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RESEARCH ARTICLE

INTERLEUKIN-1β LEVELS IN THE HUMAN GINGIVAL SULCUS: RATES AND FACTORS AFFECTING ITS LEVELS IN HEALTHY SUBJECTS

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Abstract

Background and objective: Gingival crevicular fluid (GCF) affords a exceptional window for investigation of periodontal condition as the levels of inflammatory mediators, which consequences owing to the increased local destruction of connective tissue structural elements. This study aimed to explore the interleukin 1β (IL-1β)) levels in the human gingival sulcus in healthy normal people; and the effect of host factors as age, gender, type of tooth used in pro-inflammatory biomarkers.
Methods: Eighty seven patients, 54 (62.1%) female and 33 (37.9%) male (aged 12–34 years; mean 19.58±4.4 years), participated in this study. Each subject underwent a session on professional oral hygiene and received oral hygiene instructions. Gingival crevicular fluid (GCF) sampling was conducted (baseline). GCF was collected from the Central incisor, the Lateral incisor, the Canine, the First premolar and the second premolar in this study.
Results: In total, the mean±SD of central incisor IL-1β was 32.16±4.83 pg/ml, with a mode equal to 28.01 pg/mL, the median was 32.71 pg/mL, and ranged from 20.98 to 41.25 pg/ml with the 75% interquartile range (IQR) equal to 35.94 pg/ml. For males the mean±SD of central incisor IL-1β was 31.6±5.51 pg/ml VS 32.5±4.4 pg/ml of females. For the lateral incisor, canine, first premolar, second premolar:
Conclusion: This study provides the upper limit of normal values for interleukin 1β (IL-1β) levels for subjects aged 12–34 years in the GCF. These upper limits of normal values will guide dentists in Yemen when they consider the diagnosis of periodontal disease, as well as its role during orthodontic tooth movement where they play important role in osteocyte activities (e.g, osteoclasts and osteoblasts), and will provide useful baseline data for future studies of interventions against periodontal disease, and teeth movement by orthodontics appliances, in Yemen.
Keywords: gingival crevicular fluid (GCF), Interleukin 1β (IL-1β), normal level, pro-inflammatory cytokine.

INTRODUCTION

Interleukin-1 is a polypeptide with a diversity of activities and tasks in tissue homeostasis, inflammation, immunity, and tissue breakdown. After activation, Interleukin-1 is synthesized by different types of cells, involving macrophages, monocytes, T lymphocytes, fibroblasts, vascular cells, brain cells, and skin cells. There are two subtypes of IL-1, elected IL-1α and IL-1β, that are produced primarily by monocytes and macrophages but also by other cell types[1]. IL-1β is synthesized as a protein precursor only after motivation; on the contrary to IL-1α in which expression is persuaded by the transcription factor NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells) after exposure of innate immune cells

to the agonist. This take place, for example, after macrophages and dendritic cells have been exposed to lipopolysaccharide (LPS), which binds to (Toll-like receptor 4) TLR4 and acts as a pathogen-associated molecular pattern, another set of alarms[2,3]. Synthesis of IL-1β (and IL-18) precursors is induced by stimulation of innate immune cells by RIG-like receptors (RLRs) or Toll-like receptors (TLRs), however to acquire the capacity to bind to the IL-1 receptor, IL-1β precursors must be cleaved by a cysteine protease named caspase-1. Caspase-1 requires to be activated by a configuration named inflammasome mediated by cytoplasmic pattern recognition receptor signaling. Therefore, IL-1β production requires these two actions and the activation of other receptors. Beneath particular circumstances, IL-1β can also be processed by other proteases, such as during elevated neutrophilic inflammation[2,4]. At present, blockade of IL-1 (particularly IL-1β) activity is a standard treatment for patients with autoimmune diseases or lymphomas. Anakinra (IL-1Ra) is FDA-approved as a treatment for rheumatoid arthritis[5] for the reason that it decreases symptoms and slow joints destructions of rheumatoid arthritis. IL-1β has also been recommended to indolent or smoldering myeloma patients with a elevate hazard of progression to multiple myeloma. In combination with other drugs, IL-1Ra provides a significant increase in the number of years of progression-free disease in its recipients with absence of toxicity or other disorders[6].

Interleukin-1-β is described as a "pro-inflammatory" because it stimulates the activity of genes involved in inflammation and immunity. This protein plays an important role in protecting the body from foreign invaders such as bacteria and viruses. It also participates in bone resorption, especially for teeth when orthodontics or implants are under taken, as well as plays a role in the breakdown and removal of bone tissue that is no longer needed[1]. Any categorization of periodontitis as a risk factor for other diseases must measure the periodontal tissue inflamed in order to determine the burden of inflammation. Therefore, gingival inflamed surface area has been proposed as a classification of periodontitis that quantifies the amount of inflamed gingival tissue and, as such, quantifies the burden of systemic inflammation[7]. Gingival crevicular fluid (GCF) provides a unique window to analyze periodontal status as the levels of inflammatory mediators, which result due to increased local destruction of connective tissue structural elements that are ideal markers of disease activity can be estimated within the framework of the GCF[1].

Understanding the pro-inflammatory and normal level of interleukin-1 is useful in assessing the pathological level or inflamed surface area index (PISA) by determining its GCF level is fundamental to advancing the understanding and treatment of periodontal and oral diseases. A large amount of cross-sectional studies have been conducted in Yemen to investigate various dental problems[8-30], but no previous study dealing with the normal level of IL-1β in GCF has been conducted in Yemen or even anywhere else in the world. Thus, the aim of this study was to Knowing the normal level of interleukin-1β in GCF, which will be useful in assessing the pathological level or index of inflamed surface area (periodontal inflamed surface area, PISA) by determining its level in the GCF.

MATERIALS AND METHODS

The study was in persons whom they were treated with fixed orthodontic appliances (in clinics of orthodontic department, Faculty of dentistry, Sana'a University, and Azal dental center in Sana'a city). Demographic data and those connected with basic management were collected. Moreover the evaluation concerning dentition, oral hygiene, medical history, and intra-oral examination were performed.

Study Design: This is a longitudinal prospective clinical randomized selected cohort study, comparing the Pro-inflammatory (IL1-β) cytokines levels in gingival crevicular fluid (GCF), and host and materials factors might effect in their levels.

Inclusion criteria: Yemeni female or male, aged from 12 to 35 years, free from any apparent genetic disorders or dental anomalies, apparently healthy, non-pregnant, non-smoker, non-Khat chewer, and free from any systemic or chronic diseases, and not undergoes to antibiotics, corticosteroids therapy, and/or anti-inflammatory drugs, for at least one month ago.

Collecting of Gingival Crevicular Fluids (GCF) for Detection of Cytokines: Subjects were informed in advance not to eat or drink (except for water) or chew gum, or teeth brushing for one hour before sample collection. Gingival crevicular fluid was collected by sterile Paper-points strips (PAPER POINTS DIA-PROT, DiaDent Group, Choongchong Buk Do Rep. Of Korea) which placed into the gingival crevice of the teeth until gentle resistance is felt. Sampling was performed only from inside the gingival crevice of the tooth, to prevent salivary contamination (sample sites were isolated with cotton rolls), dental plaque were removed by cotton and the tooth surfaces were dried with an air syringe of dental chair. Paper-point strips were placed into the sulcus gently and care was taken to avoid mechanical injury and bleeding, then allowed to remain there for 30 seconds until the strips absorbed the gingival fluids, and the gingival crevicular fluids were collected. Contamination of strips with saliva and/or blood is important, and give incorrect results, so contaminated samples were being excluded from the study. All gingival crevicular fluid samples were collected in a pre-labeled sterile containers (Eppendorf tube - volume 1.5ml – CITOTEST, China), and stored at -35ºC for subsequent assay and analysis.

Detection and Quantification of pro-inflammatory (IL-1β) Cytokine: Each strip was eluted into 200 µl sterile Phosphate Buffered Saline PBS (pH 7.4) which used to assist in elution of cytokines from each filter paper. The samples were centrifuged for 20 minutes at 1000×g. then carried out the assay immediately. The concentration of the pro-inflammatory (IL1-β) mediators present in the GCF was evaluated by enzyme-linked immunosorbent assay (ELISA), following manufacturers' recommendations (Wuhan Fine Biotech Co., Ltd. Wuhan, Hubei, China).

Data analysis: Data were entered and analyzed using Epi-info software (version 7). The data for IL-β1 with a normal distribution were expressed as the mean and standard deviation (SD) for its levels in different time periods of collecting GCF. This procedure computes the difference between the means observed in two independent samples.

Ethical Consideration: Ethical approval No:699 dated January 24, 2021 was taken from the Medical Ethics and Research Committee of the Faculty of Medicine and Health Sciences, Sana'a University. The trial was according to the ethical guidelines of the review committee.

RESULTS

The study included 87 healthy individuals, 37.9% male and 62.1 female, ranging in age from 12-34 years, with a mean±SD equal to 19.58±4.4 years old. Most of the participants were in the age group 16-25 years (71.3%) (Table 1). In total, the mean±SD of central incisor IL-1β was 32.16±4.83 pg/ml, with a mode equal to 28.01 pg/mL, the median was 32.71 pg/mL, and ranged from 20.98 to 41.25 pg/ml with the 75% interquartile range (IQR) equal to 35.94 pg/ml; the variance in all individual values was significantly distributed on the normal curve with t-test of 62.1 and $p<0.001$.

Table 1: Characteristics of patients, tested for Interleukine-1β Levels in the human gingival sulcus during orthodontic treatment.

Characteristics	N (%)
Gender	
Male	33 (37.9)
Female	54 (62.1)
Age groups in Years	
<16	16 (18.4)
16 -25	62 (71.3)
26 -34	9 (10.3)
Total	87 (100)
Mean	19.58 years
SD	4.4 years
Mode	17 years
Median	18 years
Min –Max	12-34 years

For males the mean±SD of central incisor IL-1β was 31.6±5.51 pg/ml, with a mode equal to 20.9 pg/mL, the median was 31.6 pg/mL, and ranged from 20.96 to 41.25 pg/ml with the 75% interquartile range (IQR) equal to 36.7 pg/ml (t-test of 32 and $p<0.001$). For females the mean±SD of central incisor IL-1β was 32.5±4.4 pg/ml, with a mode equal to 28.01 pg/mL, the median was 33.1 pg/mL, and ranged from 21.1 to 40.1 pg/ml with the 75% interquartile range (IQR) equal to 35.3 pg/ml (test of 54.5 and $p<0.001$) (Table 2). In total, the mean±SD of lateral incisor IL-1β was 32.43±5.18 pg/ml, with a mode equal to 28.6 pg/mL, the median was 33.54 pg/mL, and ranged from 20.9 to 46.9 pg/ml with the 75% interquartile range (IQR) equal to 36.08 pg/ml; the variance in all individual values was significantly distributed on the normal curve with t-test of 58 and $p<0.001$. For males the mean±SD of central incisor IL-1β was 31.07±4.87 pg/ml, with a mode equal to 34.7 pg/mL, the median was 31.95 pg/mL, and ranged from 20.92 to 38.4 pg/ml with the 75% interquartile range (IQR) equal to 34.59 pg/ml (test of 36 and $p<0.001$). For females the mean±SD of central incisor IL-1β was 33.2±5.2 pg/ml, with a mode equal to 32.3 pg/mL, the median was 34.1 pg/mL, and ranged from 21.04 to 46.9 pg/ml with the 75% interquartile range (IQR) equal to 36.5 pg/ml (test of 46.6 and $p<0.001$).

For canine: In total and for males and females. The mean±SD of canine IL-1β level , mode, median, range, 75% interquartile range (IQR), was approximately similar to that of the central and lateral incisor (Table 2). For the first premolar: Total, males and females; the mean±SD of canine IL-1β level , mode, the median, range, 75% interquartile range (IQR), was roughly similar to that of the central and lateral incisor, and canine (Table 2). For the second premolar: Total, males, and females; The mean±SD of canine IL-1β level, mode, the median, range, 75% interquartile range (IQR), was roughly similar to that of the central, lateral incisor, canine and first premolar (Table 2). Considering age groups effects on the IL-1β concentrations in GCF, a lower levels were occurred in <16 years age group in which, the mean±SD of central incisor IL-1β concentration was 26.83±4.29 pg/ml. These findings are different from that of 16-25 years age group in which a higher values recorded; the mean±SD of central incisor IL-1β concentration was 33.3±4.13 pg/ml, with a mode equal to 33.25 pg/mL, the median was 33.34 pg/mL, and ranged from 20.98 to 41.25 pg/ml with the 75% interquartile range (IQR) equal to 36.62 pg/ml. Also, the lower levels occurred in <16 years age group, are different from that of 26-34 years age group in which a higher values recorded; the mean±SD of central incisor IL-1β concentration was 33.77±3.96 pg/ml, with a mode equal to29.13 pg/mL, the median was 32.78 pg/mL, and ranged from 29.13 to 40.14 pg/ml with the 75% interquartile range (IQR) equal to 36.65 pg/ml (Table 3).

DISCUSSION

Interleukin 1β (IL-1β) levels as measures of central propensity for interleukin 1β (IL-1β) in gingival crevicular fluid, are shown in Table 1. The mean±SD of IL-1β in the current study was 32.16±4.83 pg/mL. All values including mode (the most common value), minimum, maximum (range), and 75% interquartile range (IQR) are exemplified in Table 1. The normal values for IL-1β were found in this study to be approximately similar to those reported by Basaran *et al.*, (37.4 pg/ml)[31] and by Jayaprakash *et al.*,[32] (0.37 pg/µl=37 pg/ml). But compared to the data reported by Tuncer *et al.*,[33].

Table 2: IL-1β concentrations (pg/ml) for total patients and a comparison of males and females, before orthodontic treatment of 5 different teeth.

Statistc	Baseline IL-1β concentrations (pg/ml)		
	Total n=87	Males n=33	Females n=54
Central incisor			
Mean	32.16	31.6	32.5
SD	4.83	5.51	4.4
Mode	28.01	20.9	28
Median	32.71	31.6	33.1
Min -	20.98	20.96	21.1
Max	41.25	41.25	40.1
75%ile	35..94	36.7	35.3
T-test	62.1	32	54.5
p	<0.0001	<0.001	<0.001
Lateral incisor			
Mean	32.43	31.07	33.2
SD	5.18	4.87	5.2
Mode	28.06	34.7	32.3
Median	33.54	31.95	34.1
Min	20.9	20.92	21.04
Max	46.94	38.4	46.9
75%ile	36.08	34.59	36.5
T-test	58	36	46.6
p	<0.001	<0.001	<0.001
Canine			
Mean	33.84	34.05	33.73
SD	4.4	3.99	4.68
Mode	33.05	27.97	33.1
Median	33.7	33.1	33.78
Min -	23.1	27.97	23.14
Max	42.95	42.59	41.47
75%ile	37.1	37.1	37.05
T-test	52.4	34	40.1
p	<0.001	<0.001	<0.001
First premolar			
Mean	34.59	32.2	35.7
SD	5.72	3.68	6.2
Mode	34.56	26.7	34.56
Median	34.59	30.56	35.91
Min -	23.92	26.7	23.9
Max	50.83	40.09	50.83
75%ile	38.2	35.01	38.76
T-test	35.7	29	28
p	<0.001	<0.001	<0.001
Second premolar			
Mean	34.34	33.32	34.72
SD	4.2	3.62	4.56
Mode	25.36	29.89	25.36
Median	35.01	32.97	35.5
Min -	25.36	29.89	25.36
Max	41.05	37.12	41.05
75%ile	37.12	37.12	37.04
T-test	27	15.9	21
p	<0.001	<0.001	<0.001

The mean: the average value, the median: the middle value (it's a robust alternative to mean), and the mode: the most frequent value and the interquartile range (IQR) (75%): it gives the full spread of the data in 75% of the values.

Lower interleukin 1β (IL-1β) values were found as the mean interleukin 1β (IL-1β) was 0.0013 mg/ml=13 pg/ml[33]. Also in the current study, the mean±SD for IL-1β was slightly higher than that reported from Brazil by Chamil *et al.*, where the mean±SD for IL-1β concentration in GCF was-15.2±10.03 pg/ml [34]. The difference in interleukin-1β levels in the previous study may be due to genetic factors or the presence of unnoticed periodontitis disease among the study participants in Kaya *et al.*, study (72.14±22.1 pg/ml) [35]. Determination of the normal level of IL-1 β in GCF can be used to detect periodontal disease if there is a significant elevation of IL-1 β in GCF; Because the critical problem in periodontal disease is the lack of any reliable criteria for determining the extent of disease activity or the rate of disease progression at a given time[34,36]. The radiographic profile of the alveolar bone does not provide accurate diagnostic aids[35] and it is believed that there is a strong local mediator of tissue destruction associated with inflammatory diseases such as rheumatoid arthritis and periodontitis[37].

Table 3: IL-1β concentrations (pg/ml)) of difrent age groups of patients tested for Interleukine-1β levels before orthodontic treatment for 5 different teeth.

Statistc	IL-1β concentrations (pg/ml)		
	< 16 years group n=16	16-25 years n=62	26-34 years n=10
Central incisor			
Mean	26.83	33.3	33.77
SD	4.29	4.13	3.96
Mode	21.06	33.25	29.13
Median	26.32	33.34	32.78
Min -	21.06	20.98	29.13
Max	35.27	41.25	40.14
75%ile	28.62	36.62	36.65
T-test	25	63.3	25.5
p	<0.0001	<0.001	<0.001
Lateral incisor			
Mean	26.78	33.41	35.80
SD	5.08	4.39	2.79
Mode	20.92	28.06	30.81
Median	26.78	33.68	36.45
Min	20.92	21.31	30.81
Max	37.77	46.94	39.78
75%ile	28.73	36.08	36.89
T-test	21.1	59	38
p	<0.001	<0.001	<0.001
Canine			
Mean	28.45	34.43	36.18
SD	3.79	4.06	2.41
Mode	23.14	33.05	32.25
Median	28.02	33.68	36.92
Min -	23.14	26.92	32.25
Max	35.89	42.59	39.14
75%ile	28.73	37.14	37.92
T-test	19.8	47	42
p	<0.001	<0.001	<0.001
First premolar			
Mean	28.91	35.68	36.86
SD	4.64	5.79	2.81
Mode	23.92	29.32	34.56
Median	27.04	33.97	36.88
Min -	23.92	29.32	33.25
Max	35.81	50.83	42.08
75%ile	35.17	39.23	38.36
T-test	16.4	27.5	37
p	<0.001	<0.001	<0.001
Second premolar			
Mean	30.7	35.51	33.91
SD	7.55	3.67	1.55
Mode	25.3	29.89	32.81
Median	30.7	36.49	33.91
Min -	25.36	29.89	32.81
Max	36.04	41.05	35.01
75%ile	36.04	37.59	35.01
T-test	5.7	25.6	30
p	0.10	<0.001	<0.001

The mean: the average value the median: the middle value (it's a robust alternative to mean), and the mode: the most frequent value and the inter quartile range (IQR) (75%): it gives the full spread of the data in 75% of the values.

Because of the possibility that IL-1 β has a major regulatory effect on periodontal disease processes, a significant elevation of this factor has been found in these diseased gum tissues in GCF and can be used as a marker for confirmed the periodontal disease level and severity[37,38]. In the current study, IL-1β concentrations (pg/ml) for total patients and a comparison between males and females, in a research test of Interleukine-1β levels in human gingival Sulcus from 5 different teeth. There is no significant correlation between sex and IL-1β concentrations, suggesting that the female cut is equal to the male. In addition, the comparison of IL-1β levels in periodontal fluid has not been discussed by other researchers before and this study is one of the first to address this issue. In the current study, IL-1β concentrations (pg/ml) for total patients and a comparison between different age groups, for Interleukine-1β levels in human gingival Sulcus from 5 different teeth showed significant differences between values of <16 years group (26.83±4.29 pg/ml) and

older age groups 26-34 years (33.77±3.96 pg/ml) (Table 2); so there is a need for a large sample size of people used and for wide age groups as the results may be artificially affected by the choice of age groups limited, especially when the caliber has a complex pattern of change with age. That is why this study will recommend that clinicians use the cut-off values of the individual upper limit of the normal cut-off value for ages <16 years, rather than age subgroups, such as 11 to 12 year, 13 to 14 years old, and 15 years old. This is because there is a slight variation in annual values that have been found in children aged 12 to 16 years. There is no significant correlation between different types of teeth and IL-1β concentrations (Table 1, Table 2), indicating that the central incisor tooth is roughly equal to the lateral incisor teeth and to other teeth tested. Note that this issue has not been discussed by other researchers before.

Limitation of the study

There may be limitations in this study due to limitations in the research design as the research included only healthy subjects and it was preferable to enter a diseased group for comparison. There may be other factors that were not addressed in this study, and these factors may affect the results of this study. However, putting forward these limitations should not undermine its research value in the eyes of readers and reviewers.

CONCLUSIONS

Gingival crevicular fluid (GCF) provides a unique window for analysis of periodontal condition as the levels of inflammatory mediators, which results due to the increased local destruction of connective tissue structural elements represent the ideal markers of disease activity can be estimated in the GCF. This study explore the interleukin 1β (IL-1β)) levels in the human gingival sulcus in healthy normal people; and the effect of host factors as age , gender, type of tooth used in pro-inflammatory biomarkers; and can be initial study for using this pro-inflammatory biomarker in determination and confirming the periodontal disease level and severity. This study also provides the upper limit of normal values for interleukin 1β (IL-1β) levels for subjects aged 12–34 years in the GCF. These upper limits of normal values will guide dentists in Yemen when they consider the diagnosis of periodontal disease and will provide useful baseline data for future studies of interventions against periodontal disease in Yemen. This data can also be applied to the surrounding Yemeni cities and other adjacent countries.

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AUTHOR'S CONTRIBUTIONS

This article is part of a research conducted by Dr. Omar Ahmed Ismael Al-Dossary for his Ph.D., who carried out clinical and laboratory works with the assistance and supervision of Professor Hassan Al-Shamahy. Both contributed to the evaluation of clinical and laboratory findings, data analysis, and writing of the manuscript with help of other authors.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

None to declare.

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