International Journal of Dentistry and Oral Science (IJDOS)
ISSN: 2377-8075

Semi-Quantifying Oral Polymorphonuclear Neutrophils In Subjects with Healthy Periodontium - An In Vivo Study

Research Article

Prithvi Shetty[1], K.V.V. Prasad[2], Shravan Shetty[3*]

[1] Assistant Professor, Department of Public Health Dentistry, A.J.Institute of Dental Sciences, N.H.17, Kuntikana, Mangalore, Karnataka. India.
[2] Professor, Department of Public Health Dentistry, SDM College of Dental Sciences and Hospital, A constituent unit of Shri Dharmasthala Manjunatheswara University, Dharwad, Karnataka, India.
[3] Assistant Professor, Department Of Orthodontics and Dentofacial Orthopaedics, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India.

Abstract

Background: The innate immune response of an individual depends on the critical role played by the neutrophils. They migrate into the oral cavity at an increased rate in the presence of any inflammatory condition and act as the sentinels of oral immune system. They are present both in diseased as well as healthy periodontium in varied numbers respectively.
Aim: To quantify and determine oral polymorphonuclear neutrophils (oral-PMNs) range in subjects with healthy periodontium.
Settings and Design: Forty two subjects were recruited for the study initially based on convenient sampling technique. Subjects were then standardized, monitored and imparted with rigorous oral hygiene measures over a period of three weeks until absolute no signs of gingival inflammation was observed. A standardized 10ml saline rinse for 30 seconds duration were collected from subjects before examination and instrumentation. Clinical examination was performed with the help of Loe-Silness's Gingival Index. Neutrophils were observed under the blue light of Fluorescent microscope, quantified and then correlated with the clinical parameters.
Statistical Analysis Used: Descriptive statistics was generated. Mean and Standard deviation was calculated for the continuous variables gingivalindex score and oral-PMNsvalues.
Results: When the subjects attained an average Gingival Index score of less than 0.5, oral-PMNs counts determined on subjects in absolute oral health was seen in the range from 0.04 – 1.05 x 105/ml.
Conclusions: Oral-PMNs counts obtained through a 30 seconds oral rinse served as a good marker of oral inflammatory load in subjects with healthy periodontium, which in turn would provide a healthy norm for advent of any oral inflammatory condition in future.

Keywords: Neutrophils; Periodontium; Inflammation.

Introduction

Today, oral diseases constitute a major public health problem. Nearly every other person in India is suffering from some form of periodontal disease [1] which can be prevented and reversed majorly in its initial stages [2]. Neutrophil recruitment and their anti-microbial action are essential for maintenance of absolute periodontal and oral health. They form the first line of defense against any invading pathogen, reason for which they are called the 'sentinels of oral immune system'. This particular response of neutrophils is very essential in maintaining a healthy environment in the oral cavity.

Research over the years has shown continuous presence of neutrophilsin our oral cavity both in normal as well as diseased conditions, with varying numbers [2, 3]. Quantification of oral polymorphonuclear neutrophil (oral-PMNs) levels and its respective role in absolute periodontal and oral health is very important in un-

***Corresponding Author**:
Dr. Shravan Shetty,
Assistant Professor, Department Of Orthodontics and Dentofacial Orthopaedics, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India.
Tel: +91 7411197827
E-mail: shravan.shetty@manipal.edu

Received: October 24, 2020
Accepted: February 05, 2021
Published: February 16, 2021

Citation: Prithvi Shetty, K.V.V. Prasad, Shravan Shetty. Semi-Quantifying Oral Polymorphonuclear Neutrophils In Subjects with Healthy Periodontium - An In Vivo Study. *Int J Dentistry Oral Sci.* 2021;8(2):1623-1626. **doi:** http://dx.doi.org/10.19070/2377-8075-21000321

derstanding periodontal disease and other similar oral inflammatory conditions which occur in the oral cavity. However,literature search showed very less information in this arena.In patients with suspected systemic infections, healthy norms(specific range) have been determined for circulating blood Neutrophils. However, in absolute periodontal and oralhealth, the levels of oral polymorphonuclear neutrophils are still not ascertained. Hence, the present study was carried out to quantify and determine a specific range for oral polymorphonuclear neutrophils in patients with healthy periodontium following rigorous oral hygiene measures.

Subjects and Methods

This descriptive cross-sectional single-centre study was carried out in the Department of Public Health Dentistry. The study subjects comprised of adult population above 18 years of agewho fulfilled the inclusion and exclusion criteria mentioned below.

Inclusion criteria-Subjects who gave their written consent for participation. Exclusion criteria-Subjects exhibiting any visible signs and symptoms of clinical oral inflammation(I.e. ulceration, redness, swelling, dental caries, periodontal diseaseetc.); medically compromised subjects; subjects on any sort of medication; subjects with any adverse habits (Smoking, Alcohol, etc.). The ethical clearance was obtained from the Scientific and Ethics Institutional Review Board.

Sample size and sampling procedure: the present study was based on convenient sampling technique through which 42 study subjects who fulfilled the eligibility criteria were randomly selected.

Clinical Protocol - The study protocol was explained to each participant and a written informed consent was obtained from them. Medical history was carefully reviewed and taken into consideration. Subjects who fulfilled the eligibility criteria were enrolled. A careful and meticulous oral examination was performed while recruiting the study subjects.

The criteria for determining healthy periodontium was based on the criteria given by Mancini et.al. [4]. It states that for a periodontium to be considered healthy it should have absolutely no signs of clinical inflammation i.e. no loss of clinical attachment, no bleeding on probing and a Loe and Silness's Gingival Index [5] score of less than 1 on all the tooth surfaces.Subjects when recruited had some mild signs of gingival inflammation. They were then standardized, monitored and imparted with rigorous oral hygiene measures (daily two times brushing, thorough scaling initially followed by its repetition every week) until absolute no signs of gingival inflammation were seen in the study subjects.

Sample collection, cell isolation and PMNs counting protocol: Subjects before brushing or rinsing their mouth were asked to rinse with a standardized 10ml of normal saline rinse for 30 seconds duration before patient examination and instrumentation. All samples were processed within 2-3 hours of collection.Sample collected was vertexed for a period of 15-20 seconds and 500 ul of this sample was dispensed into a falcon tube with the help of a pre-adjusted dispenser, following which 500 ul of acridine dye solution was added thereby exhibiting a 1:1 ratio. Acridine orange is a fluorescent nucleic acid marker, which allows neutrophils to be distinguished from other cells using fluorescence microscopy. The falcon tube was shaken and after 1 minute the sample was loaded onto the Neubauer's chamber until its capacity. Neutrophils were then counted and quantified under the blue light of the fluorescent microscope using the standard criteria.

Clinical examination was performed with the help of Loe-Silness's Gingival Index and the Gingival Index (GI) scores were correlated with the neutrophil counts subsequently every week. In the current study at the end of two weekssubjects had reached a stage which fulfilled the criteria for having healthy periodontium after which the normal neutrophil count for each study subject was determined.

Statistical Analysis

Descriptive statistics was generatedusing mean and SD for continuous variables and Log transformation was used to normalize the distribution.

Results

The present study was conducted to know the healthy norms of Oral PMNs in 40 subjects with absolute oral health. The study results showed that when the study subjects attained a mean GI score of <0.5,the neutrophils were in the range of 0.04 – 1.05 X 105/ml (Table 1 and Figure 1) in absolute oral health.

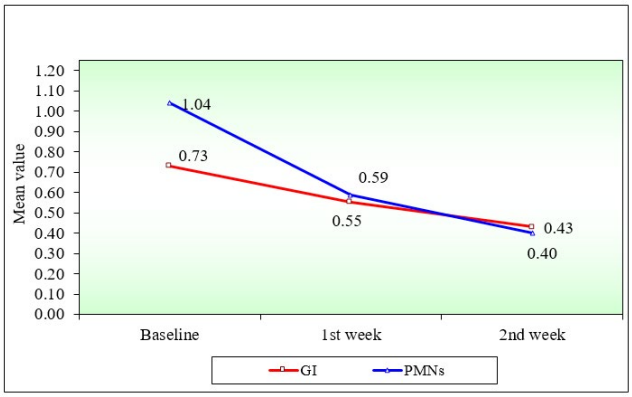
Discussion

Concept of neutrophils as a biomarker has emerged as an important aspect in terms of monitoring general health. They are the key cells of the innate immune system and have been studied extensively as early markers of inflammation. Since the inception of this concept many attempts have been made to correlate its level in overall health and disease. In humans among the leukocytes,

Table 1. Showing the values of Gingival Index (GI) and Oral polymorphonuclear neutrophils (Oral PMNs) scores at baseline, 1 week and 3 week's time points.

Summary	GI			Oral PMNs		
	Baseline	1st week	2nd week	Baseline	1st week	2nd week
N	42	40	40	42	40	40
Minimum	0.44	0.39	0.29	0.33	0.04	0.04
Maximum	1.12	0.77	0.5	2.29	1.11	1.05
Range	0.68	0.38	0.21	1.97	1.08	1.02
Mean ± SD	0.73 ± 0.22	0.55 ± 0.10	0.43 ± 0.04	1.04 ± 0.52	0.59 ± 0.30	0.40 ± 0.28

Figure 1. Values of Gingival Index (GI) and Oral Polymorphonuclear neutrophils (Oral PMNs).



Polymorphonuclear neutrophils are the most abundant, accounting for 50–70% of all circulating white blood cells [6]. Quantification of neutrophils in blood is often used as a primary screen to identify patients with acute infections [7]. Specific norms have been determined for circulating blood neutrophils in both health and disease. However, there is no specific literature available that gives us a specific norm for neutrophils in absolute oral health and disease. Several attempts have been attempted in the past to quantify Oral-PMNs using different approaches in order to facilitate comparison between periodontal health and disease [2, 8].Information available through literature is very sparse in the context of knowing the specific norms when it comes to determining levels of Oral-PMNs in absolute oral health which the current study made an attempt to do so.

As per the World Health Organisation, dental caries and periodontal disease are the most common and preventable inflammatory conditions that affect the oral cavity [9]. The basic nidus for the occurrence of both these conditions is the presence of microbial plaque.Any infection or inflammatory condition in the body starts with the advent of microbes. When these microbes enter our body or our oral cavity, our body's defensive mechanism gets activated.Our body's immune system is made of the innate and specific type of immunity. The innate immune system provides the host with an immediate but nonspecific response to any infection. The specific type of immune system provides a delayed but specific type of response to any infection. Neutrophils play a pivotal role as a part of the innate immune response and form the first line of defence against any pathogenic microbe entering into the body through the host anatomical barriers such as the skin, mucosa or the oral cavity. The more the number of bacteria, the more neutrophils will be sent by the body to fight the infection. This process will continue until either of the two supersede each other. This clearly shows that number of neutrophils can be correlated well with the degree of inflammation in our body. Thus, neutrophils play a pivotal role in the initial prevention of any disease in our body. Hence, the current study chose to assess the levels of neutrophils in absolute oral health as a parameter for assessment.

The idea of using neutrophil quantification to assess periodontal disease status and the effectiveness of therapy was first proposed in 1978 by Raeste and Aura [10]. PMNs recruitment into the oral cavity is from two sources, one being the gingival crevicular fluid and the other being the saliva.In the past, studies concentrated more on quantification of neutrophils in the gingival crevicular fluid which very well correlated with the level of periodontal inflammation.However, research now focuses more on saliva as a rapid, non-invasive and a less cumbersome procedure in quantifying oral inflammatory load.

A study conducted by Landzberg [11] compared the number of neutrophils in the oral cavity in health as well as disease by using systematically a standardized rapid rinse test that could be easily administered to dental patients in the clinical setting. This study also showed that the collection of PMNs from the oral cavity is consistently reproducible and the levels of PMNs can be increased in the presence of various oral inflammatory conditions. It also showed that a mere diagnostic rinse could be more than useful in screening various oral inflammatory conditions.

Any screening procedure unless substantiated with clinical evidence is not considered to be reliable. In the present study, only healthy subjects with absolute systemic and oral health were recruited so as to avoid any possible effects of the immune system. However, to get and maintain absolute periodontal health in the study subjects they had to follow strict oral hygiene measures and were under constant supervision of the examiner. They were given proper oral hygiene instructions, were asked to brush twice a day and thorough scaling was performed on them not as an intervention measure but only to remove the presence of any local factor. They were then recalled every week to make sure there is no presence of local factor and even if present,it was removed again by rescaling with the help of ultrasonic or hand scalers. In the current study, all the study subjects at the end of 2 weeks fulfilled the criteria of having healthy periodontium (i.e. no loss of attachment,no bleeding on probing and a gingival index score of less than 1) [4].

The adjunct of clinical examination would help the examiner in knowing the active or inactive stage of disease on the basis of increased levels of oral-PMNs. In most of the conditions, increased oral inflammatory load correlates very well with an active oral inflammatory condition. However in some situations in the absence of active oral diseases, non-oral diseases or conditions should be suspected for which the word semi quantification is used in place of quantification as the exact level of neutrophils is difficult to ascertain in such cases. In the current study, 42 subjects were selected initially for the study from which 2 subjects had to be excluded for developing viral infection during the study period supposedly in whom the neutrophil count was very high in comparison to other subjects which was in accordance with the study conducted

byGalani and Andreakos [12].

According to a review by Loos [13] oral-PMNs levels are a better generalized screening tool for oral inflammation and periodontal disease than circulating neutrophil levels. Oral-PMNs counts obtained through a 30-s oral rinse served as a good marker of oral inflammatory load in subjects with healthy Periodontium, which in turn would provide a healthy norm for advent of any oral inflammatory condition in future.

Public health dentistry specifically emphasises on the prevention of oral diseases. Quantification of oral polymorphonuclear neutrophils by public health specialists could possibly tell in future the beginning or end of any active oral inflammatory condition which could open new horizons in the prevention of oral diseases. However, further trial with a larger sample size would give a more coherent picture of the study results.

Conclusion

• This study demonstrated that oral polymorphonuclear neutrophils could be quantified through a single, rapid, non-invasive 30 second saline mouth rinse and the acridine dye technique which could be a very useful tool for monitoring oral health in patients.
• Knowing the levels of oral-PMNs could be a good indicator of oral inflammatory load for periodontal diseases and dental caries status and could be correlated well with the disease activity.
• If the method is considered reliable by many other researches in future, probably dentists could screen for oral diseases just as their medical counterparts screen for systemic infections.

References

[1]. Shaju JP, Zade RM, Das M. Prevalence of periodontitis in the Indian population: A literature review. J Indian Soc Periodontol. 2011 Jan;15(1):29.
[2]. Scott DA, Krauss J. Neutrophils in periodontal inflammation. Periodontal disease. 2012;15:56-83.
[3]. Moosani A, Sigal MJ, Glogauer M, Lawrence HP, Goldberg M, Tenenbaum HC. Evaluation of periodontal disease and oral inflammatory load in adults with special needs using oral neutrophil quantification. Spec Care Dentist. 2014 Nov-Dec;34(6):303-12.Pubmed PMID: 24961943.
[4]. Mancini S, Romanelli R, Laschinger CA, Overall CM, Sodek J, McCulloch CA. Assessment of a novel screening test for neutrophil collagenase activity in the diagnosis of periodontal diseases. J Periodontol. 1999 Nov;70(11):1292-302.Pubmed PMID: 10588492.
[5]. Löe H. The gingival index, the plaque index and the retention index systems. J Periodontol. 1967 Nov;38(6):610-6.
[6]. Guyton AS, Hall J E. Textbook of Medical Physiology, 10t h edition.Pennsylvania : Elsevier ;2000.
[7]. von Vietinghoff S, Ley K. Homeostatic regulation of blood neutrophil counts. J Immunol. 2008 Oct 15;181(8):5183-8.Pubmed PMID: 18832668.
[8]. Hajishengallis E, Hajishengallis G. Neutrophil homeostasis and periodontal health in children and adults. J Dent Res. 2014 Mar;93(3):231-7.Pubmed PMID: 24097856.
[9]. Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ. 2005;83:661-9.
[10]. Raeste AM, Aura A. Rate of migration of oral leukocytes in patients with periodontitis. Scand J Dent Res. 1978 Jan;86(1):43-51.Pubmed PMID: 273302.
[11]. Landzberg M, Doering H, Aboodi GM, Tenenbaum HC, Glogauer M. Quantifying oral inflammatory load: oral neutrophil counts in periodontal health and disease. J Periodontal Res. 2015 Jun;50(3):330-6.Pubmed PMID: 25040400.
[12]. Galani IE, Andreakos E. Neutrophils in viral infections: Current concepts and caveats. J Leukoc Biol. 2015 Oct;98(4):557-64.Pubmed PMID: 26160849.
[13]. Loos BG. Systemic markers of inflammation in periodontitis. J Periodontol. 2005 Nov;76:2106-15.