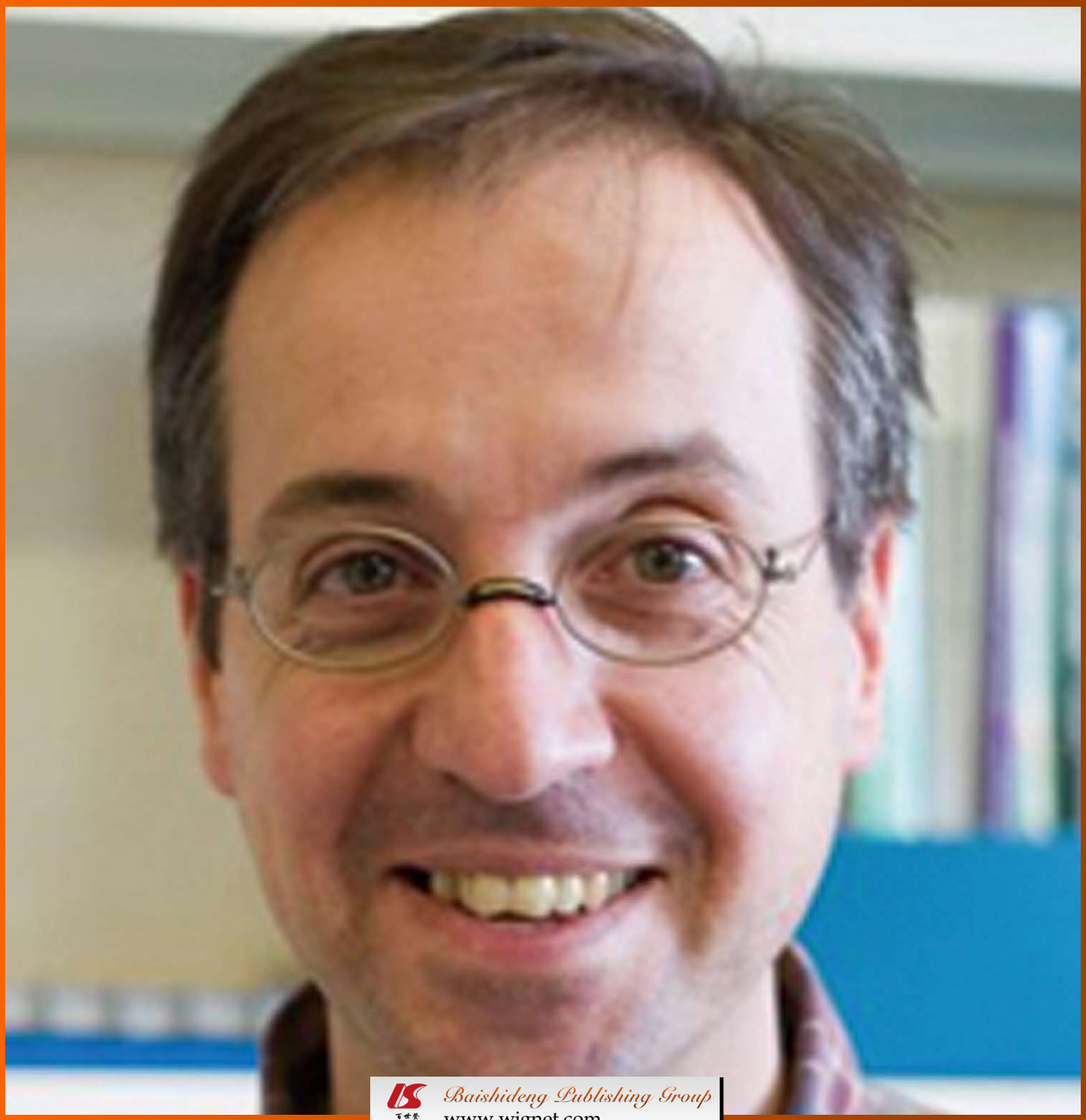
ISSN 1949-8454 (online)

World Journal of *Biological Chemistry*

World J Biol Chem 2012 October 26; 3(10): 180-183



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NAME OF JOURNAL
World Journal of Biological Chemistry

ISSN
ISSN 1949-8454 (online)

LAUNCH DATE
January 26, 2010

FREQUENCY
Monthly

EDITING
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Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpg@baishideng.com
http://www.wjgnet.com

PUBLICATION DATE
October 26, 2012

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1949-8454/g_info_20100316155305.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/esps/
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doi:10.4331/wjbc.v3.i10.180

World J Biol Chem 2012 October 26; 3(10): 180-183
ISSN 1949-8454 (online)

BRIEF ARTICLE

Salivary immunoglobulin classes in Nigerian smokers with periodontitis

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Received: July 2, 2012 Revised: August 10, 2012
Accepted: August 17, 2012
Published online: October 26, 2012

Abstract

AIM: To determine the levels of salivary immunoglobulin classes in Nigerian smokers and non-smokers with periodontitis.

METHODS: Sixty-nine individuals were recruited into this study after obtaining informed consent. They were subdivided into three groups that consisted of 20 (aged 46 ± 11 years) cigarette smokers with periodontitis (S+P); 24 (40 ± 12 years) smokers without periodontitis (S-P); and 25 (53 ± 11 years) non-smokers with periodontitis (NS+P). An oral and maxillofacial surgeon used radiographs for periodontal probing for the diagnosis of periodontitis. The smokers included subjects who smoked at least six cigarettes per day and all the periodontitis patients were newly diagnosed. About 5 mL of unstimulated saliva was expectorated by each subject into plain sample bottles. Salivary immunoglobulin levels were estimated using enzyme linked immunosorbent assay. Student's *t* test was used to determine significant differences between the means. Values of $P < 0.05$ were regarded as significant.

RESULTS: No significant differences were observed in the mean salivary levels of the immunoglobulin classes (IgG, IgA, IgM and IgE) when S+P was compared with S-P. Mean salivary levels of IgA (520.0 ± 155.1 ng/mL *vs* 670.0 ± 110 ng/mL, $P = 0.000$) and IgM (644.5 ± 160.0 ng/mL *vs* 791.4 ± 43.7 ng/mL, $P = 0.000$) were significantly lower in the S+P compared with NS+P group. Salivary IgA (570.4 ± 145.6 ng/mL *vs* 670.0 ± 110 ng/mL, $P = 0.008$) and IgM (703.1 ± 169.3 ng/mL *vs* 791.4 ± 43.7 ng/mL, $P = 0.012$) levels were significantly lower in the S-P compared with NS+P group. Only one (5%) periodontal patient had detectable levels of salivary IgE (0.20 IU/mL). Similarly, only one smoker (4.17%) had detectable levels of salivary IgE (0.04 IU/mL) and two non-smokers (9.52%) had detectable levels of IgE (0.24 IU/mL).

CONCLUSION: Our study suggests that reduced salivary IgA and IgM levels in smokers with periodontitis could enhance increased susceptibility to periodontitis.

Key words: Cigarette smoke; Immunoglobulin; Periodontitis; Saliva; Smokers

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Olayanju OA, Rahamon SK, Joseph IO, Arinola OG. Salivary immunoglobulin classes in Nigerian smokers with periodontitis. *World J Biol Chem* 2012; 3(10): 180-183 Available from: URL: http://www.wjgnet.com/1949-8454/full/v3/i10/180.htm DOI: http://dx.doi.org/10.4331/wjbc.v3.i10.180

INTRODUCTION

Tobacco smoking has consistently been identified as an important risk factor for the development of periodontal disease[1]. Cross-sectional studies have demonstrated that smoking leads to epidemiologically and biologically enhanced risk of periodontal disease[2]. The role of cigarette smoke in periodontal disease development was further established by a United States National Health Survey that showed that about 42% cases of periodontal diseases were associated with cigarette smoking[3].

Heat from cigarette smoke increases calculus formation. Nicotine deposition from cigarette smoking is associated with local effects such as attachment loss, recession on the lingual surfaces of maxillary teeth and mandibular incisors, plaque retention, reduced collagen synthesis, fibroblast growth inhibition, and damage to cell membranes[4,5]. The cellular and humoral immune systems are both affected by smoking. It exerts effects throughout the cytokine network and suppresses both chemotactic and phagocytic functions of polymorphonuclear leukocytes in saliva and tissues[6,7]. Suppression of immune responses, impaired stromal cell function, and amplified inflammatory responses have been reported in smokers. Changes in molecular and cellular mechanisms at both the local and systemic levels explain the strong association between tobacco smoke and the severity of periodontal disease[1]. Smokers with periodontal disease typically have less clinical inflammation and gingival bleeding than non-smokers. This has been attributed to nicotine with a vasoconstrictive effect that culminates in reduced blood flow, edema, and clinical signs of inflammation, which mask serious periodontal disease in smokers[1].

Smoking decreases levels of salivary IgA and serum IgG to *Prevotella intermedia* and *Fusobacterium nucleatum*. It also creates an imbalance between T-lymphocyte subset ratios, hindering T-helper-cell function and compromising antibody production. A study among Nigerian cigarette smokers has reported increased levels of serum IgG and IgM[1,8]. Smoking suppresses the production of IgG2, which is predominantly required for the protection against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. This suggests that suppression of IgG2 antibodies may be the main mechanism inducing severe periodontitis[9].

Humoral immune response has been reported to have a protective role in periodontal disease pathogenesis[10]. Alterations in specific IgG and IgA responses both locally at inflamed sites and systemically have relevance in disease progression[11,12].

Saliva contains immunoglobulins that might have originated from the salivary glands and serum[13] but previous studies on salivary immune parameters are scarce, and to the best of our knowledge, no such study has been carried out among Nigerians. The present study assessed the levels of salivary immunoglobulin classes in cigarette smokers with or without periodontitis. This would probably, explain the contributory role of cigarette smoke in the development of periodontitis.

MATERIALS AND METHODS

Study subjects

Sixty-nine individuals were recruited for this study after obtaining informed consent and ethical approval from the University of Ibadan/University College Hospital Joint Ethics Review Committee. They were subdivided into various groups that consisted of 20 (aged 46 ± 11 years) cigarette smokers with periodontitis (S+P); 24 (40 ± 12 years) smokers without periodontitis (S-P); and 25 (53 ± 11 years) non-smokers with periodontitis (NS+P). An oral and maxillofacial surgeon used radiographs for periodontal probing for the diagnosis of periodontitis. The smokers smoked at least six cigarettes per day and all the periodontitis patients were newly diagnosed. Pregnant women and patients with diabetes or human immunodeficiency virus infection were excluded from the study. Patients with other forms of oral disease were also excluded from the test group. A short structured questionnaire was administered to each subject to obtain information on age, sex, occupation, cigarette smoking, and drug consumption.

Spitting was used for saliva collection. About 5 mL of unstimulated saliva was expectorated by each subject into plain sample bottles. The samples were collected between 09:00 and 11:00 h, 1 h after eating or mouth washing. The samples were centrifuged at 3000 × *g* for 5 min and the clear supernatant was gently pipetted into another clean plain bottle and stored at -20 ℃ until analyzed.

Immunoglobulin levels were estimated using enzyme linked immunosorbent assay (ELISA) (Immunology Consultant Laboratory, Portland, OR, United States). The IgE kit was supplied by Leinco Technologies (St Louis, MO, United States). The assay was carried out following the manufacturer's instructions.

Statistical analysis

The data were presented as mean and standard deviation. Student's *t* test (unpaired) was used to determine significant differences between the means. Values of $P < 0.05$ were regarded as statistically significant.

RESULTS

The mean levels of salivary immunoglobulin classes were lower in the S+P group compared with the S-P group, although the differences were not significant (Table 1). Mean salivary levels of IgA and IgM were significantly lower in the S+P group when compared with the NS+P group ($P = 0.000$, $P = 0.000$, respectively) (Table 2). No significant differences were observed in the mean levels of IgG and IgE. In Table 2, salivary IgA and IgM levels were significantly lower in the S-P group when compared with the NS+P group. IgG and IgE levels were not significantly different.

Only one (5%) periodontitis patient had detectable levels of salivary IgE (0.20 IU/mL). Similarly, only one smoker (4.17%) had detectable levels of salivary IgE (0.04 IU/mL) and two non-smokers (9.52%) had detectable

Table 1 Levels of salivary immunoglobulin classes in smokers with periodontitis and smokers without periodontitis

	S+P (n = 20)	S-P (n =24)	P values
IgG (ng/L)	349.4 ± 91.5	355.7 ± 40.8	0.734
IgA (ng/L)	520.0 ± 155.1	570.4 ± 145.6	0.203
IgM (ng/L)	644.5 ± 160	703.1 ± 169.3	0.111
IgE (IU/mL)	0.2 ± 0.9	0.04 ± 0.2	0.519

S+P: Smokers with periodontitis; S-P: Smokers without periodontitis.

Table 2 Levels of salivary immunoglobulin classes in non-smokers with periodontitis, smokers with periodontitis, and smokers without periodontitis

	NS+P (n = 25)	S+P (n = 20)	S-P (n = 24)	P^1 value	P^2 value
IgG (ng/L)	370.0 ± 60.9	349.4 ± 91.5	355.7 ± 40.8	0.268	0.42
IgA (ng/L)	670.0 ± 110	520.0 ± 155.1	570.4 ± 145.6	0.000	0.008
IgM (ng/L)	791.4 ± 43.7	644.5 ± 160.0	703.1 ± 169.3	0.000	0.012
IgE (IU/mL)	0.2 ± 1.0	0.2 ± 0.9	0.04 ± 0.2	1.000	0.494

[1]NS+P *vs* S+P; [2]NS+P *vs* S-P. S+P: Smokers with periodontitis; S-P: Smokers without periodontitis; NS+P: Non-smokers with periodontitis.

levels of IgE (0.24 IU/mL). This could be an indication that the level of IgE was low in the saliva of smokers and periodontitis patients, and therefore immeasurable by ELISA.

DISCUSSION

Periodontal diseases are infectious diseases caused by anaerobic Gram-negative bacteria[14]. Cigarette smoking is a significant risk factor for the initiation and progression of periodontal disease. Studies have reported altered inflammatory cytokine levels in serum and gingival crevicular fluid in smokers[15]. Nicotine in cigarette smoke affects the host inflammatory response to oral pathogens by upregulating release of prostaglandin and interleukin-2 leading to accelerated periodontal tissue destruction[1].

Reduced, but nonsignificant levels of immunoglobulin classes were observed when we compared S+P with S-P groups. This observation suggests that cigarette smoking might not have a profound effect on periodontitis at the early stage because all our patients were newly diagnosed. However, the interaction between cigarette smoke and periodontitis was reflected in the lower levels of salivary IgA and IgM in smokers with periodontitis (S+P) when compared with periodontitis patients who were non-smokers (NS+P). Al-Ghamdi and Sukumaran[15] have reported reduced IgA in the serum of smokers with periodontitis. Our observation corroborates earlier reports[16,17] that cigarette smoking is associated with suppression of B-cell function and immunoglobulin production. This further explains the potential mechanism by which cigarette smoking exacerbates periodontal disease.

In order to understand the independent effects of smoking and periodontitis on the levels of salivary immunoglobulin classes, the S-P group was compared with the NS+P group. It was observed that smokers without periodontitis had significantly lower levels of IgA and IgM when compared with non-smokers with periodontitis. This indicates that cigarette smoke could be involved in suppression of oral immunity, thus making smokers more prone to Gram-negative bacterial infections that cause periodontal diseases. This observation could partly explain the mechanism through which smoking enhances increased susceptibility to periodontitis and its subsequent poorer response to treatment.

The small sample size was a major limitation of this study. Therefore, further research with a larger sample is required to confirm the differences observed in this study.

In conclusion, our study suggests that cigarette smoking could enhance initiation and progression of periodontitis due to its associated immunosuppression. Mechanisms to increase the levels of these salivary immunoglobulin classes may be therapeutic in the control and prevention of oral diseases.

COMMENTS

Background

Studies on salivary immune parameters have been scarce and no such study has been carried out among Nigerians.

Research frontiers

This study assessed the levels of salivary immunoglobulin classes in cigarette smokers with or without periodontitis. This could explain the contributory role of cigarette smoke to the development of periodontitis.

Innovations and breakthroughs

It was observed that salivary IgA and IgM were decreased in smokers with periodontitis compared with those who did not smoke.

Applications

By understanding how cigarette smoke causes reduced salivary immunoglobulin levels, increasing the levels of these salivary immunoglobulins may be therapeutic in the control and prevention of periodontitis.

Peer review

This work is interesting and merits publication. It is clearly written and the study was adequately carried out.

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Acknowledgments to reviewers of *World Journal of Biological Chemistry*

We acknowledge our sincere thanks to our reviewers. Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of our World Series Journals. Both the editors of the journals and authors of the manuscripts submitted to the journals are grateful to the following reviewers for reviewing the articles (either published or rejected) over the past period of time.

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ISSN 1949-8454 (online)

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World J Biol Chem 2012 October 26; 3(10): I-V
ISSN 1949-8454 (online)

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World Journal of Biological Chemistry

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ISSN 1949-8454 (online)

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Editorial office

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Room 903, Building D, Ocean International Center,
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Beijing 100025, China
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Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

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Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
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