Sana'a University Journal of Medical and Health Sciences https://journals.su.edu.ye/index.php/jchm
Vol. 17 | No. 1 | Page 10 – 20 | 2023 | ISSN: 2958-7476
Doi:10.59628/jchm.v17i1.210

Tumor necrosis alpha (tnf-α) levels in the human gingival sulcus: rates and factors affecting its levels in healthy subjects

Hassan Abdulwahab Al-Shamahy[2,3,4,*], Omar Ahmed Ismael Al-dossary[1,2,4], Ikram Ayad Anwar Al-Adhami [5], Khalid Al-dhorae[6]

[1]Orthodontics, Pedodontics and Prevention Department Faculty of Dentistry, Sana'a University, Yemen.
[2]Departement of Basic Sciences, Faculty of Dentistry, Sana'a University, Republic of Yemen.
[3]Medical Microbiology department, Faculty of Medicine, Genius University for Sciences & Technology, Dhamar city, Yemen.
[4]Medical Microbiology and Clinical Immunology Department, Faculty of Medicine and Health Sciences, Sana'a University, Yemen
[5]Biochemistry Department, Faculty of Medicine and Health Sciences, Sana'a University, Yemen
[6]Department of Orthodontics, college of Dentistry, University of Ibn al-Nafis for medical sciences, Sana'a, Yemen
***Corresponding author:** *h.shmahe15@su.edu.ye*

ARTICLE INFO
Article history:
Received: March 2, 2023
Accepted: May 30, 2023
Published: June, 2023

KEYWORDS
1. Gingival crevicular fluid (GCF)
2. normal level
3. pro-inflammatory cytokine
4. tumor necrosis factor-α (TNF-α)

ABSTRACT

Background and objective: Gingival crevicular fluid (GCF) provides an exceptional window for investigation of the periodontal condition in which levels of inflammatory mediators, which, as a consequence of the increased local destruction of connective tissue structural elements, are ideal markers of disease activity can be appreciated in the GCF. This study aimed to investigate the levels of tumor necrosis factor-alpha (TNF-α) in the human gingival sulcus of healthy subjects and the influence of host factors such as age, sex, and tooth type used on pro-inflammatory biomarkers.

Methods: Eighty-seven patients, 54 (62.1%) female and 33 (37.9%) males (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. The concentration of the pro-inflammatory cytokine (TNF-α) present in the GCF was evaluated by Enzyme-Linked Immunosorbent Assay (ELISA), following manufacturers' recommendations. Differences in mean TNF-α concentrations (pg/ml) of the teeth selected for sample collection for each individual with variables that included age, sex, and tooth type for GCF samples, were determined.

Results: In total, the mean ± SD of central incisor TNF-α was 31.88 ± 4.99 pg/ml, and ranged from 20.22 to 40.47 pg/ml; the variance in all individual values was significantly distributed on the normal curve with t-test of 59 and $p < 0.001$. For males the TNF-α concentration (mean ± SD) of central incisor was 39.25 ± 7.26 pg/ml Vs. 40.98 ± 9.24 pg/ml for females. For the lateral incisor, Canine, first premolar and Second premolar: total, males and females; the mean ± SD of TNF-α level were roughly similar to that of the central incisor. A lower level of TNF-α was in <16 years (37.54 ± 9.5 pg/ml). These findings differ from those of the 16-25 years age group and 26-34 age group in which a significantly higher value was recorded ($p=0.035$).

Conclusion: This study provides the upper limit of normal values for TNF-α levels for people aged 12-34 years in the GCF. These upper limits of normal values will guide dentists in Yemen when considering the diagnosis of periodontal disease, as well as its role during orthodontic tooth movement as they play an

important role in the activities of Osteocytes, and will provide useful baseline data for future studies of interventions against periodontal disease; and the movement of teeth by orthodontic appliances, in Yemen. Also, this data can also be applied to neighboring Yemeni cities and neighboring countries.

1. Introduction:

Tumor necrosis factor (TNF, cachexin, or cachectin; called tumor necrosis factor-alpha, or TNF-α) is an adipokine and cytokine. TNF-α is a member of the TNF Superfamily, which consists of various transmembrane proteins with a homologous TNF domain. TNF-α exists as a transmembrane form (mTNF-α) and as a soluble form (sTNF-α). sTNF-α results from the enzymatic cleavage of mTNF-α, [1] through a process called substrate presentation. mTNF-α is mainly found in monocytes/macrophages where it interacts with tissue receptors via cell-to-cell communication [1]. sTNF-α selectively binds to TNFR1, while mTNF-α binds to both TNFR1 and TNFR2 [2]. The binding of TNF-α to TNFR1 is irreversible, while the binding to TNFR2 is reversible [3]. The primary role of TNF is to regulate immune cells. TNF, as an endogenous pyrogen, is capable of inducing fever, apoptosis, cachexia, and inflammation, inhibits tumorigenesis and virus replication, and responds to sepsis via IL-1 and IL-6-producing cells. Dysregulated TNF production has been implicated in many human diseases including Alzheimer's disease [4], cancer [5], major depression [6], psoriasis [7] and inflammatory bowel disease [8]. Although controversial, some studies have linked depression and IBD to increased levels of TNF [9, 10]. TNF promotes an inflammatory response, which in turn causes several clinical problems associated with autoimmune disorders such as rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, psoriasis, hidradenitis suppurativa, and treatment-resistant asthma. These disorders are sometimes treated with TNF inhibitors. This inhibition can be achieved with a monoclonal antibody such as infliximab (Remicade) that binds directly to TNF, adalimumab (Humira), or certolizumab pegol (Cimzia), or with a circulating receptor fusion protein decoy such as etanercept (Enbrel) which binds to TNF with the greatest affinity for TNFR [11]. On the other hand, some patients treated with TNF inhibitors develop a worsening of their disease or a new onset of autoimmunity. TNF appears to have an immunomodulatory side, too. One explanation for the possible mechanism is this observation that TNF has a positive effect on regulatory T cells (Tregs), due to its binding to tumor necrosis factor receptor 2 (TNFR2) [12].

Anti-TNF therapy has shown modest effects in the treatment of cancer. Treatment of renal cell carcinoma with Infliximab resulted in prolonged disease stabilization in some patients. Etanercept has been tested for the treatment of patients with breast and ovarian cancer showing prolonged disease stabilization in some patients by downregulation of IL-6 and CCL2. On the other hand, the addition of Infliximab or Etanercept to Gemcitabine for the treatment of patients with advanced pancreatic cancer was not associated with differences in efficacy when compared to placebo [13].

TNF-alpha 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 [14]. 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 [14]. 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 [14]. 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 [15].

Understanding the pro-inflammatory and normal level of TNF-alpha 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 cross-sectional study has been conducted in Yemen to investigate various dental problems [16-41], but no previous study dealing with the normal level of TNF-alpha 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 TNF-alpha 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

2. Materials And Methods

The present study was carried out on persons who were treated with fixed orthodontic appliances (in outpatient clinics of the Orthodontic Department, Faculty of Dentistry, Sana'a University, and Azal Dental Center in Sana'a City, Yemen). Demographic data and those connected with basic management were collected. Moreover, the evaluation concerning dentition, oral hygiene, medical history, and intra-oral examination performed.

Study Design: This is a longitudinal prospective cohort study, where the eligible participants selected randomly to measure the Pro-inflammatory (TNF-α) cytokines levels in gingival crevicular fluid (GCF), and host and materials factors that might effect on 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 taken 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 was removed by cotton and the teeth 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 it is leaved there for 30 seconds until the strips absorbed the gingival fluids, and the gingival crevicular fluids were collected. It is important to prevent contamination of the strips with saliva and/or blood as this may give false results, so contaminated samples excluded from the study. All gingival crevicular fluid samples were collected in a pre-labeled sterile container (Eppendorf tube - volume 1.5ml – CITOTEST, China), and stored at -35^{o}C for subsequent assay and analysis.

Detection and Quantification of pro-inflammatory (TNF-α**) Cytokine:** Each strip was eluted into 200µl 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 (TNF-α) 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 TNF-α 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 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.

3. Results

The study included 87 healthy individuals, 37.9 % male and 62% female, aged 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).

Table 1: Characteristics of patients, tested for Tumor Necrosis Factor-α (TNF-α) Levels in the human gingival sulcus during orthodontic treatment

Characteristics	Number	(%)
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**	

In total, the mean ± SD of central incisor (TNF-α) was 31.88 ± 4.99 pg/ml, and ranged from 20.22 to 40.47pg/ml; the variance in all individual values was significantly distributed on the normal curve with t-test of 59 and $p < 0.001$. Tumor necrosis factor–α (TNF-α) levels differed from each other at different treatment periods as measures of (TNF-α) central tendency were elevated after orthodontic treatment was applied. Whereas, the mean ± SD for (TNF-α) increased from 32.16 ± 4.83 pg/mL at baseline to 42.89 ± 9.69 pg/mL after 7 days, to 45.55 ± 10.45 pg/mL after 21 days, and then declined to 36.61 ± 5.61 pg/mL in 90 days after orthodontic treatment. All values including mode (the most frequent value), minimum, maximum (range), and the 25% and 75% interquartile range (IQR) increased after treatment at 7 days, 21 days, and 90 days compared to the baseline period (at day 0) (Table 2). (TNF-α) concentrations (pg/ml) for total patients, in a research testing of mean ± SD (TNF-α) levels in human gingival sulcus during orthodontic treatment of 5 different teeth are showed in (Table 2). Levels of (TNF-α) differed from each other in the different treatment periods as both measures increased according to central tendency after orthodontic treatment was applied. As the mean ± SD of concentrations increased for (TNF-α) (pg/ml) for central incisor. For (TNF-α) concentrations it increased from 31.88 ± 4.99 pg/mL at baseline to 38.41 ± 7.76 pg/mL after 7 days, to 40.33 ± 8.54 pg/mL after 21 days, and then decreased to 34.09 ± 4.23 pg/ml at 90 days after orthodontic treatment, and it was statistically significant (Table 2). All values including mode (the most frequent value), minimum, maximum (range), and the 75% interquartile range (IQR) increased after treatment at 7 days, 21 days, and 90 days compared to the baseline period (at day 0) for all types of tested teeth (see Table 2). Also, very high significant changes occurred between the baseline times comparison with 3 time periods after orthodontic treatment of 5 different teeth for the levels of (TNF-α) (Table 3,4)

Table 2: Tumor Necrosis Factor –α (TNF- α (pg/ml)) concentrations (pg/ml) for total patients and a comparison of males and females, in a research testing of tumor necrosis factor levels in human gingival sulcus during orthodontic treatment of 5 different teeth.

Total n=87					Males n=33				Female n=54			
statistc	**Baseline**	**7 d**	**21 d**	**90 d**	**Baseline**	**7 d**	**21 d**	**90 d**	**Baseline**	**7 d**	**21 d**	**90 d**
Central incisor												
Mean	31.88	38.41	40.33	34.09	32.13	37.42	39.25	34.18	31.72	39.02	40.98	34.04

SD	4.99	7.76	8.54	4.23	5.75	6.61	7.26	4.83	4.51	8.38	9.24	3.86
SE	0.53	0.83	0.916	0.45	1.00	1.15	1.26	0.842	0.615	1.14	1.25	0.52
Mode	30.77	44.09	33.46	30.06	20.22	27.06	28.08	24.91	30.77	56.95	33.46	30.06
Median	32.15	37.43	40.33	34.09	32.13	38.01	39.28	34.18	32.11	39.024	38.59	34.83
Min -	20.22	24.15	25.04	24.77	20.22	27.06	28.08	24.91	22.23	24.15	25.04	24.77
Max	**40.47**	**59.64**	**63.45**	**42.57**	**40.47**	**59.64**	**63.45**	**42.57**	**40.28**	**58.53**	**62.34**	**40.81**
25%ile	28.39	**33.49**	**34.83**	**31.18**	**28.39**	**33.43**	**34.61**	**30.47**	**28.42**	**33.99**	**35.17**	**31.55**
75%ile	**35.39**	**41.31**	**43.93**	**37.09**	**37.03**	**40.62**	**43.05**	**38.54**	**35.09**	**42.75**	**44.17**	**36.61**
T-test	59	46	44	75	32	32	31	40	51	34	32	64
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Lateral incisor												
Mean	30.93	40.176	42.74	34.03	29.90	38.59	41.19	33.29	31.56	41.13	43.67	34.48
SD	5.13	8.14	9.35	4.43	4.96	7.36	8.66	4.48	5.17	8.50	9.70	4.38
SE	0.55	0.88	1.01	0.48	0.87	1.3	1.53	0.79	0.71	1.168	1.33	0.60
Mode	35.45	28.13	38.17	25.95	36.19	38.09	40.08	35.01	34.28	36.99	38.17	35.07
Median	31.36	39.06	40.87	34.86	29.77	38.09	39.95	33.46	31.84	39.75	41.07	34.97
Min -	20.88	23.37	24.62	22.92	20.88	25.01	26.28	22.92	21.45	23.37	24.62	24.21
Max	40.67	58.17	61.98	44.51	40.15	56.08	60.61	41.31	40.67	58.17	61.98	44.51
25%ile	26.35	34.99	36.17	31.77	25.85	33.87	35.04	31.08	27.79	36.99	38.07	32.14
75%ile	34.81	44.68	50.16	36.78	33.83	42.38	47.92	36.11	35.45	46.05	50.79	37.67
T-test	55	45	42	70	34	29	26.89	41.9	44	35	32	57
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Canine												
Mean	32.60	39.90	42.75	35.38	31.81	39.25	41.56	34.80	33.03	40.26	43.40	35.67
SD	5.82	5.46	7.29	4.56	5.71	4.42	5.89	4.04	5.994	6.0	7.98	4.85
SE	0.86	0.81	1.088	0.688	1.43	1.10	1.47	1.04	1.10	1.11	1.48	0.91
Mode	22.66	27.32	28.05	25.02	22.66	31.02	32.8	27.32	24.15	27.32	28.05	25.02
Median	32.553	40.25	41.74	35.1	32.43	40.06	41.21	33.69	33.65	40.25	41.93	35.45
Min -	22.66	27.32	28.05	25.02	22.66	31.02	32.18	27.32	24.15	27.32	28.05	25.02
Max	42.88	52.17	57.02	44.04	40.97	45.67	54.46	41.38	42.88	52.17	57.02	44.04
25%ile	28.39	36.74	37.92	32.35	27.37	35.32	36.63	32.63	28.39	37	38.18	31.63
75%ile	37.28	43.23	47.52	39.48	35.10	42.51	45.46	39.38	39.11	43.72	51.24	40.27
T-test	37	48	39	51	22	35	28	33	29	36	29	39
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
First premolar												
Mean	31.48	39.23	42.06	34.56	27.54	39.20	42.82	32.11	33.45	39.24	41.68	35.78
SD	6,51	6.91	8.38	5.33	3.71	7.00	9.06	2.86	6.77	7.03	8.22	5.89
SE	1.13	1.20	1.46	0.92	1.11	2.11	2.73	0.86	1.44	1.5	1.75	1.25
Mode	22.66	27.44	28.06	25.01	22.66	29.86	31.02	26.45	25.31	27.44	28.06	25.01
Median	29.98	41.34	43.09	33.43	27.73	41.47	45.39	32.45	31.52	41.13	42.94	34.68
Min -	22.66	27.44	28.06	25.01	22.66	29.86	31.02	26.45	25.31	27.44	28.06	25.01
Max	45.09	57.84	61.65	46.25	32.15	49.03	53.58	35.81	45.09	57.84	61.65	46.25
25%ile	25.86	33.05	34.68	31.08	23.16	32.99	34.44	31.32	26.77	34.01	35.28	31.04

75%ile	36.48	43.32	47.32	37.64	30.79	44.79	52.62	33.72	40.05	42.84	46.05	41.02
T-test	27	32	28	37	24	18	15	37	23	26	23.77	28
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Second premolar												
Mean	30.64	44.16	48.76	35.12	30.07	44.15	48.86	34.19	30.89	44.16	48.72	35.52
SD	5.63	5.15	6.50	3.29	4.52	9.76	10.68	2.11	6.36	2.84	5.03	3.76
SE	1.78	1.63	2.05	1.04	2.61	5.63	6.17	1.2	2.40	1.07	1.9	1.42
Mode	24.27	36.01	37.55	31.09	26.89	36.01	37.55	32.22	24.27	39.62	41.07	31.09
Median	28.93	44.39	50.46	34.53	28.08	41.47	50.26	33.95	29.79	44.42	50.66	35.12
Min -	24.27	36.01	37.55	31.09	26.89	36.01	37.55	32.22	24.27	39.62	41.07	31.09
Max	40.44	54.98	58.79	41.06	35.26	54.98	58.79	36.42	40.44	47.51	53.16	41.06
25%ile	26.77	41.28	42.73	32.41	26.89	36.01	37.55	32.22	25.67	41.28	42.73	32.41
75%ile	35.26	46.85	53.16	36.42	35.26	54.98	58.79	36.42	38.78	46.85	53.16	39.94
T-test	17	27	23	33	11.5	7.8	7.9	28	12.8	41	25.62	24
P	<0.001	<0.001	<0.001	<0.001	0.007	0.015	0.015	0.0012	<0.001	<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) (25% and 75%): it gives the full spread of the data in 25% or 75% of the values.

The standard error of the mean, or simply standard error, indicates how different the population mean is likely to be from a sample mean. It tells you how much the sample mean would vary if you were to repeat a study using new samples from within a single population.

A t-test is a statistical test that compares the means of two samples. It is used in hypothesis testing, with a null hypothesis that the difference in group means is zero and an alternate hypothesis that the difference in group means is different from zero.

P-value is the level of marginal significance within a statistical hypothesis test, representing the probability of the occurrence of a given event.

Table 3: Tumor Necrosis Factor-α (TNF-α) concentrations (pg/ml) for 87 patients in the baseline time comparison with 3 time periods after orthodontic treatment of 5 different teeth.

Teeth	Baseline	7 days	21 days	90 days
Central incisor				
Mean ± SD	31.88 ±4.99	38.41± 7.76	40.33± 8.54	34.09± 4.23
t-test	Refrance	6.59	7.96	3.15
Significance	Refrance	<0.0001	<0.0001	0.0019
Lateral incisor				
Mean ± SD	30.93± 5.13	40.176± 8.14	42.74± 9.35	34.03 ±4.43
t-test	Refrance	8.95	10.3	4.26
Significance	Refrance	<0.0001	<0.0001	<0.0001
Canine				
Mean ± SD	32.60 ±5.82	39.90 ±5.46	42.75 ±7.29	35.38 ±4.56
t-test	Refrance	8.5	10.14	3.51
Significance	Refrance	<0.0001	<0.0001	0.006
First premolar				
Mean ± SD	31.48± 6,51	39.23± 6.91	42.06 ±8.38	34.56±5.33
t-test	Refrance	7.61	9.3	3.41
Significance	Refrance	<0.0001	<0.0001	0.0008
Second premolar				
Mean ± SD	30.64± 5.63	44.16 ±5.15	48.76 ±6.50	35.12 ±3.29
t-test	Refrance	16.5	19.65	6.408
Significance	Refrance	<0.0001	<0.0001	<0.0001

4. Discussion

TNF-α levels as measures of central propensity for TNF-α in gingival crevicular fluid, are shown in Table 1. The mean ± SD of TNF-α in the current study was 31.88 ± 4.99 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 TNF-α were found in this study to be significantly higher than this reported by Pramustika *et al.* (8.25± 1.67 pg/ml) [15]. But compared to the data reported by Giacomelli, et al. [42] very high TNF-α values were found as the mean TNF-α was 1µg /ml = 1000 pg/ml. The difference in TNF-α levels in the previous study may be due to genetic factors or the presence of unnoticed periodontitis disease among the study participants [43]. Determination of the normal level of TNF-α in GCF can be used to detect periodontal disease if there is a significant elevation of TNF-α 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 [44]. The radiographic profile of the alveolar bone does not provide accurate diagnostic aids [45] and it is believed that there is a strong local mediator of tissue destruction associated with inflammatory diseases such as rheumatoid arthritis and periodontitis [46]. Because of the possibility that TNF-α 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 [44].

In the current study, TNF-α concentrations (pg/ml) for total patients and a comparison between males and females, in a research test of TNF-α levels in human gingival Sulcus from 5 different teeth. There is no significant correlation between sex and TNF-α concentrations, suggesting that the female cut is equal to the male. In addition, the comparison of TNF-α 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, TNF-α concentrations (pg/ml) for total patients and a comparison between different age groups, for TNF-α levels in human gingival Sulcus from 5 different teeth showed significant differences between values of <16 years group (37.54 ± 9.5 pg/ml) and older age groups 26-34 years (46.89 ±11.82 pg/ml, p=0.035) (Table 4); 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 TNF-α concentrations (Table 1,2,3,4), 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.

Table 4: The effect of ages on the Tumor Necrosis Factor-α (TNF-α) (pg/ml) concentrations of in the human gingival sulcus after 21 days of orthodontic treatment for 5 different teeth.

Teeth	Mean±SD	<16 years group n=16	16-25 years n=62	26-34 years n=10
Central incisor				
	Mean	37.54	40.16	46.89
	SD	9.5	7.52	11.82
	t-test	Refrance	1.17	2.22
	significant		0.24	0.035
Lateral incisor				
	Mean	40.43	42.34	50.166

	SD	9.55	8.84	10.52
	t-test	Refrance	0.758	2.43
	significant		0.45	0.02
Canine				
	Mean	35.74	43.85	44.87
	SD	7.46	6.83	5.66
	t-test	Refrance	4.15	3.31
	significant		0.0001	0.002
First premolar				
	Mean	33.70	44.54	43.68
	SD	8.46	8.05	2.26
	t-test	Refrance	4.75	3.62
	significant		<0.0001	0.0014
Second premolar				
	Mean	52.24	47.85	32.41
	SD	1.3	7.62	0.10
	t-test	Refrance	-2.28	-47
	significant		0.025	<0.0001

Limitation of the study:

There may be limitations in this study due to limitation 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.

5. Conclusion

Gingival crevicular fluid (GCF) provides a unique window for analysis of periodontal condition as the levels of inflammatory mediators, this 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 explores the TNF-α levels in the human gingival sulcus in healthy normal people; and the effect of host factors as: age, gender, and type of tooth used in pro-inflammatory biomarkers; this can be considered as an 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 TNF-α 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 countries.

6. References

[1] Qu Y, Zhao G, Li H. "Forward and Reverse Signaling Mediated by Transmembrane Tumor Necrosis Factor-Alpha and TNF Receptor 2: Potential Roles in an Immunosuppressive Tumor Microenvironment". Frontiers in Immunology 2017; 8: 1675. doi:10.3389/fimmu.2017.01675.

[2] Probert L. "TNF and its receptors in the CNS: The essential, the desirable and the deleterious effects". Neuroscience 2015; 302: 2–22. doi:10.1016/j.neuroscience.2015.06.038

[3] Szondy Z, Pallai A. "Transmembrane TNF-alpha reverse signaling leading to TGF-beta production is selectively activated by TNF targeting molecules: Therapeutic implications". Pharmacological Research 2017; 115: 124–132. doi:10.1016/j.phrs.2016.11.025.

[4] Swardfager W, Lanctôt K, Rothenburg L, Wong A, Cappell J, Herrmann N. "A meta-analysis of cytokines in Alzheimer's disease". Biol Psychiatry 2010; 68 (10): 930–941. doi:10.1016/j.biopsych.2010.06.012.

[5] Locksley RM, Killeen N, Lenardo MJ. "The TNF and TNF receptor superfamilies: integrating

mammalian biology". Cell 2001; 104 (4): 487–501. doi:10.1016/S0092-8674(01)00237-9.

[6] Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctôt KL. "A meta-analysis of cytokines in major depression". Biol Psychiatry 2010; 67 (5): 446–457. doi:10.1016/j.biopsych.2009.09.033.

[7] Victor FC, Gottlieb AB. "TNF-alpha and apoptosis: implications for the pathogenesis and treatment of psoriasis". J Drugs Dermatol 2002;1 (3): 264–75. PMID 12851985.

[8] Brynskov J, Foegh P, Pedersen G, Ellervik C, Kirkegaard T, Bingham A, Saermark T. "Tumour necrosis factor alpha converting enzyme (TACE) activity in the colonic mucosa of patients with inflammatory bowel disease". Gut 2002; 51 (1): 37–43. doi:10.1136/gut.51.1.37.

[9] Mikocka-Walus AA, Turnbull DA, Moulding NT, Wilson IG, Andrews JM, Holtmann GJ. "Controversies surrounding the comorbidity of depression and anxiety in inflammatory bowel disease patients: a literature review". Inflammatory Bowel Diseases 2007; 13 (2): 225–234. doi:10.1002/ibd.20062.

[10] Bobińska K, Gałecka E, Szemraj J, Gałecki P, Talarowska M. "Is there a link between TNF gene expression and cognitive deficits in depression?". Acta Biochim. Pol 2017; 64 (1): 65–73. doi:10.18388/abp.2016_1276.

[11] Haraoui B, Bykerk V. "Etanercept in the treatment of rheumatoid arthritis". Therapeutics and Clinical Risk Management 2007; 3 (1): 99–105. **doi:10.2147/tcrm.2007.3.1.99**.

[12] Salomon BL, Leclerc M, Tosello J, Ronin E, Piaggio E, Cohen JL (2018). "Tumor Necrosis Factor α and Regulatory T Cells in Oncoimmunology". Front. Immunol 2018; 9: 444. doi:10.3389/fimmu.2018.00444.

[13] Korneev KV, Atretkhany KN, Drutskaya MS, Grivennikov SI, Kuprash DV, Nedospasov SA. "TLR-signaling and proinflammatory cytokines as drivers of tumorigenesis". Cytokine 2017; 89: 127–135. doi:10.1016/j.cyto.2016.01.021.

[14] Walsh LJ, Trinchieri G, Waldorf HA, Whitaker D, Murphy GF. "Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocyte adhesion molecule 1". Proceedings of the National Academy of Sciences of the United States of America 1991; 88 (10): 4220–4. Bibcode:1991PNAS...88.4220W. doi:10.1073/pnas.88.10.4220.

[15] Pramustika A, Soedarsono N, Krisnawati, Widayati R. Comparison of tumor necrosis Factor-a concentrations in gingival crevicular fluid between self-ligating and preadjusted edgewise appliances in the early leveling stage of orthodontic treatment. Contemp Clin Dent 2018;9:92-6.

[16] Zabara AQMQ, Al-Kholani AIM, Alrubaidi YAS, Al-Kibsi TAM, Al-Akwa AAY, AL-Haddad KA, Al-Najhi MMA, Hassan Abdulwahab Al-Shamahy HA. Resolution of factors and pattern of permanent dental extraction in selected dental clinics in Sana'a city, Yemen. Universal Journal of Pharmaceutical Research 2022; 7(4):44-49. https://doi.org/10.22270/ujpr.v7i4.813

[17] Mutaher NJA, AL-Haddad KA, Al-Akwa AAY, Al-labani MA, Al-Shamahy HA, Zabara AQMQ, Al-deen HMS. Prevalence and causes of traumatic dental injuries to anterior teeth among primary school children in Sana'a city, Yemen. Universal J Pharm Res 2020; 5(3):38-43. https://doi.org/10.22270/ujpr.v5i3.414

[18] Abbas AM, Al-Kibsi TAM, Al-Akwa AAY, AL-Haddad KA, Al-Shamahy HA, Al-labani MA. Characterization and antibiotic sensitivity of bacteria in orofacial abscesses of odontogenic origin. Universal J Pharm Res 2020; 5(6):36-42. https://doi.org/10.22270/ujpr.v5i6.510

[19] Al-Akwa AA, Zabara A, Al-Shamahy HA, Al-labani MA, Al-Ghaffari KM, Al-Mortada AM, Al-Haddad AM, and Al-Sharani AA. Prevalence of Staphylococcus aureus in dental infections and the occurrence of MRSA in isolates. Universal J Pharm Res 2020; 5(2):1-6. https://doi.org/10.22270/ujpr.v5i2.384

[20] Al-Haddad KA, Al-dossary OE, Al-Shamahy HA. Prevalence and associated factors of oral non-candida albicans candida carriage in denture wearers in Sana'a city- Yemen. Universal J Pharm Res 2018; 3(4):7-11. 10.22270/ujpr.v3i4.176

[21] Al-Haddad KA, Al-Najhi MMA, Abbas AKM, Al-Akwa AAY, Al-Shamahy HA, Al-labani MA. Clinical features, age and sex distributions, risk factors and the type of bacteria isolated in periodontitis patients in Sana'a, Yemen. Universal J Pharm Res 2021; 6(1):1-8. https://doi.org/10.22270/ujpr.v6i1.532

[22] Alhadi Y, Al-Shamahy HA, Aldilami A, Al-Hamzy M, Al-Haddad KA, et al. Prevalence and pattern of third molar impaction in sample of Yemeni adults. On J Dent Oral Health 2019; 1(5):1-4. https://doi.org/10.33552/OJDOH.2019.01.000523

[23] Alhadi YAA, Al-Shamahi NYA, AL-Haddad KA, Al-Kholani AIM, Al-Najhi MMA, Al-Shamahy HA, Al-labani MA. Maxillary sinus septa: prevalence and association with gender and location in the maxilla among adults in Sana'a city, Yemen. Universal Journal of Pharmaceutical

Research 2022; 7(3):20-26. https://doi.org/10.22270/ujpr.v7i3.775

[24] Alhasani AH, Ishag RA, Al-Akwa AAY, Al shamahy HA, Al-labaniMA. Association between the Streptococcus mutans biofilm formation and dental caries experience and antibiotics resistance in adult females. Universal J Pharm Res 2020; 5(6):18-23. https://doi.org/10.22270/ujpr.v5i6.507

[25] Abbas AKM, Al-Shamahi NYA, Al-Shamahy HA, Al-Akwa AAY, Al-labani MA. Panorama-Radiographic Assessment of Impacted Teeth and Associated Pathosis Among Selected Males in Sana'a City, Yemen. J Dent Ora Heal Ad Re: JDOHAR 2020;101 (1):1-5. https://doi.org/10.39127/ JDOHAR:1000101

[26] Al-Kebsi AM, Othman AM, Abbas AKA, Madar EM, Al-Shamahy HA, Al-Gaffari KM, Daname SMN, Motareb FL. Oral C. albicans colonization and non-Candida albicans Candida colonization among university students, Yemen. Universal J Pharm Res 2017; 2(5):1-6. https://doi.org/10.22270/ujpr.v2i5.R2

[27] Alsamhari MMA, AlKhawlani MML,Al-Kholani AIM, Al-Najhi MMA, Al-Shamahy HA. Antimicrobial activity of Sodium hypochlorite, nano Silver and Chlorhexidine against mono-species biofilms of selected microorganisms of oral sources. Universal J Pharm Res 2022; 7(1):11-16. https://doi.org/10.22270/ujpr.v7i1.716

[28] Alsamhari MMA, Al-Najhi MMA, Al-Shamahy HA, Al-dossary OAI. Analysis of biofilms for Streptococcus mutans from dental root surfaces of adult patients with root caries. Universal J Pharm Res 2021; 6(5):19-23. https://doi.org/10.22270/ujpr.v6i5.668

[29] Al-Sanabani NF, Al-Kebsi AM, Al-Shamahy HA, Abbas AMA Etiology and risk factors of stomatitis among Yemeni denture wearers. Universal J Pharm Res 2018; 3(1):49-53. https://doi.org/ 10.22270/ujpr.v3i1.R9

[30] Al-Shamahi NYA, Abbas AKM, Al-Shamahy HA, Al-akwa AAY, Al-haddad KA. Panoramic Radiographic Analysis of Signs of Proximity to the Third Molar Roots of the Mandibular Canal in Yemen. J Med Clin Case Rep 2020; 2(3): 1-7. https://doi.org/10.47485/2767-5416.1017.

[31] Al-Shamahi NYA, Abbas Al-Kasem M, Al-Shamahy HA, Al-Akwa AAY, Al-Ghaffari KM, Al-Mortada AM, Al-labani MA. Radiographic assessment of the course and visibility of the mandibular canal by panoramic radiography. Universal J Pharm Res 2020; 5(5):17-20. https://doi.org/10.22270/ujpr.v5i5.481

[32] Al-Shamahy HA, Abbas AMA, Mahdie Mohammed AA, Alsameai AM. Bacterial and Fungal Oral Infections Among Patients Attending Dental Clinics in Sana'a City-Yemen. On J Dent Oral Health 2018; 1(1): 1-6. https://doi.org/10.33552/OJDOH.2018.01.000504

[33] Al-Shamahy HA, Mohammed Abdullah Al-labani MA, Ameen al-akwa. Biofilm formation and antifungal susceptibility of candida isolates from oral cavity of denture wearer and free denture individuals. EC Dental Sci 2020; 19(10):58-66.

[34] Al-Shami IZ, Al-Shamahy HA, Abdul Majeed ALA, Al- Ghaffari KM and Obeyah AA. Association between the salivary Streptococcus Mutans levels and dental caries experience in adult females. On J Dent Oral Health 2018; 1(1):1-5. https://doi.org/10.33552/OJDOH.2018.01.000505

[35] Bamashmoos, KAO, Alhasani AH, Al-Akwa AAY, Zabara AQMQ, Al-Shamahy HA et al.Prevalence of premature loss of primary teeth at the age of 6-10 years in Sana'a city, Yemen. Universal J Pharm Res 2020; 5(4):42-46. https://doi.org/10.22270/ujpr.v5i4.439

[36] Dahaq WAM, Al-Kholani AIM, Al-Kibsi TAM, Al-Deen HS, Al-Shamahy HA, AL-Haddad KA, Al-Akwa AAY, Al-labani MA. Tanaka and Johnston's mixed dentition validity: an analysis among Yemeni adults in Sana'a city. Universal J Pharm Res 2021; 6(6):1-5. https://doi.org/10.22270/ujpr.v6i6.691

[37] Gylan EMA, Muharram BA, Al-Kholani AIM, AL-Haddad KA, Al-Akwa AAY, Al-Shamahy HA, Al-Hamzi MA, Al-labaniMA. In vitro evaluation of the antimicrobial activity of five herbal extracts against Streptococcus mutans. Universal J Pharm Res 2022; 7(1):37-44. https://doi.org/10.22270/ujpr.v7i1.721

[38] Bin Yehia LAT, AL-Haddad KA, Al-labani MA, Al-ShamahyHA, Shaga-aldeen HM. Occlusal characteristics of the primary dentition among a sample of Yemeni pre-school children. Universal J Pharm Res 2020; 5(1):25-31. https://doi.org/10.22270/ujpr.v5i1.359

[39] Makki KIF, Abbas AMA, Alhadi YAA, Al-Shamahy HA. Clinical effects of Platelets Rich Fibrin (PRF) following surgical extraction of impacted lower third molars among a sample of Yemeni adults. Universal Journal of Pharmaceutical Research 2022; 7(6):14-21. DOI: https://doi.org/10.22270/ujpr.v7i6.864

[40] Al-dossary OAI, Al-Kholani AIM, AL-Haddad KA, Al-Najhi MMA, Al-Shamahy HA, Al-adhami IAA, Aldhorae K. Interleukin-1β levels in the human gingival sulcus: Rates and factors affecting its levels in healthy subjects. Universal

Journal of Pharmaceutical Research 2022; 7(5):42-48.
DOI: https://doi.org/10.22270/ujpr.v7i5.8384

[41] Al-Shami IZ, Alrubaidi YAS, AL-Haddad KA, Al-Najhi MMA, Al-Shamahy HA, Al-Kibsi TAM, Al-Kholani AIM. Effect of removable dentures on colonization of aerobic bacteria in the oral cavity and antibiotic pattern of the common isolated bacteria. Universal Journal of Pharmaceutical Research 2022; 7(6):1-8. DOI: https://doi.org/10.22270/ujpr.v7i6.862

[42] Giacomelli C, Natali L, Nisi M, De Leo M, Daniele S, Costa B, Graziani F, Gabriele M, Braca A, Trincavelli ML, Martini C. Negative effects of a high tumour necrosis factor-α concentration on human gingival mesenchymal stem cell trophism: the use of natural compounds as modulatory agents. Stem Cell Res Ther. 2018; 11;9(1):135. doi: 10.1186/s13287-018-0880-7.

[43] Gokul K, Faizuddin M, Pradeep AR. Estimation of the level of tumor necrosis factor- α in gingival crevicular fluid and serum in periodontal health & disease: A biochemical study. Indian J Dent Res. 2012; 23(3):348-52. Doi:10.4103/0970-9290.102221..

[44] Baik H, Kim C, Lim WH, Chun YS. Interleukin-1α and tumor necrosis factor-α expression on the compressed side of gingiva during orthodontic tooth movement. Open J Stomatol 2012;2:182-7.

[45] Davenport RH, Jr, Simpson DM, Hassell TM. Histometric comparison of active and inactive lesions of advanced periodontitis. J Periodontol 1982; 53 (5):285–9. https://doi.org/10.1902/jop.1982.53.5.285

[46] Dayer JM, de Rochemonteix B, Burrus B, Demczuk S, Dinarello CA. Human recombinant interleukin 1 stimulates collagenase and prostaglandin E2 production by human synovial cells. J Clin Invest. 1986; 77(2):645-8. https://doi.org/10.1172/jci112350.