Journal of Dentistry and Oral Hygiene

Volume 5 Number 2 February 2013

ISSN 2006-9871

Academic Journals

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Journal of Dentistry and Oral Hygiene

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Research Article

Journal of Dentistry and Oral Hygiene Vol. 5(2), pp. 7-12, February 2013
Available online at http://www.academicjournals.org/JDOH
DOI:10.5897/JDOH12.003
ISSN 2141-2472 ©2013 Academic Journals

Full Length Research Paper

Titanium particles enhanced osteoclast differentiation and osteoclast bone resorption activity *in vitro*

Bo Meng* and Xiaoyu Yang

Oral Implant Center, Guangdong Provincial Stomatological Hospital, Affiliated Stomatological Hospital of Southern Medical University, Guangzhou, 510280, China.

Accepted 26 February, 2013

The aim of this study was to evaluate the effect of titanium particles that detached from dental implants on osteoclast differentiation and their bone resorption activity. Osteoclasts were generated by titanium particles medium and conditioned medium with or without anti-receptor activator of nuclear factor kappa-B ligand (RANKL). The number of osteoclast was counted and the resorption areas on dentin slices were measured. Moreover, the expression of RANKL and the phagocytosis of titanium particles by cells were evaluated by western blot and transmission electron microscopy (TEM), respectively. Osteoclast differentiation and osteoclast bone resorption activity were enhanced both in titanium particles medium and conditioned medium. In addition, titanium particles promote the expression of RANKL in osteoblasts and the phagocytosis of titanium particles by osteoblasts was demonstrated by TEM. In conclusion, titanium particles enhanced osteoclast generation and their activity and could induce bone resorption around dental implants.

Key words: Titanium particles, dental implants, osteoclast, bone resorption.

INTRODUCTION

Titanium is a particular suitable material for dental implants due to its better mechanical properties and better biocompatibility. Osseointegration between dental implant surface and alveolar bone play a crucial role in the stability of titanium implants (Castellani et al., 2010; Vidigal et al., 2009). In clinical use of dental implant, many methods were used to modify dental implant surfaces to enhance osseointegration (Triplett et al., 2003). It has been demonstrated that a rough implant surface could enhance osseointegration as compared to a smooth implant surface (Cheng et al., 2010; Schwarz et al., 2009). To enhance osseointegration, many methods were used to create rough surfaces such as titanium plasma sprayed (TPS), sandblasted large-grit acid-etched (SLA), physical vapor deposition, etc (Le Guehennec et al., 2007).

However, many *in vivo* studies have demonstrated that titanium particles were generated in the implant-bone interface. Some researchers pointed that the detachment of titanium particles were due to the frictional force during implant insertion and micro-motion during functional activity of implant (Franchi et al., 2007; Martini et al., 2003). For example, TPS surface was made by spraying titanium fluid to titanium and the cohesive force of titanium plasma with titanium implant was weak (London et al., 2002). Therefore, titanium particles could easily be detached from implant surfaces and be released to the implant-bone interface. Titanium particles that exited around dental implants would have effects on cell's activity, which would finally influence the osseointegration around dental implants.

Osseointegration was a dynamic balance between bone formation by osteoblasts and bone resorption by osteoclast (Mavrogenis et al., 2009). Many factors could influence this balance, such as bacteria, occlusion force, etc (Renvert et al., 2009). Once this balance is destroyed, bone loss would happen and dental implant would be a failure. Therefore, in this study, we evaluated the effect of titanium particles on osteoclast generation and activity *in vitro*.

*Corresponding author. E-mail: mengboo@gmail.com. Tel: +86-20-84408890. Fax: +86-20-84433177.

MATERIALS AND METHODS

Preparation of titanium particles

Titanium particles (Alfa Aesar, Milwaukee,WI, USA) with 93% of the particles <20 μm in diameter were suspended in deionized water, vortexed and separated according to the variable sedimentation rates of the variously sized particles. Then, titanium particles were examined using oil-immersion-lens microscopy and the particles with diameter less than 10 μm were separated. After sedimentation, titanium particles were passivated with a 25% nitric acid wash at 70°C for 1 h, washed three times with sterile phosphate-buffered saline (PBS) and then autoclaved at 130°C for 20 min to minimize endotoxin contamination. In preparation for the cell culture, titanium particles were mixed with culture medium (a-MEM, Hycolne, Logan, UT, USA) under sterile conditions. The titanium particles culture media were ultrasonicated for 30 min in sealed sterile containers before being added to the cell culture.

Conditioned medium preparation

Conditioned medium were obtained by culturing osteoblasts with titanium particles medium. Briefly, osteoblasts were cultured with titanium particles medium for 48 h. Then, the culture media were collected, centrifugated and passed through a 0.2 μm filter, and stored were at -80°C. Conditioned medium was obtained by mixing the collected medium and the fresh new medium at the ratio of 1:1.

Osteoclast culture

Osteoclast were generated by six culture media: (A) common culture medium (containing 10^{-6} mol/L prostaglandin E_2 (PGE_2) and 10^{-8} mol/L 1,25$(OH)_2D_3$), (B) common culture medium + antibody of receptor activator of nuclear factor kappa-B ligand (anti-RANKL), (C) conditioned culture medium (CM), (D) conditioned culture medium + anti-RANKL, (E) titanium particles culture medium, and (F) titanium particles culture medium + anti-RANKL.

Osteoclasts were generated by co-culturing osteoblast and bone marrow macrophages as described by Helfrich and Ralston (2003). Bone marrow cells were obtained from the femora and tibiae of 6-week-old male imprinting control regions (ICR) mice and suspended in a-minimum essential medium (a-MEM)/10% fetal calf serum (FCS, Hyclone). After bone marrow cells were cultured on culture dishes for 48 h, the culture medium was removed and most of the adherent cells on culture dishes were bone marrow macrophages. Osteoblasts were prepared from growing calvarial cells from neonatal ICR mice in a-MEM (Hyclone, Logan, UT, USA) containing 10% (v/v) FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Then bone marrow macrophages and osteoblasts were co-cultured in a culture medium with 10% FCS (Hyclone), a-MEM, 100 U/ml penicillin, and 100 μg/ml streptomycin and 10^{-8} M of 1,25-$(OH)_2$ vitamin D_3 (Sigma, St. Louis, MO, USA) and 10^{-6} M PGE_2 in humidified atmosphere of 5% CO_2 at 37°C. After cultured for 2 days, culture medium was removed and six culture media were added separately. The culture medium was changed every other day. After 6 days, the culture medium was removed and cells were stained with tartrate-resistant acid phosphatase (TRAP). The cells with 3 or more cells were considered as osteoclast.

Osteoclast bone resorption ability

Osteoclasts were generated on dentin slices by co-culturing osteoblasts and bone marrow macrophages. After 7 days, the culture medium was removed and conditioned medium and titanium particles medium were added. After cultured for 48 h, attached cells were completely removed from the dentin slices by ultrasonic cleaning. Then dentin slices were stained with toluidine blue and viewed under an inverted phase contrast microscope. Five images were selected seldom and the resorption area was measured by image pro plus 6.0.

Western blot

Osteoblasts were cultured with titanium particles for 48 h. Then, culture medium were removed and osteoblasts were homogenized in a lysis buffer containing 20 mM Tris-HCl, 0.5 mM ethylenediaminetetraacetic acid (EDTA) , 0.5 mM ethylene glycol tetraacetic acid (EGTA), 100 mM NaCl, 1% Triton X-100, 100 μM sodium orthovanadate and 1 mM protease inhibitor PMSF. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. After blocking with 5% non-fat dry milk in PBS/0.15% Tween for 1 h at room temperature, blots were incubated overnight at 4°C with a monoclonal anti-RANKL antibody pv diluted 1:150 to detect RANKL. The secondary antibody (Jackson Immuno Research Labs, Westgrove, PA, USA) was detected. β-actin expression served as an internal control for protein loading.

Transmission electron microscopy (TEM)

After osteoblasts cultured with titanium particles for 48 h, the culture medium were removed and cells were washed three times with PBS. Then 2% glutaraldehyde was added as the primary fixative. Then cells were collected by a cell scraper, centrifugated and fixed by 5% formalin. Then cells were fixed by 1% OsO_4 in PBS. The samples were then embedded in Epon 812 and Ultrathin sections cut with a diamond knife. Sections were mounted on copper grids, stained with 1% uranyl acetate and lead citrate, and examined with a Philips electron microscope (Philips, Leicester, UK) at 60 kV.

Statistic analysis

All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed by analysis of variances (ANOVA), and $P < 0.05$ was considered as significant.

RESULTS

To evaluate the effect of titanium particles on osteoclast differentiation, we studied osteoclast differentiation which was induced by conditioned medium, titanium particles medium and common culture medium, separately. We found that both conditioned medium and titanium particles medium generated more osteoclast when compared with common culture medium, indicating titanium particles promoted osteoclast differentiation. However, after adding anti-RANKL, the numbers of osteoclast were decreased in all groups (Figure 1).

As shown in Figure 2, titanium particles increased bone resorption area on dentin slices, indicating titanium particles could enhance osteoclast bone resorption activity *in vitro*. Moreover, conditioned medium also increased bone resorption area than in the control.

In the western blot test, we found that both titanium

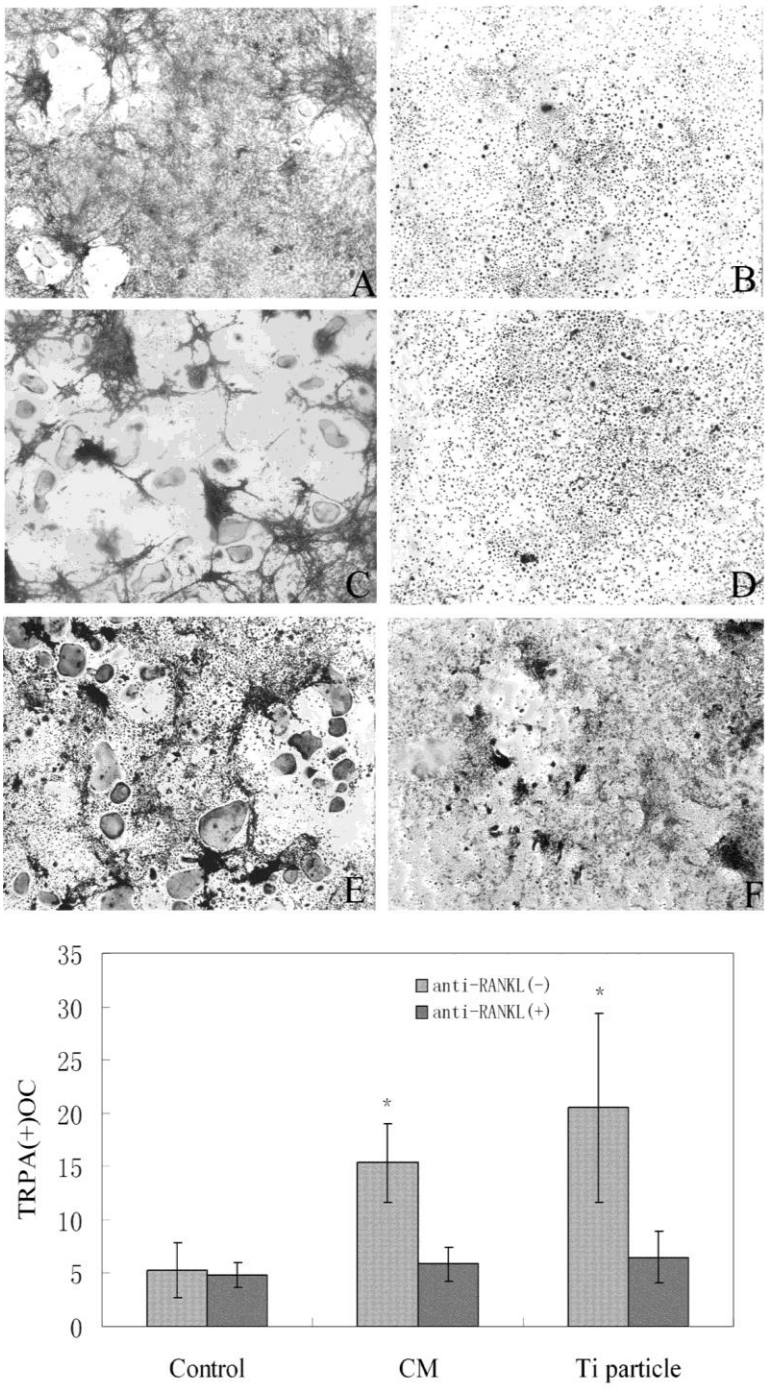

Figure 1. Effect of titanium particles on osteoclast differentiation. (A) control, (B) control+anti-RANKL, (C) CM (conditioned medium), (D) CM+anti-RANKL, (E) titanium particles, (F) titanium particles+anti-RANKL. *P < 0.05.

particles and conditioned medium enhanced the expression of RANKL in osteoblasts (Figure 3). From the TEM examination, we found that many titanium particles were inside cells and some were even inside nucleus, indicating the phagocytosis of titanium particles by cells (Figure 4).

DISCUSSION

In this study, we studied the effect of titanium particles on osteoclast differentiation and osteoclast activity. Our results showed that both titanium particles medium and conditioned medium enhanced osteoclast differentiation

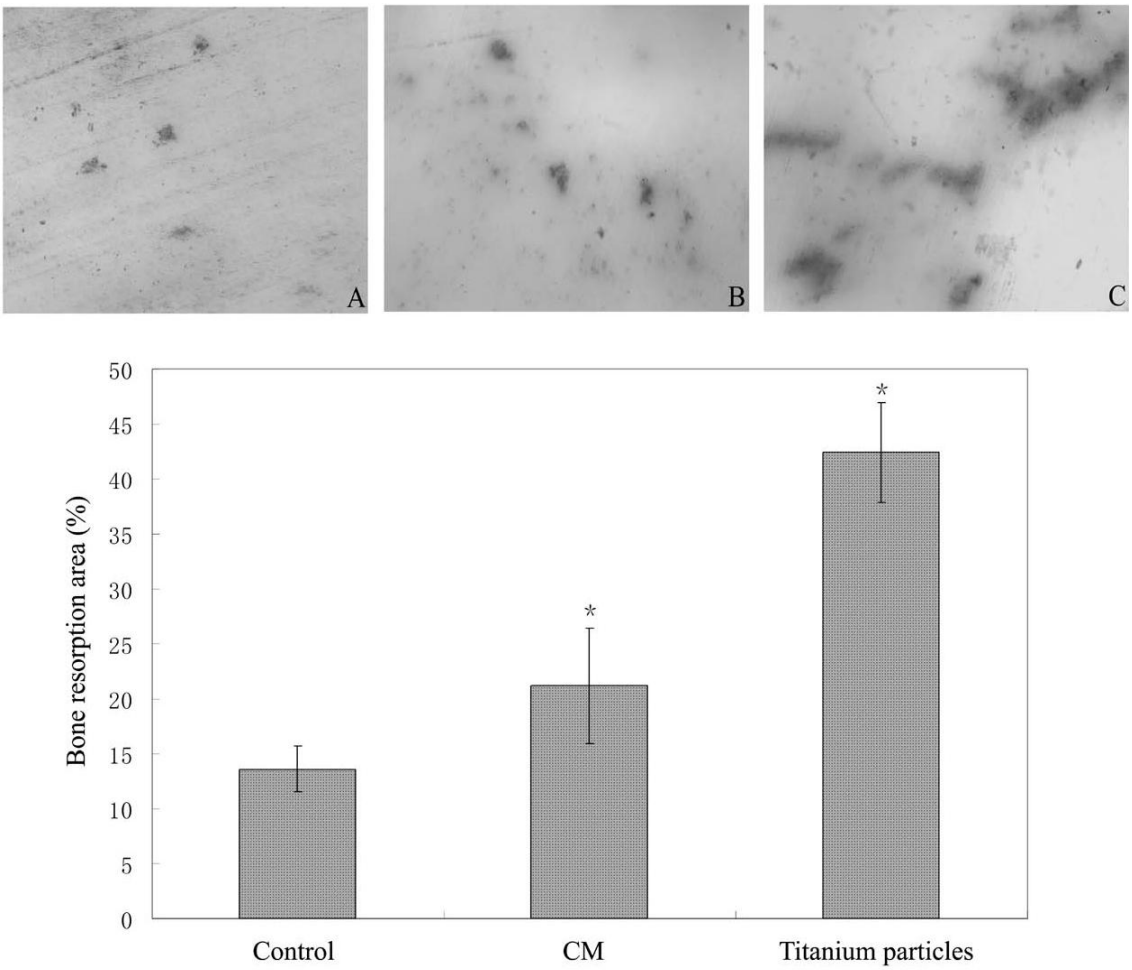

Figure 2. Effect of titanium particles on osteoclast bone resorption activity. Both conditioned medium and titanium particles medium enhanced osteoclast bone resorption area on dentin slices. * P < 0.05.

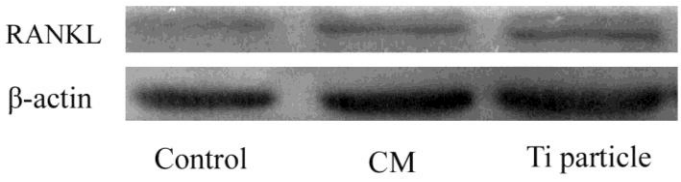

Figure 3. Expression of RANKL in osteoblasts after treated with conditioned medium and titanium particles medium for 48 h.

and resorption activity. Therefore, titanium particles around dental implants have an adverse effect on the osseointegration of dental implants.

The results showed that osteoclast differentiation were enhanced by both titanium particles and conditioned medium, which may be partly because titanium particles promote some cell factors released from cells after they phagocytosed titanium particles. When exposed to titanium particles, many cells such as osteoblasts and fibroblasts, could release inflammatory cytokines including interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-a (Bukata et al., 2004; Nakashima et al., 1999; Okafor et al., 2006). In *in vivo* research, cytokines such as IL-6, PGE_2 were also detected around titanium implants (Suska et al., 2005). It is widely accepted that these cytokines could stimulate osteoclast differentiation and promote bone resorption (Colucci et al., 2007; Wei et al., 2005; Yago et al., 2009).

RANKL, as an essential factor for osteoclast differentiation, is usually secreted by osteoblasts and bone marrow stem cells (Anandarajah, 2009; Kobayashi et al., 2009). Therefore, in this study, we studied the effect of titanium particles on the expression of RANKL in osteoblasts and found that titanium particles enhanced the expression of RANKL. The increased expression of RANKL could enhance the fusion of mononuclear cells and finally promote osteoclast differentiation (Lu et al., 2010).

Besides osteoclast generation, we also studied the effect of titanium particles on osteoclast bone resorption

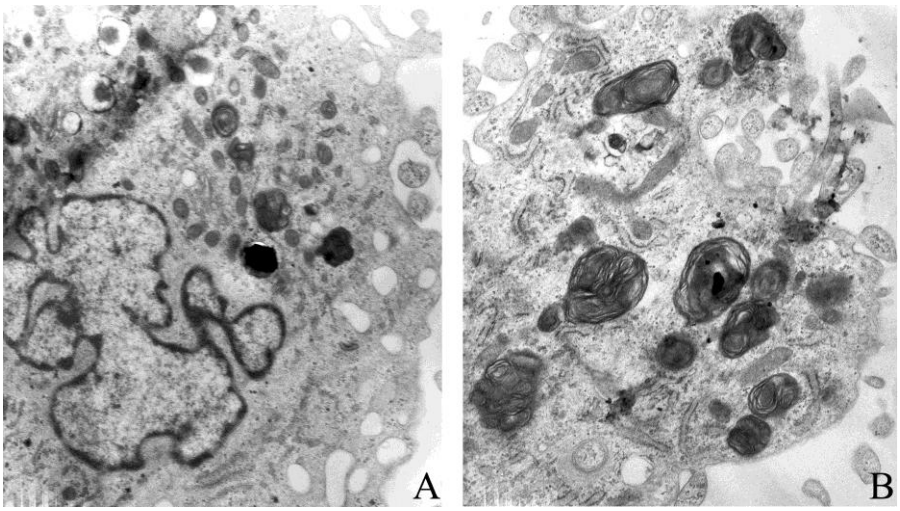

Figure 4. Transmission electron micrographs (×200,000) of osteoblasts after treated with titanium particles for 48 h.

activity. We found that both titanium particles medium and conditioned medium enhanced osteoclast resorption area on dentin slices. The exact mechanism by which titanium particles take their effects on osteoblasts and osteoclast is not clearly known at present. In the TEM image, it was discovered that titanium particles were inside osteoblasts, indicating the phagocytosis of titanium particles by cells. The diameter of titanium particles around dental implants was about 1 to 10 μm and they could be easily phagocytosed by cells. This phagocytosis could enhance the expression of RANKL, which could enhance osteoclast' activity (Leibbrandt and Penninger, 2009). Furthermore, titanium particles could promote osteoclast' activity as an inflammatory factor. This is consistent with the result that macrophages could absorb bone after phagocytosing titanium particles (Fujikawa et al., 2005). Therefore, titanium particles could enhance bone resorption around dental implants due to excessive osteoclast resorption activity.

The enhanced osteoclast generation and their activity would disrupt the balance between bone formation and bone resorption in the bone-implant interface. Moreover, it has been reported that titanium particles could inhibit osteoblasts' and bone marrow stem cells' activity (Choi et al., 2005). As a result, there may be bone loss around dental implants due to the effects of titanium particles, which would finally induce dental implant failure.

Conclusively, titanium particles could promote osteoclast differentiation both in direct and indirect contact and the enhanced osteoclast generation and osteoclast activity could finally induce bone resorption around dental implants.

ACKNOWLEDGEMENT

Thi study was supported by the National Natural Science Foundation of China (No.81100780).

REFERENCES

Anandarajah AP (2009). Role of RANKL in bone diseases. Trends Endocrinol. Metab. 20:88-94.

Bukata SV, Gelinas J, Wei X, Rosier RN, Puzas JE, Zhang X, Schwarz EM, Song XY, Griswold DE, O'Keefe RJ (2004). PGE2 and IL-6 production by fibroblasts in response to titanium wear debris particles is mediated through a Cox-2 dependent pathway. J. Orthop. Res. 22:6-12.

Castellani C, Lindtner RA, Hausbrandt P, Tschegg E, Stanzl-Tschegg SE, Zanoni G, Beck S, Weinberg AM (2010). Bone-implant interface strength and osseointegration: Biodegradable magnesium alloy versus standard titanium control. Acta Biomater. 7:432-440.

Cheng Z, Zhang F, He F, Zhang L, Guo C, Zhao S, Yang G (2010). Osseointegration of titanium implants with a roughened surface containing hydride ion in a rabbit model. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 110:e5-12.

Choi MG, Koh HS, Kluess D, O'Connor D, Mathur A, Truskey GA, Rubin J, Zhou DX, Sung KL (2005). Effects of titanium particle size on osteoblast functions *in vitro* and *in vivo*. Proc. Natl. Acad. Sci. U. S. A. 102:4578-4583.

Colucci S, Brunetti G, Cantatore FP, Oranger A, Mori G, Quarta L, Cirulli N, Mancini L, Corrado A, Grassi FR, Grano M (2007). Lymphocytes and synovial fluid fibroblasts support osteoclastogenesis through RANKL, TNFalpha, and IL-7 in an *in vitro* model derived from human psoriatic arthritis. J. Pathol. 212:47-55.

Franchi M, Orsini E, Martini D, Ottani V, Fini M, Giavaresi G, Giardino R, Ruggeri A (2007). Destination of titanium particles detached from titanium plasma sprayed implants. Micron 38(6):618-625.

Fujikawa Y, Itonaga I, Kudo O, Hirayama T, Taira H (2005). Macrophages that have phagocytosed particles are capable of differentiating into functional osteoclasts. Mod. Rheumatol. 15:346-351.

Helfrich MH, Ralston S (2003). Bone research protocols. Humana Press, Totowa NJ. p 448.

Kobayashi Y, Udagawa N, Takahashi N (2009). Action of RANKL and OPG for osteoclastogenesis. Crit. Rev. Eukaryot. Gene Expr. 19:61-72.

Le Guehennec L, Soueidan A, Layrolle P, Amouriq Y (2007). Surface treatments of titanium dental implants for rapid osseointegration. Dent. Mater. 23:844-854.

Leibbrandt A, Penninger JM (2009). RANKL/RANK as key factors for

osteoclast development and bone loss in arthropathies. Adv. Exp. Med. Biol. 649:100-113.
London RM, Roberts FA, Baker DA, Rohrer MD, O'Neal RB (2002). Histologic comparison of a thermal dual-etched implant surface to machined, TPS, and HA surfaces: bone contact *in vivo* in rabbits. Int. J. Oral Maxillofac. Implants 17:369-376.
Lu SY, Li M, Lin YL (2010). Mitf induction by RANKL is critical for osteoclastogenesis. Mol. Biol. Cell 21:1763-1771.
Martini D, Fini M, Franchi M, Pasquale VD, Bacchelli B, Gamberini M, Tinti A, Taddei P, Giavaresi G, Ottani V, Raspanti M, Guizzardi S, Ruggeri A (2003). Detachment of titanium and fluorohydroxyapatite particles in unloaded endosseous implants. Biomaterials 24:1309-1316.
Mavrogenis AF, Dimitriou R, Parvizi J, Babis GC (2009). Biology of implant osseointegration. J. Musculoskelet. Neuronal Interact. 9:61-71.
Nakashima Y, Sun DH, Trindade MC, Maloney WJ, Goodman SB, Schurman DJ, Smith RL (1999). Signaling pathways for tumor necrosis factor-alpha and interleukin-6 expression in human macrophages exposed to titanium-alloy particulate debris *in vitro*. J. Bone Joint Surg. Am. 81:603-615.
Okafor CC, Haleem-Smith H, Laqueriere P, Manner PA, Tuan RS (2006). Particulate endocytosis mediates biological responses of human mesenchymal stem cells to titanium wear debris. J. Orthop. Res. 24:461-473.
Renvert S, Polyzois I, Maguire R (2009). Re-osseointegration on previously contaminated surfaces: A systematic review. Clin. Oral Implants Res. 20(4):216-227.
Schwarz ML, Kowarsch M, Rose S, Becker K, Lenz T, Jani L (2009). Effect of surface roughness, porosity, and a resorbable calcium phosphate coating on osseointegration of titanium in a minipig model. J. Biomed. Mater. Res. A. 89:667-678.
Suska F, Gretzer C, Esposito M, Emanuelsson L, Wennerberg A, Tengvall P, Thomsen P (2005). *In vivo* cytokine secretion and NF-kappaB activation around titanium and copper implants. Biomaterials 26:519-527.
Triplett RG, Frohberg U, Sykaras N, Woody RD (2003). Implant materials, design, and surface topographies: Their influence on osseointegration of dental implants. J. Long Term Eff. Med. Implants. 13:485-501.
Vidigal GM, Groisman M, Gregorio LH, Soares Gde A (2009). Osseointegration of titanium alloy and HA-coated implants in healthy and ovariectomized animals: a histomorphometric study. Clin. Oral Implants Res. 20:1272-1277.
Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL (2005). IL-1 mediates TNF-induced osteoclastogenesis. J. Clin. Invest. 115:282-290.
Yago T, Nanke Y, Ichikawa N, Kobashigawa T, Mogi M, Kamatani N, Kotake S (2009). IL-17 induces osteoclastogenesis from human monocytes alone in the absence of osteoblasts, which is potently inhibited by anti-TNF-alpha antibody: A novel mechanism of osteoclastogenesis by IL-17. J. Cell Biochem. 108:947-955.

Journal of Dentistry and Oral Hygiene Vol. 5(2), pp. 13-20, February 2013
Available online at http://www.academicjournals.org/JDOH
DOI:10.5897/JDOH2013.0079
ISSN 2141-2472 ©2013 Academic Journals

Full Length Research Paper

Self report of adverse gingival conditions among pregnant South-Western Nigerian women

Joy Ucheonye Ifesanya* and Gbemisola Aderemi Oke

[1]Department of Child Oral Health, College of Medicine, University of Ibadan, Ibadan, Nigeria.
[2]Community Dentistry, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Accepted 22 February, 2013

Reports have associated the occurrence of periodontal diseases, of which gingivitis is the earliest and commonest form in pregnancy with an increased risk for poor pregnancy outcome. There is dearth of information on the oral status of pregnant women in Nigeria. This study assessed self reported adverse gingival changes and its relationship with observed gingival health status among pregnant women in a South-Western Nigerian locality. Responses about self observed gingival status were obtained from 405 pregnant women attending two primary health care antenatal clinics from a local government area within a municipality in South-Western Nigeria. In addition to demographic data, pregnancy and social history, oral hygiene practice and history of professional dental care were obtained using an interviewer administered questionnaire. An intra-oral examination was also performed on each woman and data obtained were analysed using the Statistical Package for the Social Sciences (SPSS) Version 17. The mean age was 25.35 (±5.02) years. Married women accounted for 96.8% of the study population. None of the women reported having ever smoked, while 23.5% of them reported taking alcohol in the form of local herbal preparations both before and during the course of their pregnancy. Older women were more likely to report adverse gingival changes in pregnancy. The association between reported adverse gingival changes and observed severity of gingivitis was significant (P < 0.05). Those who had visited a professional dental care giver were more likely to report adverse gingival changes. Women who chewed kolanuts or bitter kola were also more likely to report adverse gingival changes. This study highlights the low awareness of pregnant Nigerian women about their gingival health status and exposes the need to explore the effect of locally available stimulant nuts on gingival health.

Key words: Self-report, gingivitis, oral hygiene, pregnancy, Nigeria.

INTRODUCTION

Pregnancy has far-reaching systemic effects extending beyond the reproductive organs. In the mouth, the greatest effect of pregnancy is seen in the gingiva (Eley and Manson, 2004). Women with previous chronic gingivitis which attracted no attention before pregnancy become aware of their gingival status as the previously inflamed areas become enlarged and oedematous and more noticeably discoloured with an increased tendency to bleeding (Carranza, 1990). It was reported that this occurs as a result of factors introduced in pregnancy that aggravate gingival response to local irritation by bacterial plaque (Laine, 2002). There is partial resolution of this exaggerated response by the second month post-parturition, with complete resolution to the pre-pregnant state by the first year post-partum (Laine, 2002), except with persistence of local irritants such as plaque (Laine, 2002). The aforementioned findings corroborate the findings in the study of Taani et al. (2003), who also observed an increase in severity of gingivitis with increased parity of the women. Also increased in pregnancy are tooth mobility (Diaz-Guzman and Castellanos-Suarez, 2004), as well as pocket depth and amount of gingival crevicular fluid (Carranza, 1990; Machuca et al., 1999).

*Corresponding author. E-mail: joyifesanya@yahoo.co.uk. Tel: +2348055623129.

The factors implicated in pregnancy gingivitis are bacterial plaque, lowered immunity in pregnancy and pregnancy hormones. Microbiologic studies have documented a change in sub-gingival micro-flora to a more anaerobic flora during pregnancy. Significantly increased organisms include *Bacteroides intermedius* (Carranza, 1990) and *Porphyromonas gingivalis* (Mascarenhas et al., 2003). In addition, pregnancy hormones are thought to stimulate growth of some bacterial species which have been implicated in gingivitis such as *Bacteroides* species and *Prevotella intermedia* (Zachariasen, 1991).

Changes in maternal immune-responsiveness expressed in terms of decrease in T_8, T_4 and B-cells in peripheral blood and gingival tissues as well as decreased neutrophil chemotaxis and depression of cell mediated immunity and phagocytosis in pregnancy have been reported (Tandon and D'Silva, 2003). Also, *in vitro* studies have demonstrated a decrease in response of peripheral blood lymphocytes to bacterial antigens and a decrease in the absolute number of CD_4 positive cells in pregnant women (Barak et al., 2003). The aforementioned make the gingiva more susceptible to irritation which it otherwise would have combated without clinical expression of disease. However, an increase in the number of T-cells in gingival specimens from pregnant women during experimental gingivitis has also been documented (Laine, 2002).

High levels of progesterone and estradiol β-17 in the gingiva of pregnant women have been documented to effect the development of localized inflammation by stimulating the production of prostaglandins especially prostaglandin E_2 (PGE_2), a mediator of inflammation (Zaki et al., 1984). The pregnancy hormones have also been found to destroy gingival mast cells leading to release of histamine and proteolytic enzymes which contribute to tissue destruction (Carranza, 1990).

Plaque can be controlled by maintaining good oral hygiene (Jin et al., 2003). This is achieved by brushing the teeth at least twice daily using a tooth brush or chewing stick with appropriate techniques (Aderinokun et al.,1999; Al-Otaibi et al., 2004) preferably with the addition of inter-dental cleaning aids such as the dental floss. In addition, regular visits to the dentist or other professional dental care giver for routine professional cleaning of the mouth (scaling and polishing) aids good oral hygiene.

The prevalence of gingivitis in pregnancy has been reported to range between 30 to 100% of pregnant women (Christensen et al., 2003; Al Habashneh et al., 2005). A previous study of Danish pregnant women showed that one-third of their subjects reported gingival changes during pregnancy with bleeding gums being the most frequent symptom (Christensen et al., 2003). The women who were regular users of dental services were more likely to report any gingival changes than those who had not visited the dentist. The proportion of pregnant women who make use of dental services in more developed countries is high: about 90% (Christensen et al., 2003; Al Habashneh et al., 2005). However, less than 15% utilization has been reported among Nigerian pregnant women (Bassey et al., 2010) and less than 10% among South-Western Nigerian students with no significant gender predominance (Bamise et al., 2008). Data on individual assessment of gingival health among Nigerian pregnant women are not available in literature.

Research suggests that women do not seek professional help if they perceive that their gingival status is normal (Christensen et al., 2003). Women were more likely to use dental services in pregnancy if married, educated, had dental insurance, previously used dental services when not pregnant, or had knowledge about the possible connection between oral health and pregnancy outcome (Al Habashneh et al., 2005). The previously established link between periodontal diseases and poor pregnancy outcome, such as pre-mature birth and low birth weight, as well as pre-eclampsia (Boggess et al., 2003; Buduneli et al., 2005; Yiorgos et al., 2006), makes the need to assess how pregnant women in our environment view their gingival health relevant. Though a cause-effect relationship is yet to be established between periodontal status and pregnancy outcome, individuals' view of their oral health status is of great value as it is likely to affect their oral health seeking behaviour (Lopez et al., 2002; Marin et al., 2005). This study assessed self report of gingival changes, the relationship it bears with socio-demographic factors and observed gingival health in pregnant women of South-Western Nigeria.

METHODOLOGY

Ethical approval was obtained from the Oyo State Ethical Review Board. This survey was carried out in Ibadan South-East Local Government Area; one of the five off-shoots of the split of the Ibadan Municipal Government Area (IMG) of Oyo state in 1989. The Primary Health Care Department of the local government has six outreach health centres; two of which have established antenatal clinics. This study sample consisted of 405 consecutive and consenting attendees at the two primary health care antenatal clinics from the local government area between June and August of the year 2007. Interviewer-administered questionnaires (Appendix 1) were used to obtain demographic data, pregnancy and social history, oral hygiene practices and history of professional dental care from the pregnant women. Questions about self observed gingival status were also asked. Two research assistants were recruited and trained using a translated version (in Yoruba) of the questionnaire to enable them administer the questionnaires appropriately. Each woman had an intra-oral examination with a mirror and periodontal probe which was conducted by the first author (IJU), under natural light with particular attention to their oral hygiene and gingival health status. The Oral Hygiene Index-Simplified of Green and Vermillion (1960) was used as a measure of oral hygiene, while the Gingival Index of Löe and Silness (1963) was used as a measure of gingival health. Mild gingivitis was defined as slight reddening and swelling of the gingival, but no bleeding on probing. Moderate gingivitis was defined as red, swollen and shinny gums with bleeding on probing. While severe gingivitis was defined as red, swollen and shiny gums which bleed spontaneously.

The data obtained were entered into a computer spread sheet analyzed using the Statistical Package for the Social Sciences (SPSS) version 17. Frequency tables and measures of central and

tendency were generated and statistical relationships for categorical data was obtained using Chi-square (χ^2) test, while the paired sample t-test was used for continuous variables.

RESULTS

Four hundred and five (405) pregnant women attending the two primary health care antenatal clinics of a South-Western locality in Nigeria were studied. The mean age was 25.35 (±5.02) years. The socio-demographic distribution of the women is shown in Table 1.

None of the women reported having ever smoked cigarette, 138 (34.1%) chewed either kolanuts or bitter kola, and 96 (23.5%) of them reported taking alcohol in the form of local herbal preparations *'agbo'*, both before and during the course of their pregnancy. 267 women (66.2%) brushed their teeth once daily, while a 132 (33.8%) brushed twice. The most commonly used brushing implement was the toothbrush and toothpaste reported by 361 (89.1%), 43(10.6%) of the women used chewing stick, while 1 (0.2%) cleaned her mouth with cotton-wool and salt.

Most of the women, 389 (96.0%) had never visited a dentist or any other oral health care provider. Of the 16 (4%) women who had been attended to by dental professionals, 12 (75.0%) were for toothache related issues, 2 (12.5%) were for trauma and 2 (12.5%) for professional dental cleaning.

140 (34.6%) women reported to have noticed any swelling, bleeding or pain from their gums. The swollen, bleeding or painful gums had been present before pregnancy in 101 (72.1%) of them and 12 (11.9%) of these attested to a worsening in their gingival condition since they got pregnant. The remaining 39 (27.9%) women who noticed gingival changes did so after they got pregnant. Almost half of these women 17 (43.6%) noticed the gingival changes in the second trimester, 6 (15.4%) in the first and third trimesters, respectively, while 10 (25.6%) of them were not sure of the time the gingival changes took place in the course of their pregnancies. Of the 279 multigravid women, only 19 (6.8%) reported any gingival changes in previous pregnancies.

All the women had gingivitis (100.0%). The distribution of gingival health and oral hygiene status is presented in Table 2. Paired sample t-test on individual gingival and oral hygiene scores showed statistically significant variation between oral hygiene and gingivitis ($P < 0.001$). Women who reported adverse gingival changes were observed to have more severe gingivitis than those who did not ($P = 0.039$). This association was stronger in women who reported having swollen, painful or bleeding gums in previous pregnancies ($P = 0.032$) as shown in Table 3. The older women were more likely to report adverse gingival changes in pregnancy than the younger ones ($P=0.035$). It was also observed that those who had visited a professional dental care giver were more likely to report any adverse gingival change in pregnancy than those who had never visited a dental care giver ($P = 0.001$). Women who chewed kolanuts or bitter kola were also more likely to report adverse gingival changes than those who did not ($P = 0.003$) as shown in Table 4. There was no significant relation between the report of adverse gingival condition and the marital status ($P = 0.77$), level of education ($P = 0.12$) and occupational status ($P = 0.43$) of the women studied. The oral hygiene status did not vary significantly with the socio-demographic variables age ($P = 0.27$) and marital status ($P = 0.95$), but oral hygiene significantly improved with the level of education

Table 1. Socio-demographic distribution of sample studied.

Variable	Frequency (%)
Age group	
< 20	81 (20.0)
21-30	277 (68.4)
>31	47 (11.6)
Marital status	
Single	13 (3.2)
Married	392 (96.8)
Level of education	
Post secondary education	7 (1.7)
Secondary education	242 (59.8)
Primary education	146 (36.0)
No formal education	10 (2.5)
Occupation	
Unskilled workers	288 (71.1)
Skilled workers	81 (20.0)
Professionals	5 (1.2)
Students	31 (7.7)
Parity	
1st child	126 (31.1)
2-4 children	257 (63.5)
Greater than 4 children	22 (5.4)

Table 2. Distribution of gingival and oral hygiene status.

Variable	Frequency (%)
Gingivitis	
Mild gingivitis	152 (37.5)
Moderate gingivitis	253 (62.5)
Oral hygiene	
Good oral hygiene	159 (39.3)
Fair oral hygiene	246 (60.7)

Table 3. Relationship between reported gingival changes and observed severity of gingivitis among Ibadan South East LGA pregnant women.

Reported adverse gingival change	Severity of gingivitis		Total	P value
	Mild gingivitis	Moderate gingivitis		
In index pregnancy				
Yes	43 (30.7)	97 (69.3)	140 (100.0)	P = 0.039*;
No	109 (41.1)	156 (58.9)	265 (100.0)	χ^2 = 4.24
Total	152 (37.5)	253 (62.5)	405 (100.0)	
In previous pregnancy				
Yes	4 (16.7)	19 (83.3)	24(100.0)	P = 0.032*;
No	99 (38.8)	156 (61.2)	255(100.0)	χ^2 = 4.62
Total	103 (36.9)	176 (63.1)	279(100.0)	

χ^2= Chi square.

Table 4. Relationship between other variables and reported gingival changes.

Variable	Reported gingival change		Total	P value
	Yes	No		
Age (years)				
11-20	20 (24.7)	61 (75.3)	81 (100.0)	χ^2 = 6.687; P = 0.035*
21-30	98 (35.4)	179 (64.6)	277 100.0)	
6>30	22 (46.8)	25 (53.2)	47 (100.0)	
Total	140 (34.6)	265 (65.4)	405 (100.0)	
Use of dental care				
Yes	12 (75.0)	4 (25.0)	16 (100.0)	χ^2 = 12.04; P = 0.001*
No	128 (32.9)	261 (67.1)	389 (100.0)	
Total	140 (34.6)	265 (65.4)	405 (100.0)	
Kolanut/bitter kola consumption				
Yes	61 (44.2)	77 (55.8)	138 (100.0)	χ^2 = 8.591; P = 0.003*
No	79 (29.6)	188 (70.4)	267 (100.0)	
Total	140 (34.6)	265 (65.4)	405 (100.0)	

χ^2= Chi square value; *Significant.

education (P < 0.01). In addition, the oral hygiene was better in women using the toothbrush and toothpaste than in those using chewing stick or cotton wool (P = 0.01).

The severity of gingivitis did not vary significantly with the age (P = 0.40), level of education (P = 0.06), or marital status of the women, nor did it vary with the types of tooth brushing implements (P = 0.83) or the frequency of tooth brushing (P = 0.15).

DISCUSSION

In spite of the high prevalence of gingivitis in pregnancy observed, just over a third of the women studied reported adverse gingival changes and this finding is comparable with previous findings in a Danish population (Christensen et al., 2003), further showing that majority of the women are not aware of their gingival health status. This may be an underlying reason for the extremely low utilization of dental services among these women when compared to other reports (Christensen et al., 2003; Al Habashneh et al., 2005). Gingivitis among the women worsened as their oral hygiene scores worsened and this is in congruence with known documentation on the aetiology of gingivitis (Eley and Manson, 2004; Carranza, 1990). The fact that most of the women noticed gingival changes in the second trimester of pregnancy is similar to

the findings from a previous research (Kornman and Loesche, 1980). This is probably a cumulative effect of the increasing circulating hormones on the gums which increase the dilation and increases the tortuosity of gingival micro-vasculature, encouraging circulatory stasis and increasing susceptibility to irritation by metabolic by products in the static blood. This also favours leakage of fluid into the peri-vascular tissues (Carranza, 1990). It was also observed that women who had ever visited the dentist were more likely to report adverse gingival changes than those who had not. This is similar to findings from Christensen et al. (2003) and may be attributed to the ample education the women had received at their previous dental visits, making it easier for them to recognise any deviation from normal appearance of their gingivae. The women who reported this gingival change had more severe gingivitis than those who did not and this is probably as a result of worsening of pre-existing gingivitis. This has been documented in previous literature (Carranza, 1990). This relationship was stronger among those who reported gingival changes in previous pregnancies and may be due to the poorer oral hygiene observed among the multiparous women. Older women were more likely to report adverse gingival changes and this may be due to the fact that their perceived gingival status has been of longer duration than that of the younger women. The limitations of recall bias on the aforementioned should however be borne in mind. In addition, the fact that a significant number of these women had never visited a dentist may mean that the observed gingival conditions were not primarily a result of pregnancy. However, since most of them reportedly observed the adverse gingival condition after they got pregnant, it is safe to say that the pregnancy had aggravated the pre-existing gingivitis.

The significant relationship between kolanut and/or bitter kola consumption and self report of adverse gingival changes may be derived from some effect of these commodities on oral hygiene status, though no significant relationship was found between the latter two in this study. Further studies are necessary to define the possible effect(s) of these two commonly chewed stimulant nuts on oral tissues and oral health in this environment as none were found. On the other hand, these items tend to be sources of extrinsic stains which attract the women to their discoloured teeth, hence inadvertently discovering their gingival status.

A number of reports have associated the occurrence of periodontal diseases in pregnant women with an increased risk for poor pregnancy outcome (Lopez et al., 2002; Marin et al., 2005); hence, the findings from this study serve as a call for the incorporation of routine dental check up and care into the antenatal programme in developing countries.

Contrary to known reports (Al Habashneh et al., 2005), this sample of women showed no significant variation in gingival health status with respect to marital status, level of education and occupational status. This may be due to the fact that majority of the women studied are of similar educational and professional status, thus masking the possible statistical relationship between the groups.

Conclusion

Periodontal disease and low utilization of dental care facilities are common occurrences in developing nations of the world. In view of the aforementioned, it is advocated that every pregnant woman be referred for a dental check-up and necessary treatment at first contact with the antenatal care facility. There is also need for policy formulation with regards to incorporating routine oral health care into antenatal care in this environment. Proper health education is also advocated to improve the use of the available oral health care facilities.

REFERENCES

Aderinokun GA, Lawoyin JO, Onyeaso CO (1999). Effect of two common Nigerian chewing sticks on gingival health and oral hygiene. Odontostomatol. Trop. 22(87):13-18.

Al-Otaibi M, Al-Harthy M, Gustafsson A, Johansson A, Claesson R, Angmar-Månsson B (2004). Subgingival microbiota in Saudi-Arabians after use of Miswak chewing stick and toothbrush. J. Clin. Periodontol. 31:1048-1053.

Bamise CT, Bada TA, Bamise FO, Ogunbodede EO (2008). Dental Care Utilization and Satisfaction of Residential University Students. Libyan J. Med. 3(3):140-143.

Barak S, Oettinger-Barak O, Oettinger M, Machtei EE, Peled M, Ohel G (2003). Common oral manifestation during pregnancy: a review. Obstet. Gynecol. Surv. 58(9):624-628.

Bassey GO, Anyanechi CE, Ekabua KJ, Ekabua JE (2010). Oral health among antenatal care attendees in Calabar, Nigeria. J. Obstet. Gynaecol. 30:143-146.

Boggess KA, Lieff S, Murtha AP, Moss K, Beck J, Offenbacher S (2003). Maternal periodontal disease is associated with an increased risk for preeclampsia. Obstet. Gynecol. 101(2):227-231.

Buduneli N, Baylas H, Buduneli E, Tu¨rkog˘lu O, Ko¨se T, Dahlen G (2005). Periodontal infections and pre-term low birth weight: A case-control study. J. Clin. Periodontol. 32:174-181.

Carranza FA (1990). Endocrinologic influences on the periodontium. In: Glickman's Clinical Periodontology, 7th edition, WB Saunders Company, Philadelphia pp. 452- 455.

Christensen LB, Jeppe-Jensen D, Petersen PE (2003). Self-reported gingival conditions and self-care in the oral health of Danish women during pregnancy. J. Clin. Periodontol. 30(11):949-953.

Diaz-Guzman LM, Castellanos-Suarez JL (2004). Lesions of the oral mucosa and periodontal disease behavior in pregnant patients. Med. Oral Patol. Oral Cir. Bucal 9:430-437.

Eley BM, Manson JD (2004). The effect of systemic factors on the periodontal tissue;. Periodontics 5th ed. Elsevier Limited, Toronto. pp. 90-91.

Al Habashneh R, Guthmiller JM, Levy S, Johnson GK, Squier C, Dawson DV, Fang Q (2005). Factors related to utilization of dental services during pregnancy. J. Clin. Periodontol. 32(7):815-821.

Jin LJ, Chiu GKC, Corbet EF (2003). Are periodontal diseases risk factors for certain systemic disorders? - What matters to medical practitioners. Hong Kong Med. J. 9:31-37.

Kornman KS, Loesche WJ (1980). The Subgingival Microbial Flora during Pregnancy. J. Periodont. Res. 15:111-122.

Laine MA (2002). Effect of pregnancy on periodontal health. Acta Odontol. Scand. 60(5):257-264.

Lopez NJ, Smith PC, Gutierrez J (2002). Higher risk of pre-term and low birth weight in women with periodontal disease. J. Dent. Res. 81:58-63.

Marin C, Segura-Egea JJ, Martinez-Sahuquillo Á, Bullon P (2005).

Correlation between infant birth weight and mother's periodontal status. J. Clin. Periodontol. 32(3):299-304.
Mascarenhas P, Gapski R, Al-Shammari K, Wang HL (2003). Influence of sex hormones on the periodontium. J. Clin. Periodontol. 30(8):671-681.
Taani DQ, Habashneh R, Hammad MM, Batieha A (2003). The periodontal status of pregnant women and its relationship with socio-demographic and clinical variables. J. Oral Rehab. 30(4):440-445.
Tandon S, D'Silva I (2003). Periodontal physiology during pregnancy (Guest editorial). Indian J. Physiol. Pharmacol. 47(4):367-372.
Yiorgos AB, Silvana PB, Steven O (2006). Exploring the relationship between periodontal disease and pregnancy complications. J. Am. Dent Assoc. 137(10 Suppl):7S-13S.
Zachariasen RD (1991). Ovarian hormones and gingivitis. J. Dent. Hyg. 65(3):146-150.
Zaki K, el Hak R, Amer W, Saleh F, El Faras A, Ragab L, Nour H (1984). Salivary female sex hormone levels and gingivitis in pregnancy. Biomed. Biochem. Acta 43(6):749-754.

APPENDIX 1 (QUESTIONNAIRE)

The prevalence of gingivitis in pregnant women attending primary health care ante-natal clinic in Ibadan South-East Local Government Area

Section A: Demographic data

1. Serial no _____
2. Initials _____
3. Age _____
4. Marital status 1. Single 2. Married 3. Divorced
4. Widowed 5. Others
5. Religion 1. Christian 2. Islam 3. Traditional
4. Others (specify)
6. Occupation _____
7. Level of education 1. Primary school. 2. Secondary school. 3. Teacher training
4. University 5. Others (specify) 6. None

Section B: Pregnancy history

8. Is this your first pregnancy? 1. Yes 2. No
8b.If no, how many times have you been pregnant? Specify number _____

9. How many months old is this pregnancy? (1) 1-3 months. (2) 4-6 months.
(3) 7-9 months

10. Do you have swollen, painful or bleeding gums? 1. Yes 2. No

11. If yes, did it you notice it before or after you got pregnant? 1. Before 2. After

12. If before, has it become worse since you got pregnant? 1. Yes 2. No

13. If after, how many months was this pregnancy when it started? (1) 1-3 months. (2) 4-6 months
(3) 7-9 months (4) Not sure.

14. If you have had previous pregnancies, were your gums swollen, painful or bleeding then?
(1) Yes (2) No (3) Can't remember

15. If yes to above, during which pregnancies? (1) At least one of previous
(2) All previous pregnancies. (3) Can't remember

Section C: Social history

16. Do you smoke cigarettes presently? (1) Yes (2) No

16b. If No, have you ever smoked cigarettes? (1) Yes (2) No

16c. If yes, how long ago did you stop? ……

17. Do you take beer or any local brew containing alcohol either before or during this pregnancy? (1) Yes (2) No

18. Do you chew Kola nuts or bitter cola? (1) Yes (2) No

19. How often do you brush your teeth? (1) Twice daily 2. Once daily
(3) Alternate days (4) Once a week 5. Others (specify)

20. What do you use to brush your teeth? (1) Toothbrush and paste (2) Chewing stick (3) Cotton wool/foam and salt (4) Cotton wool/foam and ash (5) Others (specify)

21. Have you ever visited a dentist? 1. Yes 2. No

22. If yes what for? 1. To remove or take care of a painful tooth
2. To clean my teeth
3. I had an accident/ fall
4. Others (specify)

Section D: Intra oral examination

Hard tissue:

Present teeth ----------

Missing teeth ----------

Caries ----------

Debris index
Mean debris score ----------

Calculus index
Mean calculus score----------

Oral hygiene score----------

Gingival index
Mean gingival score ----------

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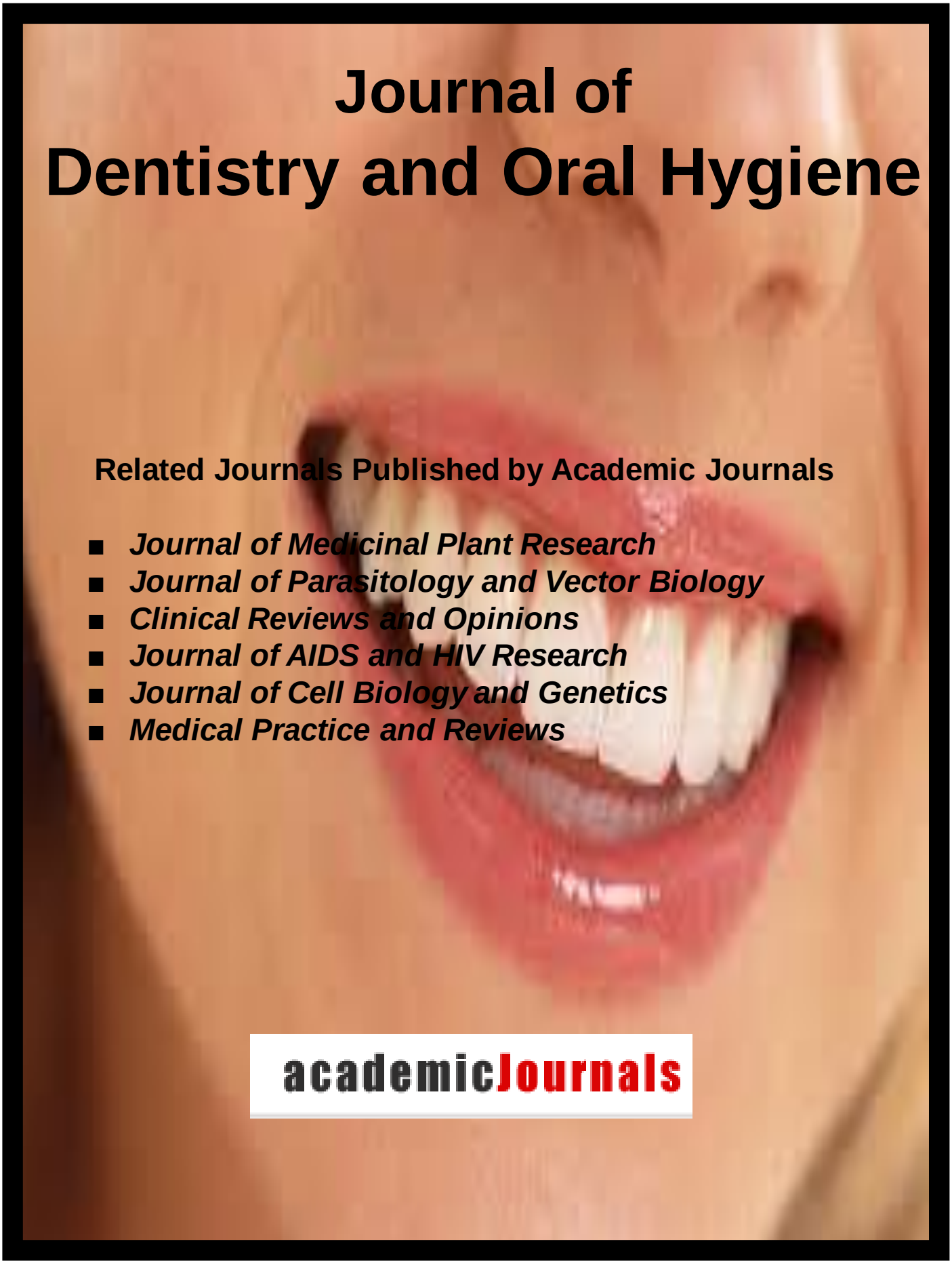

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