immune responses hyper-reactivity, like cytokines and chemokines with local and systemic consequences. Such similar situations also happen in Coronavirus disease (COVID-19) such as hypersecretion of "Cytokine Storm" like IL (Interleukin) -1, IL-2 and IL-6, C Reactive Protein (CRP), acute phase proteins, TNF-(Tumor necrosis Factor- Alpha) and Interferon gamma (INF-gamma) which can exaggerated by a multiplicity of factors such as some medications, infections etc.[1] This is an indication of path mechanism of severe viral infections such as corona virus that cause Coronavirus disease (COVID-19), characterizing a pandemic.[2] Thus, it is suggestive to consider the probability of pre-existing periodontal disease with the severity of COVID-19 rather than viral pathogenesis alone. Some experimental studies revealed that pre-exposure of airway epithelial cells to bacteria aggravates the release of cytokines in response to further viral infection and also, promotes microbial growth on airway epithelial cells which may include the hyperproduction of interleukin-6 which futher can make COVID-19 more critical.[3]

It is a renowned fact that Periodontitis is linked to heart disease and chronic lung diseases, both of which also put people at risk for becoming severely ill from or dying of COVID-19. People affected with chronic periodontitis raises levels of an immune protein called interleukin-6, which can

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lead to inflammation in the gums as well as the blood vessels and lung. Researchers in California and Brazil confirmed in a review of studies that COVID-19 patients show increase in interleukin-6 (IL-6) level which is a part of 'Cytokine storm'.[4] Coronavirus disease 2019 (COVID-19) is a viral pandemic disease, due to the SARS-associated coronavirus 2 (SARSCoV-2), first defined as 2019-nCoV. In December 2019, the local Centres for Disease Control and Prevention (CDC) in Wuhan City, Hubei Province, China reported a cluster of unexplained pneumonia cases, named as coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO), and were considered to be caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) from bats;5 which is a novel enveloped RNA beta-coronavirus.[6] The pandemic of SARS-CoV-2, as declared by the WHO, is showing no signs of slowing down with over 40,000 new confirmed cases every day in India and >121,969,223 cases worldwide till 20,March,2021.[7] Early detection and treatment for severe and critically ill patients are crucial issues requiring urgent investigation. By summarizing the clinical characteristics, abnormal immunologic features were identified among COVID-19 patients with a higher serum concentration of proinflammatory cytokines.[8]

Inflammatory IL-6 proteins flood the site of gum disease, but can then enter the bloodstream and travel to the lungs by one of several pathways, leaving the organs more vulnerable to severe COVID-19, increasing inflammation there, too, and therefore raising risks for life-threatening coronavirus infection..[9] Elevated IL-6 level is a hallmark inflammatory signature seen in serum of patients with severe COVID-19 acute respiratory distress (ARDS).[10] The entry of the virus can subvert the immune system and oral microbiota of host triggering a dysbiosis that allows a superinfection, understanding of the association of the PD with severe COVID-19.[11] Numerous cytokines have been identified at sites of chronic inflammation during periodontitis. One of these, interleukin-6 (IL-6), is a major mediator of host response to tissue injury and infection. Spontaneous production of IL-6 has been reported in mononuclear cells isolated from inflamed gingival tissues of patients with periodontitis. IL-6 levels may correlate with the severity of periodontal disease. IL-6 is elevated in sites of refractory periodontitis compared to sites of stable, advanced

periodontitis, which suggests that it could be a diagnostic marker for sites of active periodontal disease.[12] This has led to the hypothesis that dysregulated activation of the mononuclear phagocyte (MNP) compartment contributes to COVID-19-associated hyperinflammation.[13-14] Previous retrospective studies indicated that an elevated level of Interleukin-6 (IL-6) was associated with high case fatality in COVID-19 patient.[15]

Aims and Objectives:

The aim of the present study was to investigate the association of systemic levels of INTERLEUKIN-6 & COVID-19 patients with and without chronic periodontitis, in comparison to recovered COVID-19 patient with and without chronic periodontitis by monitoring cytokines blood levels during disease progression and evolution.

Materials and Methods:

Patients with SARS-CoV-2 infection with confirmed cases of COVID-19 was defined by a positive reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay. The nasopharyngeal swab was taken in Hazaribag Collage of Dental Sciences and Hospital, Demotand, Hazaribagh, Jharkhand, India for confirming COVID-19, till March 31, 2020. Of total 200 consecutive confirmed cases with both males and females within the age group of 20-55 years were included and categorized equally into four groups-group1A & group1B (COVID-19 positive), group2A & group2B (COVID-19 recovered) respectively. Each group contains 50 subjects on the basis of chronic and healthy periodontal status alongwith Interleukin-6 elevation on admission and/or during hospital stay in Hazaribag college of dental sciences and hospital, Demotand, Hazaribagh, Jharkhand, India and written informed consent was obtained from each patient or next of kin. The diagnostic criteria for COVID-19 followed the Revised Guidelines on Clinical Management of COVID-19 from Government of India, Ministry of Health & Family Welfare, Directorate General of Health Services (EMR Division).16This document aims to provide clinicians with updated interim guidance on timely, effective, and safe supportive management of patients with COVID-19, particularly those with severe acute respiratory illness and

critically ill which is mainly includes epidemiological history and clinical symptoms. The serum IL-6 was detected at least one time in patients with SARS-CoV-2 infection alongwith clinical examination of oral hygiene and with demographic data, information on comorbidities and laboratory. Pregnant women and patients unwilling to give written informed consent were excluded from the study. The self-reported oral health indicators of painful and bleeding gums alongwith loosened teeth were utilized as surrogates for periodontal disease as they have demonstrated their validity in the absence of a clinical diagnosis. Gingival index and bleeding on probing was recorded. Subjects with occurrence of probing depths $\geq$ 5 mm, bleeding on probing and bone loss $\geq$ 2mm were considered as chronic periodontitis. The clinical periodontal parameters used were the Gingival Index (Loe and Silness, 1963) and Clinical Attachment Level (CAL). Criteria followed for Diagnosing Periodontitis. (1999, AAP International workshop) were:

i. Patients with >30% of sites with loss of attachment were classified as having generalized periodontitis.
ii. Patients with <30% of sites with attachment loss were classified as having localized periodontitis.

Fig. 1(a) Clinical view of chronic periodontitis with COVID 19 patient

Fig. 1(b) Clinical view of patient without chronic periodontitis in COVID 19 patient

Methods:
Laboratory measurements:

At 08:00 a.m., a fasting venous blood was collected from each subject in ethylenediamine tetraacetic acid (EDTA)-containing tubes. Tubes were immediately put on ice, centrifuged, and serum isolated within two hours.

Fig. 11(a) Sample collection from COVID 19 patient

Fig. II(b) Blood samples to keep in centrifuged machine for isolation

Blood samples were stored in a storage vial box containing a dry ice pack, before being transferred and stored in a freezer at $-70°C$. Serum IL-6 assays were determined in plasma samples by Chemiluminescence immunoassay using Roche Company (Made in Japan) in Pathkind Lab, Ranchi, Jharkhand, India. The lower detection limit of this kit is 1.5 pg/ml,
and the upper detection limit is 5000 pg/ml without dilution. The normal upper limit is 7 pg/ml.

Results:
Data Analysis:

The data was entered in Microsoft excel format and was analysed using SPSS version 21(IBM SPSS Corp. Ltd. Armonk, N.Y). Summarized data was presented using Tables and Graphs. Descriptive data was reported for each variable. Descriptive statistics such as mean and standard deviation for continuous variables was calculated. Shapiro Wilk test was used to check the normality of the data. As the data was found to be normally distributed bivariate analyses was performed using One way ANOVA followed by Tukey's test for post hoc comparison. Level of statistical significance was set at p-value less than 0.05.

Table I: Assessment of parameters

Parameters	Group 1A	Group 1B	Group 2A	Group 2B	P value
Gingival index	1.62	0.80	1.81	0.72	0.01
Probing Depth	7.9	3.10	5.06	3.00	0.002

Table I, shows that mean gingival index score was 1.62, 0.20,1.58 and 0.60 and Probing depth score was 7.9, 3.10, 5.06, 3.00 in Group 1A, Group 1B, Group 2A and Group 2B respectively. The difference was seen significant (P< 0.05).

Table 2: Distribution according to mean age

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group 1A	50	40.80	10.394	1.470	37.85	43.75	20	55
Group 1B	50	38.02	10.358	1.465	35.08	40.96	20	55
Group 2A	50	41.06	10.013	1.416	38.21	43.91	20	55
Group 2B	50	38.98	8.412	1.190	36.59	41.37	20	55
P VALUE	0.349							

Table 2 and Fig. (III) showed the mean age was 40.80,38.02, 41.06,38.98 in group (1A,1B,2A & 2B) respectively and the difference was not significant (P>0.05).
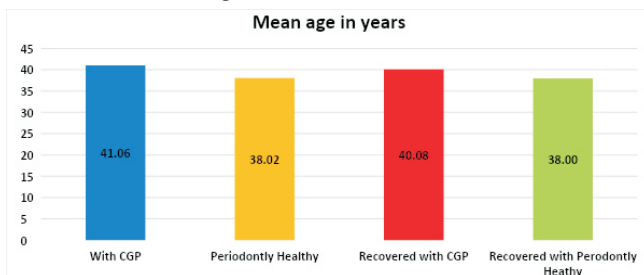

Fig. (III) Graphical distribution a/c to mean age

Table 3: Mean interleukin-6 pg/ml

	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Group 1A: COVID-19 pt. with chronic periodontitis	26.0752	22.04345	3.11741	19.8105	32.3399	11.36	105.20
Group 1B: COVID pt. with periodontally healthy	1.5306	.07003	.00990	1.5107	1.5505	1.73	1.89
Group 2A: Recovered pt. with chronic periodontitis	4.1290	1.51585	.21437	3.6982	4.5598	2.16	9.16
Group 2B: Recovered pt. without periodontitis	.7514	3.49928	.49487	-.2431	1.7459	1.50	1.53
P VALUE	0.0001*						

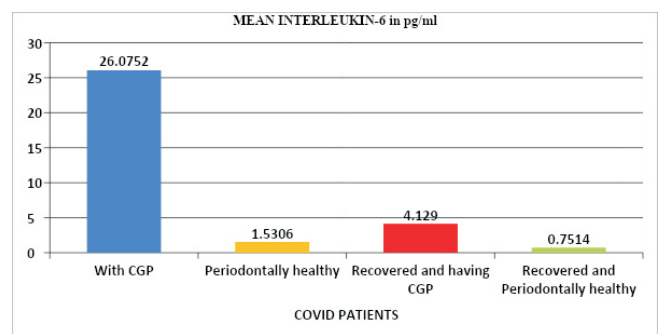Fig. (IV) Graphical presentation of Mean interleukin-6 pg/ml

Table 3 & Fig. IV showed the mean interleukin-6 pg/ml level was (26.0752, 1.5306, 4.1290, 0.7514) pg/ml in group(1A,1B,2A,2B) respectively and the difference was significant (P-0.0001*).

Table 4: POST HOC COMPARISON of mean IL-6pg/ml

Inter-group comparison	Mean Difference (I - J)	Std. Error	Significant value	95% Interval Lower Bound	Confidence Upper Bound
Group1A vs Group1B	24.54460*	2.23710	.000*	18.7478	30.3414
Group1A vs Group2A	21.94620*	2.23710	.000*	16.1494	27.7430
Group1A vs Group2B	25.32380*	2.23710	.000*	19.5270	31.1206
Group1B vs Group2A	-2.59840	2.23710	.652	-8.3952	3.1984
Group1B vs Group 2B	.77920	2.23710	.985	-5.0176	6.5760
Group2A vs Group2B	3.37760	2.23710	.434	-2.4192	9.1744

Post Hoc Comparison (Table 4), using one way ANOVA followed by Tukey's test showed the mean difference in inter-

group comparison between group (1A versus 1B) was 24.54460*, group (1A versus 2A) was 21.94620* & group (1A versus 2B) was 25.32380* respectively and the difference was significant (P<0.05), but inter-group comparison between group (1B versus 2A) was -2.59840, group (1B versus 2B) was 0.77920 & group 2A versus 2B was 3.37760 respectively and difference was non-significant (P>0.05).

Discussion:

An observational short study was conducted in total 200 participants affected with SARS-CoV-2 infection and symptoms ranging from mild to critically ill patients. Serum Interleukin-6 concentration was tested and analyzed in all included cases. Some teams had reported IL-6 expression in COVID-19 patients, suggesting that elevated IL-6 and other cytokine levels correlated with severity of this disease.[17,18,19] Drastically elevated IL-6 levels (> 100 pg/ml), were closely associated with detectable serum SARS-CoV-2 viral load.[20] Distribution according to mean age (Table 2 & Fig. III) of group 1A was 40.80 years, group 1B was 38.02 years, group 2A was 41.06 years, group 2B was 38.02 years respectively no significant difference (P>0.05)) was seen when compared using One way ANOVA test revealed that age may not be the prime factor in Interleukin-6 elevation in the COVID-19 patients with poor periodontal status. The mean interleukin-6 pg/ml level (Table 3 & Fig. IV) was (26.0752, 1.5306, 4.1290, 0.7514) pg/ml in group (1A,1B,2A,2B) respectively and the difference was seen significant (P-0.0001*) when compared using one-way Anova test which showed the highest mean InterleukinL-6 level in COVID-19 cases with chronic periodontitis and lowest in COVID-19 recovered cases without chronic periodontal condition. As per given data (Table 3), the highest elevated Interleukin-6 level reported in group 1A cases was 105.20 pg/ml which further turned to more severe and critical condition while such emergency was not seen in the other rest groups revealed that those patients infected with the novel coronavirus with chronic periodontitis having more elevated Interleukin-6 faced more complications like life threatening respiratory complications, ICU admission and ventilation rather than the patients without periodontitis. The Post Hoc Comparison (Table 4), as the inter-group comparison among

groups (1A vs 1B, 1A vs 2A ,1A vs 2B) with significant difference (P-.0001*) and the groups (1B vs 2A, 1B vs 2B and 2A vs 2B respectively with non- significant difference (P>0.05). The significant value revealed the risk of mortality in the participants with chronic periodontitis following COVID-19 infections.

According to an International Team of Dental Researchers, hospitalized COVID-19 patients with periodontitis, with elevated levels of interleukin-6, more than 80 pg/ml were 22 times more likely to suffer from acute respiratory distress and placed on a ventilator.21 Zhao et al.22 compared IL-6 levels between COVID-19 patients and other pneumonia patients, and found that they did not differ significantly (19.34 pg/ml versus 15.06 pg/ml, P = 0.7). Giofoni et al.23 identified a cut-off value of 25 pg/ml of serum IL-6 as an independent risk factor of progression for severe COVID-19 and/or in-hospital mortality in a cohort of 77 patients. In another cohort study by Munich, elevated IL-6 (> 80 pg/ml) was strongly associated with a 22 times higher need for mechanical ventilation compared with patients with lower IL-6 levels in a cohort involving 40 patients, suggesting that high IL-6 level might predict the critical illness.24 The Periodontitis as well as COVID-19 the host immune response appears central to delineate the course of the disease. It is crucial that the health care professionals must be aware to assess oral health of the patients, since periodontal disease is, sometimes, neglected. Besides, it is mandatory to stimulate the oral care and oral hygiene practices to reduce the risk of local and systemic complications during the pandemic, particularly in patients with higher risk to COVID-19.

Conclusion:

To our knowledge, this is a short observational study regarding the strong inter-relationship among COVID-19, periodontal diseases and serum IL-6 levels. It is a genuine and on time attempt with certain limitations and urges the need for large case series to support this association. However, we may conclude that periodontitis is able to cause adverse systemic inflammation with excessive host response production of IL-6 that further has an important role in the contribution in the severity of systemic disease as well as probably, the progression of COVID-19 which may make worsen the

prognosis. Hence, Chronic periodontitis must be included in diagnostic work-up to stratify SARS-CoV-2 severity.

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