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# The assessment of CD4 lymphocyte counts in patients with chronic periodontitis in Benin City, Nigeria

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## ABSTRACT

**Objective:** To determine the CD4 lymphocyte counts in patients diagnosed with chronic periodontitis. **Methods:** A total of eighty patients and twenty control subjects attending Central Hospital and University of Benin Teaching Hospital (UBTH), Nigeria were recruited for the study. Three millilitres of blood sample was collected from each subject, put into ethylene diaminetetra-acetic acid (EDTA) bottle and mixed thoroughly. CD4 cell counts were determined using the Cyflow SL-3 method. **Results:** The results showed that subjects within the age of 51–60 years had the highest cases of periodontitis (32.5%). Females were more affected (61.25%) than the males (38.75%). Based on the CD4 counts among the subjects, 60 (75%) had greater than 700 cells/ $\mu$ L; 12 (15%) between 500–700 cells/ $\mu$ L and only 8(10%) had less than 500 cells/ $\mu$ L which showed that CD4 counts increased in the peripheral blood of the subjects. **Conclusions:** The need to properly brush the teeth and encourage regular cleanings by the dentist is further encouraged for the prevention of periodontal disease.

## 1. Introduction

Periodontal diseases, also called gum diseases, are serious bacterial infections that destroy the gums and the surrounding tissues of the mouth. If the inflammation is left untreated, the disease will continue and the underlining bones around the teeth will dissolve, and will no longer be able to hold the teeth in place; it is the most common cause of tooth loss in adults [1,2].

It has been reported that periodontitis bacteria like *Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Actinobacillus actinomycetemcomitans* are implicated and regarded as specific pathogens in various types of periodontitis diseases [3]. Several studies have demonstrated that the antibody titers to these organisms are increased in patients with periodontitis compared with subjects without periodontitis disease [4,5]. The CD4 count is a measurement of how many functional CD4 T-cells are circulating in the blood. The lower the CD4 count, the weaker the immune system [6]. CD4+/CD8+ ratios have been reported to be

increased in the peripheral blood of patients with adult periodontal disease and reduced in periodontal lesions compared to peripheral blood or normal gingival [7].

Most studies in Nigeria indicate that the prevalence of periodontitis is high and the occurrence of the disease is related to oral hygiene status and socio-economic class [8]. Most of the reports in our environment are on the aetiological agents of periodontitis without evaluating the immune status of the patients. The authors are not aware of any report or study in Benin City that evaluated CD4 counts of patients with chronic periodontitis. Therefore, this study was carried out to determine the CD4 count of patients clinically diagnosed to have periodontitis attending the dental clinics of University of Benin Teaching Hospital and Central Hospital, Benin City, Nigeria.

## 2. Materials and method

### 2.1. Study population and sample collection

A total of eighty patients, who have been diagnosed clinically by Dentists to have chronic periodontitis and mobile teeth were recruited for this study. Periodontitis was confirmed when a patient had some of the following

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signs and symptoms: deep pockets measuring about 4mm and above between the teeth and the gum, mobile or loose teeth, redness and swelling of gums and dental plaque. Exclusion criteria included pregnant women, patients with mobile teeth due to accident or trauma, and patients with immunocompromised ailments such HIV/AIDS and tuberculosis. Subjects consisted of 31 males and 49 females between 22 to 90 years old; mean ( $55.4 \pm 3.6$ ). The patients were attending the Dental Clinics in the University of Benin Teaching Hospital (UBTH) and the Central Hospital, Benin City, Nigeria. Twenty apparently healthy individuals served as control. Verbal informed consent was obtained from all patients prior to specimen collection. The protocol for this study was approved by the Ethical Committees of both Health Institutions.

A 3 ml of blood was collected by venipuncture from each patient and dispensed into ethylene diaminetetra acetic acid (EDTA) container, mixed and labeled. Samples were stored at 4°C until used.

The CD4 count was determined using the Cyflow method (manufactured by Partec Cooperation, Germany). Briefly, a 20  $\mu$ L of CD4PE mAb was dispensed into Rohren test tube and 20  $\mu$ L of a well mixed EDTA whole blood collected within 6 hours was added. This was mixed and incubated in the dark for 15 minutes at room temperature. A quantity of 800  $\mu$ L of CD4 buffer was added, mixed and analysed on the Cyflow. The above was determined using Cyflow SL-3 green (Partec Co-operation, Germany) following the manufacturer's instructions.

## 2.2. Statistical analysis

The Mean values as well as other statistical analysis at 95% confidence limits were carried out by using the SPSS software package. Using Pearson's correlation test ( $P < 0.01$ ), the CD4 cell counts of the study group were correlated with those of the control group.

## 3. Results

The results for the evaluation of the CD4 cell counts for patients who presented with chronic periodontitis is given below. The patients were made up of both males and females (aged between 22–90 years).

A total of 80 subjects were screened for seropositivity to chronic periodontitis, out of which, 31 (38.75%) were males with mean age of  $57.23 \pm 10.98$  years while 49 (61.25%) were female with mean age of  $54.98 \pm 14.95$  years.

Table 1 shows the age ranges of the studied subjects. The age group 51–60 years has the highest number of cases (i.e. 26 made up 11 males and 15 females) with chronic periodontitis.

Table 2 shows the CD4 cell counts as obtained among the studied subjects. The results showed that 75% of the subjects had greater than 700 cells/ $\mu$ L, 15% had between 500–700 cells/ $\mu$ L and only 10% of the subjects had less than 500 cells/ $\mu$ L.

Data collected were analysed statistically using Pearson's

correlation analysis. The CD4 cell counts were compared between the studied group and the control group. However, CD4 cell counts were not statistically significant between the studied and control groups.

**Table 1.**

Age range distribution of studied subjects.

| Age Range (Year) | No. of subjects | Control |
|------------------|-----------------|---------|
| 21–30            | 4               | 16      |
| 31–40            | 1               | 4       |
| 41–50            | 23              | –       |
| 51–60            | 26              | –       |
| 61–70            | 15              | –       |
| 71–80            | 10              | –       |
| 81–90            | 1               | –       |
| Total            | 80              | 20      |

**Table 2.**

The CD4 cell counts among the test and control subjects.

| CD4 cell count (cells/ $\mu$ L) | No. of subjects | Control |
|---------------------------------|-----------------|---------|
| 2000                            | 1               | –       |
| 1900–2000                       | 1               | –       |
| 1600–1700                       | 3               | 1       |
| 1500–1600                       | 4               | –       |
| 1400–1500                       | 2               | –       |
| 1300–1400                       | 4               | 3       |
| 1200–1300                       | 4               | 1       |
| 1000–1200                       | 16              | 9       |
| 800–1000                        | 16              | 2       |
| 700–800                         | 9               | –       |
| 500–700                         | 12              | 4       |
| >500                            | 8               | –       |
| Total                           | 80              | 20      |

## 4. Discussion

Apart from measuring the strength of the immune system, the CD4 count also determines in part, the treatment needs for a patient. A CD4 count of less than 200 cell /  $\mu$ L suggests that the patient is at risk of opportunistic infections [12]. From the results obtained in this study the female subjects had a higher number of cases of periodontitis (61.25%) than the males (38.75%). This implied that females were more affected with chronic periodontitis than their male counterparts. This was however not statistically significant ( $p > 0.05$ ). This may be due to the fact that females have more immune and hormonal changes than males and also tend to age faster than males. Females also take in more carbohydrate (sugary) substances due to their social life style.

Also, it was observed from the results that among the studied subjects, those within the age bracket of 51–60 years had the highest number of cases of subjects with chronic periodontitis 26 (32.5%). This is in agreement with the other report where chronic periodontitis was commonly observed in people who are about 60 years of age [15]. It can be concluded further that chronic periodontitis is a disease condition commonly found in elderly people than young

ones.

The results from this study based on the CD4 lymphocytes showed that most of the subjects had a high value of CD4 lymphocytes in their peripheral blood. It was observed that about 35 (43.75%) of the subjects had CD4 cell count above 1000 cells/ $\mu$ L. Hence, among all the subjects, about 90% of the subjects had high CD4 cell counts above 500 cells/ $\mu$ L while 10% of the study subjects had less than 500 cells/ $\mu$ L as their CD4 cells count. One reason for this could be that a majority of the subjects may have elicited effective immune response against the organisms known to cause periodontitis, hence the high CD4 values [16]. This is in concordance with other findings which showed that CD4+ values appear to be increased in the peripheral blood of patients with adult periodontal disease [13,15]. It has been reported that patients with chronic periodontitis presented increased frequency of T-lymphocytes and CD4+CD25+T-cells in the inflammatory infiltrate of gingival tissues [17].

In periodontitis, periodontal pathogens and their products gain access to the systemic circulation, which may result in immune responses and have adverse effects on the homeostasis of the circulatory and immune systems.

It was observed in the course of this study that almost all the subjects diagnosed clinically with chronic periodontitis at the Dental Clinics had very poor dental or oral hygiene. The primary etiology of gingivitis is poor oral hygiene which leads to the accumulation of a mycotic and bacterial matrix at the gum line, called dental plaque [18]. In some people, gingivitis progresses to periodontitis -- with the destruction of the gingival fibers, the gum tissues separate from the tooth and deepened sulcus, called a periodontal pocket. The authors further encourage individuals to adhere strictly to the twice daily brushing as recommended by the Nigerian Dental Association. This study did not establish a correlation between chronic periodontitis and CD4 levels; however, there is the need for all patients with oral disorders to be evaluated for CD4 levels so as to prevent any possible immunocompromise situation that may further arise from such diseases.

### Conflict of interest statement

We declare that we have no conflict of interest.

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### References

- [1] Listgarten M.A. Pathogenesis of Periodontitis. *J. Clin. Periodontol.* 1986; **13**:418–430
- [2] Savage A, Eaton K, Moles DR, Needleman I. A systematic review of definitions of periodontitis and methods that have been used to identify this disease. *J. Clin. Periodontol.* 2009; **36**:458–467.
- [3] Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. *J. Periodontol.* 1992; **63**:322–331.
- [4] Ready D, D' Ajuto F, Spraitt DA, Suvan J, Tonetti MS, Wilson M. Disease severity associated with presence in subgingival plaque of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia*, singly or in combination as detected by nested multiplex PCR. *J. Clin. Microbiol.* 2008; **46**:3380–3383.
- [5] Suide PT, Olsen I, Enersen M, Beiske K, Grinde B. Human cytomegalovirus and EPV in apical and marginal periodontitis: a role in pathology. *J. Med. Virol.* 2008; **80**:1007–1011.
- [6] Thomasini RL, Bonon SH, Durante, Coasta SCB. Correlation of cytomegalovirus and human herpesvirus 7 with CD3 and CD3+CD4 cells in chronic periodontitis. *J. Periodontal Research* 2012; **47**(1): 114–120
- [7] Bilichodmath S, Mangalekav SB, Sharma DCG, Prabhakar AK, Reddy SB, Kalburg NB. Herpesvirus in chronic and aggressive periodontitis patients in an Indian population. *J. Oral. Sc.* 2009; **51**:79–86.
- [8] Rotok A, Cassai E, Farina R, Caselli V, Lazzarotto T, Trombelli L. Human herpesvirus 7, EPV and human cytomegalovirus in periodontal tissue of periodontally diseased and healthy subjects. *J. Clin. Periodontol.* 2008; **35**:831–837
- [9] Lima PMA, Souza PEA, Coasta JE, Gomez RS, Gollob KJ, Dutra WO. Aggressive and chronic periodontitis correlate with distinct immunoregulatory cytokines. *J. Periodontol.* 2011; **82**:86–94
- [10] Taubman M A, Stoufi ED, Ebersole JL, Smith DJ. Phenotypic studies of cells from periodontal disease tissues. *J. Periodontal Res.* 1984; **19**:587–590.
- [11] Kojima T, Yano K, Ishikawa I. Relationship between serum antibody levels and subgingival colonization. *J. Periodontol.* 1997; **68**(7):618–625.
- [12] Mark–Cichoki R.N. Understanding absolute CD4 count and CD4percentage. *About.com Health's Dis. Con.* 2007; **1**:42–43
- [13] Mather A, Michalowicz BS. Cell-mediated immune system regulation in periodontal diseases. *Crit. Rev. Oral Biol. Med.* 1997; **8**:76–89.
- [14] Akpata ES. Oral Health in Nigeria. *Int. Dental J.* 2004; **54**:361–366.
- [15] Kaye EK, Valencia A, Baba N, Spiro IA, Dietrich T, Garcia R I. Tooth Loss and Periodontal Disease Predict Poor Cognitive Function in Older Men. *J. Am. Geriatrics Society.* 2010; **58** (4): 713–718.
- [16] Andrukhov O, Ulm C, Reischl H, Nguyen P Q, Matejka M, Rausch–Fan X. The Serum Cytokine levels in Periodontitis Patients in relation to the bacterial load. *J. Periodontol* 2011; **82**(6) 885–892.
- [17] Cardoso CR, Garlet GP, Ana –Paula– Moreira AP, Martins WJ, Rossi MA, Silva JS. Characterization of CD4+/CD25+ natural regulatory T cells in the inflammatory infiltrate of human chronic periodontitis. *J. Leukocyte Biol.* 2008; **84**:311–318.
- [18] Urzúa B, Hermosilla G, Gamonal J, Morales–Bozo I, Canals M., Barahona S, Cóccola C, Cifuentes V. Yeast diversity in the oral microbiota of subjects with periodontitis: *Candida albicans* and *Candida dubliniensis* colonize the periodontal pockets. *Med. Mycol.* 2008; **46** (8): 783–793.