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Editorial Office

Office of the SLDA,
Professional Centre,
275/75, Bauddhaloka Mawatha,
Colombo - 7.

Correspondence regarding editorial matters, articles, reviews and news items should be addressed to the Editor, SLDJ, Dr. Deepthi Nanayakkara, Dept. of Basic Sciences, Faculty of Dental Sciences, University of Peradeniya.
Tele: 08/387999.

Correspondence regarding advertisements should be addressed to Dr. Ranjith Weerasinghe, Dental Surgery, Y. M. B. A. Building, Dehiwala.
Tele: 01/713217, 723741.

Correspondence concerning financial matters should be addressed to Dr. Gamini de Silva, 22, Wijerama Mawatha, Colombo - 07.
Tele: 01/685443, 685755

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Sri Lanka Dental Journal

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EDITORIAL

International Dental Congress and 69th Annual Scientific Sessions

The growth of the Sri Lanka Dental Association (SLDA), especially over the past two decades has been truly remarkable. The SLDA is 69 years old this year and it continues to grow in strength and scope. The Association is almost 500 members strong, has 3 branch Associations, the Kandy branch, Southern Branch and the North Western Branch. It is heartening to note the numerous accomplishments and activities of the SLDA over the past years.

One of the prime objectives of the SLDA is the promotion of dental and allied sciences. In order to achieve this objective the Association organizes several professional activities at a national level. Among them are the publication of the Sri Lanka Dental Journal, conducting lectures and seminars on a regular basis and organizing the Annual Scientific Sessions.

The International Dental Congress and the 69th Annual Scientific Sessions, are two major events that the SLDA is organizing this year. Year. They will undoubtedly be very stimulating learning experiences to all participants. The theme of the Congress " Development of oral health care systems in the next decade" is very timely and well chosen and will focus on the recent advancements in the field of dentistry. The highlights of the Scientific Programme include guest lectures, seminars, panel discussions and free

paper presentations by distinguished speakers. This Congress will be a great opportunity to all partcipants to update themselves and to widen the horizon of their knowledge and professional skills.

The first issue of the 30th volume of the Sri Lanka Dental Journal carrying these thoughts about the activities of the Sri Lanka Dental Association will be presented to you on the day of the inauguration of the International Congress.

Deepthi Nanayakkara
Editor

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LEADING ARTICLE

Dental Informatics - the gateway for Dentistry of the 21st century

S. Fernando and M T M Jiffry
Faculty of Medical Sciences, University of Sri Jayawardenepura

Introduction

Information technology or IT is the systematic and effective utilization of electronic systems used to create, acquire, process, mix, store, retrieve, select, transform and disseminate a wide variety of audio, video, graphical, textural or numerical inputs. Medical informatics means the use of computers, computerized equipment and IT oriented software in medical care whereas dental informatics is the application of the same in dentistry. Dental informatics is a rapidly developing scientific field that deals with the use of computer based applications in biomedical information processing, problem solving and decision making events. Greenes and Shortliffe [1] have defined Medical Informatics as the field that 'concerns itself with the cognitive, information processing and communication tasks of medical practice, education, and research, including information science and technology to support these tasks'.

Modern technological advancement has facilitated the global transfer of information of any nature quickly and accurately in its entirety and extent. The use of computers with multimedia facilities, multitask oriented user friendly software, networking facilities and the advances in the satellite communication system have tremendously contributed to the evolution of this new technology in information transfer. Similarly, the specific use of IT in all the fields of dental practice and education is identified as dental informatics. Dental informatics is concerned with the systematic processing of data, information and knowledge in dental education, research and dental healthcare. In short, dental informatics is not just the application or use of computers in dentistry, but their systematic and organized utilization to enhance efficiency of delivery of dental care and dental education and research.

Very few advances in technology have had as broad an impact on humanity as IT. The appearance and rapid spread in the past several years of innovations such as the world wide web (www) on the internet and the emergence of the ability to network the personal computer (PC) terminals all over the world had paved the way for an unprecedented growth and advancement in the field of IT. These global events forecast a significant improvement in the delivery of health care, the conduct of biomedical research, and the undergraduate, graduate and continuing education of health professionals.

IT and medical education

IT has a longer history in medical education. Hence, it is beneficial for us to look at the available data on IT usage amongst the medical professionals and in medical education. Metcalfe[2] reported that at least half of UK hospital doctors and the majority of general practitioners had access to and use of computer facilities.

Studies from Europe suggest that at present we are in a transitional phase with rapid development towards universal IT usage by medical practitioners.[3] Furthermore, as reported by Williams et al.[4] the need for education in IT has been recognised by the Medical Informatics Committee of the Royal College of Physicians and also by the General Medical Council in 1993.[5] With the advent of the e mail, internet, network technology and data storage ability on CDs, there has been a significant revolution in the field of communication and the potential use of computers in the medical fields have tremendously increased. The use of computers in the medical fields consists of computer based information storage and retrieval in medical education, practice and research. It is a well known fact that during the last decade the global internet users have increased quite dramatically. Such unprecedented use of internet has helped in the access of published scientific literature through the electronic media. Even from the point of view of developing countries, especially with regard to access to published information in reputed journals, it is cost effective to utilize electronic media for dissemination.[6]

IT in Sri Lanka

It is encouraging to witness a satisfactory awareness and motivation amongst the general public in Sri Lanka in the use of computers. There has been a significant improvement in computer education and awareness in general in Sri Lanka, through the intervention of professional organizations like the Computer Society of Sri Lanka (CSSL), Computer and Information Technology Education Council (CINTEC) and Lanka Educational And Research Network (LEARN) which are in existence and have been functioning effectively over a decade or two. The majority of school leavers usually follow either short courses or certificate level courses in computing. In a survey conducted amongst the medical students it was observed that 82% expressed the view that computing skills should be introduced to the medical course and 84% stated that computing is essential to improve their professional knowledge.[7] The increasing trend and need of computer use amongst the dental professionals in Sri Lanka has been reported almost a decade ago.[8] The formation of a Health Informatics Society of Sri Lanka (HISSL) in the latter part of 1998 is one of the landmark events which occurred in bringing all the computer users in the health related fields under one organisation. There is a promising future for computer related fields and computer application in the higher education sector and for different professionals in Sri Lanka. This is further strengthened by the fact that IT has been identified as one of the priority areas to be developed in the reforms that have been proposed in educational sector on the recommendations of the Presidential Task Force. This would no doubt further promote the build up of human resources with adequate computer literacy in Sri Lanka. It is also heartening to note that the year 2001 has been declared as the Year of IT in education in Sri Lanka.

Information sciences in dental education

The current trend in professional education is to introduce independent learning and problem solving skills into the curriculum since the ultimate aim of the teaching/learning exercise is to produce a creative thinker. In introducing such innovative techniques into the dental curricula it is mandatory to utilize computers and computerized modern electronic instruments for the teaching/ learning exercises. With the introduction of evidence based medicine to medical education by the American Medical Association in 1992,[9] Richards and Lawrence [10] identified the need and usefulness of the concept of evidence based

dentistry in dental education. In practicing evidence based dentistry by the dental professionals, it is mandatory to have access to the available reports of evaluation of studies in a particular topic accurately and speedily. The use of IT in dentistry would definitely facilitate faster information dissemination and communication throughout the world. As a first step in introducing IT to the dental practice and amongst dental professionals, the American Association of Dental Schools started to collate nationwide information on dental education in 1988. The use of modern telecommunication facilities such as e-mail, internet, intranet and teleconferencing, has further facilitated data gathering and retrieval. Such information would be beneficial for undergraduate dental education too.[11] It is desirable to designate an academic unit in the application of information sciences and computer technology in each faculty in order to promote their effective use. Moreover, a significant impact of IT in dental education can be achieved by the introduction of IT in the dental curriculum rather than by simply establishing an IT unit which only provides a service function. However, the implementation of this needs further resources, both physical and human.[12]

The use of medical and dental informatics

IT has a wide scope of application in many disciplines. Use of IT in the medical and dental fields could be applied to;

- Patient and laboratory record keeping
- Access to references
- Data base on patient/laboratory/ inventory record keeping
- Report preparation
- Multimedia use in education and presentations
- Internet accessibilty
- E mail
- Video conferencing
- Telemedicine /Teledentistry
- Computer assisted learning
- Virtual hospital
- Clinical applications

Dentistry on the internet

A considerable number of reputed web sites in dentistry are available that could be browsed through the internet. The addresses of some useful sites are given in Table 1. One could easily visit other sites cross linked through the sites mentioned here. It is necessary to understand that the information available in all the websites may not be accurate or authentic unless the ownership of the site is claimed by a recognized institution or information source.

Table 1 : Some useful Web addresses related to dentistry and their description

Web site address	Description
http://www.derweb.ac.uk/	Dental Education Resource on the WEB
http://www.dentanet.org.uk/	Details of computer assisted learning programs
http://www.priory.co.uk/journals/dent.htm	Dentistry online
http://www.vol.it/dentistry/	Dentistry Tomorrow
http://www.mda.org.my/	Malaysian Dental Association
http://www.sci.lib.uci.edu/~martindale/Dental.html	Martindale's virtual dental center, USA
http://www.signet.com.sg/~jonfntan	Dental resources from Singapore
http://www.dentalglobe.com/	Informative web site on dentistry
http://www.bdj.org.uk/	British Dental Journal

Clinical informatics and dentistry

The use of computers and computer based applications in clinical teaching, patient care and dental reserach has made a significant impact on the quality and efficiency of such functions. The potential for the use of IT for clinical teaching in the form of interactive learning sessions and via the virtual hospital setting is unlimited and challenging. It also provides an opportunity for students to undertake self learning and offers facilities for obtaining a wide variety of integrated inputs in the learning exercise. The learning sessions of the students also could be strengthened with self assessment exercises on computers with provision for recording the performance. These are only a few examples of the unlimited potential for the use of IT in dentistry. Furthermore, dental patient information (record keeping) is one of the important tasks that could be effectively performed on an IT based clinical environment. This procedure would help in easy retrieval of patient records for future reference as well as facilitate the process of second opinion from another expert even from another country. The records maintained within a computerized environment could be easily fed in for future statistical analysis. Furthermore, research in dentistry is greatly facilitated by the free access to medline abstracts through the internet.

Future perspectives of dental informatics in Sri Lanka

The use of dental informatics by the dental surgeons is largely depended on the leadership that needs to be provided by the educational and professional institutes related to dentistry. This trend may also be facilitated by the support available from the IT industry and software experts. The whole process revolves around a cycle where the dental surgeons themselves initiate projects and start using computers in all the professional activities, as the starting point.

The availability of internet facility and e mail have certainly brought the dental professional of Sri Lanka closer to rapidly advancing dental technology and education throughout the world. However, for more effective and efficient use of dental informatics for the benefit of the dental profession, all the stakeholders of dentistry in Sri Lanka should be proactive in promoting and introducing computer based applications to dentistry.

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CLINICAL UPDATE

Systemic antibiotic therapy in the management of periodontal disease

Sunethra Rajapakse

Division of Periodontology, Faculty of Dental Sciences, University of Peradeniya,

Introduction

Periodontal diseases are pathophysiologic responses of the supporting structures of the teeth to the overgrowth of bacteria in the form of dental plaque.

Gingivitis and periodontitis comprise two major infections of the periodontium. Although all humans are susceptible to gingivitis, only about 10-15% of most adult populations is susceptible to destructive periodontal disease. The prevalence of periodontal disease in the adults of Sri Lanka is around 96%. [1]

The reduction of levels of dental caries, and improved methods of restorative treatment together with the increased life expectancy of the individuals have resulted in increased levels of dentate adults in any population. Increasing age being an unmodifiable risk factor for periodontal disease[2], there will invariably be increasing numbers of individuals with periodontal disease with concomitantly increased demand for periodontal therapy.

It has been shown using human subjects that gingivitis is completely reversible.[3] However, unlike gingivitis, the effects of periodontitis are only partially reversible. Therefore, it is necessary to control or arrest periodontitis early in order to minimize destruction that would in turn increase the longevity of the dentition of patients with destructive periodontal disease.

At present irrespective of the extent and type of the plaque associated periodontal disease, whether it be gingivitis or periodontitis of various types such as aggressive periodontitis, chronic periodontitis or a form of refractory periodontitis, intervention mainly focuses on universal application of non specific plaque control methods. The basis of this is the removal of factors, which would promote accumulation of bacterial plaque, and factors that would facilitate removal of plaque by the patients themselves. These include customized oral health instructions, removal of calculus and overhanging restorations, correction of orthodontic problems, correction of gingival architecture etc. The ultimate goal of this type of measures is to control the quality and quantity of bacterial plaque at a level compatible with periodontal health. In other words the bacterial plaque levels (quality and quantity) thus obtained will effectively be dealt with by host defense mechanisms. This treatment approach is based on the non-specific plaque hypothesis which was put forward to explain the aetiology of periodontal disease in 1960s'.[3] This hypothesis is still true with regard to gingivitis where there is maturation of dental plaque leading to selective outgrowth of anaerobic bacteria, which in turn alters the balance between the host and anaerobic bacteria. Therefore gingivitis could be considered as an endogenous infection.

However the theories on aetiology of periodontitis has changed since then. It is now agreed that human periodontitis is initiated and perpetuated by a small group of predominantly Gram negative-anaerobic or micro-aerophilic bacteria that colonize the sub-gingival area: the Specific Plaque Hypothesis. Association of *Actinobacillus*

actinomycetemcomitans with localized juvenile periodontitis[4,5] originated this concept. The specific plaque hypothesis proposes that dental plaque isolated from periodontitis lesions are qualitatively distinct from that of plaque obtained from healthy sub-gingival sites. Approximately a dozen or less of the 200 bacterial taxa, which can inhabit the human oral cavity, appear to be associated with periodontitis. Out of these specific microorganisms *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Actinobacillus actinomycetemcomitans* have now been considered as periodontal pathogens.[4,6] Earlier these bacteria were referred to as putative periodontal pathogens. In fact research data available to date have substantiated the pathogenic role of these three bacteria in periodontal disease. The absence of these bacterial species or the occurrence of them in very few numbers in the oral cavities of healthy people, transmission of these bacterial species among members of the family and production of a wide range of virulent factors support their role as periodontal pathogens. Further, existence of refractory forms of periodontitis in all categories of periodontal infections and evidence of tissue invasion in vitro and in vivo by periodontal pathogens substantiate the fact that at least some forms of periodontal disease could be considered as exogeneous infections.

Some forms of periodontitis may be opportunistic periodontal infections due to bacteria in the normal flora with low pathogenic potential. Opportunistic periodontal infections can occur in a systemically or locally impaired host. Some forms of early onset periodontitis have been associated with abnormalities in the neutrophil functions.[5] Patients with uncontrolled diabetes[7], AIDS[8], immunosuppressive drug therapy[9], stress and smoking[10] may also be associated with opportunistic periodontal infections.

The treatment of non-specific plaque infections such as gingivitis or some types of chronic slow progressing periodontitis would entail the bacterial flora to be suppressed either continuously or periodically to achieve an overall reduction of bacterial mass to a level, which is compatible with periodontal health. Mechanical debridement, oral health instructions, antiseptic mouthwashes and supportive periodontal therapy that involve professional plaque control and periodic reviews could accomplish this. Use of antibiotic therapy in such situations is unnecessary.

In conditions where there are reasons to believe that an opportunistic infection exists, improvement of hosts' immune status and systemic antibiotic therapy help the host to overcome the infection.

In the treatment of periodontal diseases due to true periodontal infections it is necessary to establish the nature of periodontal infection. As mentioned above anaerobic and micro-aerophilic infections have been implicated in the aetiology of periodontal disease. It is a prerequisite to establish the nature of infection (anaerobic /micro-aerophilic) before selecting an antibiotic to control plaque infection.[11,12] In an extensive review of literature on systemic antibiotic therapy in periodontal diseases it has established that periodontal infections are predominantly anaerobic infections.[11] The dilemma faced by clinicians is the diagnosis of anaerobic infections in the dental surgery. Various tests based on biochemical reactions such as BANA test, and other methods based on molecular biological techniques, immunological techniques and bacteriological analysis of plaque such as chair side DNA probing, and microbiological culture methods are available in this regard. However it should be noted that in a developing country like Sri Lanka the chances of routine use of these methods are remote.

The lack of resources to confirm an anaerobic infection has led to the widespread use of antibiotics in general practice in Sri Lanka. There are instances where antibiotics are prescribed even for straightforward cases of plaque-associated gingivitis and mild forms of chronic periodontitis. Antibiotics should be considered as an adjunct to mechanical debridement.[13] Further, their use should be reserved for patients with continuous periodontal breakdown despite careful mechanical debridement with maximum efforts by the patient in plaque control measures. Some forms of aggressive periodontitis may be treated with antibiotics after initial mechanical debridement. Use of antibiotics without thorough mechanical debridement and without adequate clinical diagnosis, microbiological analysis and antibiotic sensitivity testing of target organism(s) should be regarded as inappropriate.

Problems associated with indiscriminate use of systemic antibiotics

- This indiscriminate use of antibiotics could lead to emergence of antibiotic resistant strains to a particular antibiotic as well as propagation of bacterial clones with multiple drug resistance in the population. This will limit the use of a particular antibiotic(s) in the treatment of periodontal disease as well and will also lead to serious consequences with regard to life threatening bacterial infections, such as pneumonia, gangrene, septicemia and meningitis.
- It could also cause treatment failure in periodontal management due to selection of inappropriate antibiotic(s).
- It may lead to a suppression of bacteria in the normal flora of the oral cavity, which play a protective role in maintaining periodontal health. This could lead to increase pathogenic bacteria that may give rise to acute periodontal infections.
- Suppression of normal flora in the gut could lead to life threatening illnesses like acute pseudomembranous colitis (inappropriate use of clindamycin).
- The patient will under estimate the value of oral hygiene practices and they will also get a false sense of improvement of disease, which would only be temporary.

Selection of an antibiotic in the management of periodontal disease

The choice of an antibiotic depends on the periodontal pathogen(s) involved, the patient's medical status, availability and potential adverse drug reactions. It should be noted that the use of narrow spectrum antibiotics is the best approach to deal with any infection, which need antibiotic treatment. Therefore, dentists should avoid the use of combinations of antibiotics without microbiological analysis. Bactericidal drugs should be preferred to bacteriostatic since their effectiveness does not rely on functioning host defence in a periodontal pocket. Nevertheless tetracyclines (bacteriastatic) may be used in certain instances such as micro aerophilic infection.

Antibiotics active against periodontal pathogens

- Metronidazole is the drug of choice for anaerobic infections since all anaerobes are uniquely sensitive to this agent[14] and resistance to metronidazole is extremely rare.[15] Further, metronidazole would not affect the facultative flora, which is associated with health.[12] It has also been shown that the hydroxymetabolite of metronidazole has potent antimicrobial properties. It is known to be active against micro-aerophilic bacteria such as *Actinobacillus actinomycetemcomitans*.

- Doxycycline, clindamycin, and members of the penicillin family are also active against anaerobes. **Quinolones should be reserved for opportunistic periodontal infections caused by enteric bacteria, pseumonads and staphylococcus, that may occur in severe immuno- compromised patients.**
- In addition to antibiotic monotherapy, combinations of antibiotics and serial antibiotic therapy have been used in the management of periodontal disease. Combinations of metronidazole plus amoxicillin or Augmentin have been used successfully.
- Antibiotics may also be used serially in the management of periodontal disease. Serial drug regimens studied to date include systemic doxycycline therapy followed by either Augmentin or metronidazole. [16,17]

Recommended antibiotic regimens

- Metronidazole 250-500 mg/TID/ 8 days; adult dose orally. Since metronidazole is a concentration dependant & time independent drug, effectiveness is better when used after reduction of bacterial mass by mechanical debridement. Metronidazole therapy, without adequate plaque control is not effective in eliminating the disease.[18, 19]
- Tetracycline HCI : 500 mg / TFD / 21 days; adult dose orally. It is useful in periodontal infection with *Actinobacillus actinomycetemcomitans* (Aa)[20]. Tetracycline has a limited effect on sub gingival anaerobes. It may not arrest the destructive disease activity in mixed infections. [21,22]
- Ciprofloxacin 500 mg / BID / 8 days; adult dose orally is indicated only with periodontal superinfections involving enteric rods, pseudomonads or staphylococcus[23].
- Metronidazole (200 mg) and amoxcillin (250 mg) (or metranidazole & augmentirs) combination (TID / 8 days of each drug; adult doses) orally is indicated for periodontal infections associated with bacterial flora with (Aa).[24]
- Clindamycin 150 mg / QID / 7 days; adult dose orally should be used with caution since acute pseudomembranous colitis associated with the use of clindamycin[25] has been reported.

Conclusion

It is thus seen that systemic antibiotics should be used with caution in the management of periodontal disease.

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RESEARCH ARTICLES

The action of Finasteride- an inhibitor of 5α-reductase enzyme on sex hormones in human oral periosteum

[1]A. Tilakaratne and [2]Mena Soory

[1] *Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka*

[2] *Department of Periodontology, Guy's King's St Thomas' Dental Institute, King's Campus, London, UK*

Abstract

***Objective*:** Since androgens and oestrogens stimulate collagen matrix synthesis, and finasteride is a specific inhibitor for the 5α-reductase-type 2 isoenzyme activity, this study aimed to find out the effects of oestradiol (O) and finasteride (F) on 5α-reduction of androgen substrates by human periosteal fibroblasts (HPF). ***Materials and methods*:** Monolayer cultures of HPF were established in Eagle's Minimum Essential Medium. Duplicate incubations were performed with 14C-testosterone (14C-T) / 14C-4-androstenedione (14C-4-A) as substrates and optimal concentrations of O and F alone and in combination. The steroid metabolites were analysed and quantified using a radioisotope scanner. ***Results*:** With 14C-T as the androgen substrate, (O) alone showed 2%-16% increases in DHT formation over the control value, in response to its concentrations of 0.1, 0.5 and 1.0 μg/ml, respectively ($p<0.01$). Finasteride (0.1, 1.0 and 3.0 μg/ml) caused 43%, 5.9-fold and 8-fold decreases in DHT formation respectively, ($p<0.01$). O+F at 0.1, (0.5_O+1_F) and (1_O+3_F) μg/ml too caused inhibitory effects on DHT synthesis ($p<0.01$). With 14C-4-A as the substrate, all 3 concentrations of O caused increases ranging from 2%-43% in DHT synthesis ($p<0.01$). F caused 15%-80% decreases in the yields of DHT in response to its concentrations, compared to the control value. O+F, at all concentrations showed inhibitory effects on DHT synthesis ($p<0.01$). ***Conclusion*:** The specific inhibition of 5α-reductase type 2 enzyme activity by finasteride in periosteal fibroblasts is suggestive of the presence of target tissue synthetic activity in human oral periosteum. Stimulatory effects of oestradiol on the above enzyme activities indicate the hormone-mediated tissue responses during repair.

***Key words*:** 5α-reductase, fibroblasts, finasteride, androgen, oestradiol.

Introduction

It is well established that the androgens particularly 5α-dihydrotestosterone (DHT) exert anabolic effects in tissues in both animals and humans. Testosterone increased the growth of selected muscles in growing lamb.[1] The fact that testosterone could slightly augment the rate of connective tissue turnover and osteogenic capacity of the periodontium has been reviewed earlier.[2] The expression of androgen receptors has been detected in a high proportion of periodontal and gingival tissues; also in fibroblasts derived from the same.[3]

5α-DHT and 4-androstenedione are formed from testosterone metabolism by human gingival fibroblasts in culture. 5α-DHT is the most biologically active metabolite with anabolic actions in tissues. This metabolite stimulates matrix synthesis in connective tissue and bone.[4,5,6,7] When a reparatory response is required anabolic steroids such as DHT and testosterone can contribute to the synthetic activity in fibroblasts and oste oblasts.[2,5,8] 5α-reductase activity has been shown to be elevated in human gingivae and periodontal ligament in response to growth factors associated with inflammatory repair.[9,10,11]

Oestrogens show stimulatory effects on the metabolism of collagen; e.g. oestradiol increased the incorporation of proline into the collagen molecule under synthesis in cultured skin fibroblasts.[12] In uterine endometrium, oestrogen stimulated fibroblast growth factor secretion from stromal cells.[13] Similarly, treatment with oestradiol at serum concentrations similar to those observed in the luteal phase of the human menstrual cycle has shown significant increases in the epithelial proliferation of normal human breast tissue xenografts implanted into athymic nude mice.[14]

Further, the oestrogen status has been shown to have influences in alveolar bone density in post menopausal women.[15] In this study, oestradiol-17β sufficient women exhibited a higher frequency of sites demonstrating a gain in alveolar bone density in relation to periodontal lesions, while the oestradiol-17β deficient women exhibited a higher frequency of sites demonstrating loss in alveolar bone density.

Administration of pharmacological doses of oestrogen, sufficient to raise concentrations to those seen in late pregnancy, increased bone formation above the rates observed in rats with physiological - non pregnant oestrogen levels.[16] It is evident that oestrogen maintains bone volume not only through inhibition of bone resorption but also through stimulation of bone formation.[17]

Skeletal bone growth following administration of 5α-DHT and oestradiol-17β into ovariectomized osteopenic rats was studied by Coxam and others in 1996.[18] They observed that the periosteal bone formation rates in the rat skeletal bones were increased with the high dose of DHT alone and in combination with oestrogen. DHT combined with oestrogen increased endochondral growth rates relative to the group treated with oestrogen alone.[18]

The enzyme 5α-reductase catalyses the reduction of 4,5 double bonds in a variety of substrates and is thought to play both catabolic and anabolic roles in steroid hormone metabolism. 5α-reductase plays a central role in androgen action by catalysing the conversion of testosterone into the more potent hormone 5α-dihydrotestosterone. Studies carried out earlier have revealed the existence of two genes in both humans and rats that encode different 5α-reductase isoenzymes, designated as type 1 and type 2.[19] The 4-azasteroid compound finasteride is an inhibitor of the 5α-reductase type

2 isozyme which is abundantly found in male reproductive tissues and is associated with target tissues such as the prostate. Type 1 enzyme is predominantly found in peripheral tissues. It has been suggested that the type 2 enzyme has an anabolic role while type 1 has a catabolic role.[20,21] The rat type 2 isozyme has a 10-20 fold higher affinity for testosterone, androstenedione and progesterone than the type 1 isozyme and has shown to be anabolic and is responsible for DHT synthesis in androgen target tissues. 5α-reductase type 1 isozyme appears to inactivate testosterone in non androgen target tissues and plays a catabolic role in androgen metabolism.[6]

5α-DHT is thought to play an important role in the pathogenesis of benign prostatic hypertrophy (BPH), and the 5α-reductase inhibitor finasteride has recently been in use in clinical trials for the treatment of BPH. This drug offers a potentially unique advantage over other androgen withdrawal therapies in blocking the formation of 5α-DHT, the major active prostate androgen without blocking testosterone. Finasteride administration to patients with BPH has shown marked decreases in prostatic DHT concentrations[22], reflecting the inhibition of 5α-reductase activity by finasteride.

In the gingival model, 5α-reductase activity was effectively inhibited by finasteride, confirming the presence of the type 2 isozyme.[23]This study also confirmed the specificity of the anti-androgen as a 5α-reductase type 2 isoenzyme inhibitor.[23]

Our previous studies on the effects of oestradiol-17β and progesterone on androgen metabolism in human gingival fibroblasts showed a heightened 5α-reductase activity in response to oestradiol, while progesterone inhibited 5α-reductase activity.[24,25] In other studies, finasteride alone or in combination with progesterone had significant inhibitory effects on 5α-reductase activity in human gingival fibroblasts. [26]

In view of the above effects of oestradiol-17β on 5α-reductase activity, the influence of 5α-reductase inhibitor, finasteride in the presence of oestradiol on the metabolic conversion of two androgen substrates in other target cells, namely periosteal fibroblasts was our aim for investigation in this study.

Materials and methods

Authentic steroid, oestradiol-17β from Sigma Chemicals Co., Poole, Dorset, UK was dissolved and redistilled in ethanol (supplied by Merck Chemicals Ltd., Dagenham, Essex) at appropriate concentrations and stored at 4° C. 14C-testosterone and 14C-4-androstenedione (specific activity 58 μCi / μmol) were obtained from Amersham International, Amersham, Bucks. Solvents for thin layer chromatography - TLC (benzene and acetone), ethyl acetate for extraction of metabolites, and chloroform to redissolve the dry bulk of extract were all supplied by BDH (Merck). The TLC plates were precoated silicagel kieselgel 60 (20 cm x 20 cm). Finasteride used in the incubations was obtained from the pharmacy at King's College School of Medicine and Dentistry, London, UK.

The incubation medium used was Eagle's minimum essential medium (MEM), with 10% foetal bovine serum (FBS), L-glutamine, antibiotic solution (penicillin and streptomycin), and sodium bicarbonate which were all supplied by Gibco Ltd, Paisley, Scotland.

Chronically inflamed periosteal tissues were obtained from 4 periodontal patients (aged 30-50 years) undergoing vertically advanced flap procedures for the treatment of gingival recession at the Department of Periodontology, King's College Dental Institute, London, UK. A small sample of periosteum was isolated from the surface of the bone for each cell line that was

derived. They had all completed initial phase treatment comprising scaling and root planing before these procedures and subsequent isolation of the periosteal tissues.

Establishing fibroblasts in cultures

The isolated periosteal tissues were minced into small fragments, approximately 1 mm^3 and periosteal fibroblasts were established in primary culture in 25 cm^2 tissue culture flasks. Since there is evidence to support the fact that the cells derived from serial passaging could still maintain to express their in-vivo phenotype in-vitro [27, 28] the primary cultures were sub-cultured by partial digestion with 0.25% trypsin solution. Fibroblasts of the 4^{th} to 9^{th} passage in confluent monclayer culture were used for the experiments. The contents of a fully confluent 25 cm^2 flask ($2.2x10^6$ cells) were divided among 24 wells of a multiwell dish in Eagle's MEM, and the cells were allowed to become fully confluent in the multiwell dishes before setting up experiments. Duplicate incubations were performed with 14C-testosterone / 14C-4-androstenedione (0.025 μCi/ml and 0.01 μCi/ml respectively), optimal concentrations of oestradiol-17β, finasteride alone and in combination.

After a 24-48 hours of incubation in a humidified CO_2 incubator at 37°C, the experiments were removed from the incubator and the medium in each well was solvent extracted using ethyl acetate (2 ml x 2).

The extracts were evaporated to a small dry bulk in a vortex evaporator (Gyrovap, V.A. Howe LTD., Banbury, Oxon, UK), redissolved in 100 μl chloroform and spotted on TLC plates. Thus the isolated metabolites were subjected to thin layer chromatography in a benzene : acetone solvent system (4:1 v/v), for their separation. The separated metabolites were then scanned in a radioisotope scanner linked to a computer for their quantification.

Establishing optimal stimulatory / inhibitory concentrations of oestradiol-17β / finasteride, for 5α-reduction of androgen substrates in fibroblasts

Based on preliminary experiments, using 11 serial concentrations of oestradiol, the optimal stimulatory concentrations were established. Thus the optimal concentrations of oestradiol used in the incubations were 0.1, 0.5 and 1.0 μg/ml. In similar experiments, independent incubations of cultured periosteal fibroblasts with serial concentrations of finasteride have shown that 0.1, 0.5, 1.0, 3.0 and 5.0 μg/ml are effective inhibitory concentrations for 5α-reductase activity.[23]

Results

Effects of optimal concentrations of oestradiol (O)/ finasteride (F) alone and in combination on the metabolism of testosterone by periosteal fibroblasts The metabolism of 14C-T by periosteal fibroblasts in the presence of optimal concentrations of O / F and O+F was compared with the control incubations in each experiment. Figure 1 compares this metabolic conversion of 14C-T to DHT in response to control and test incubations.

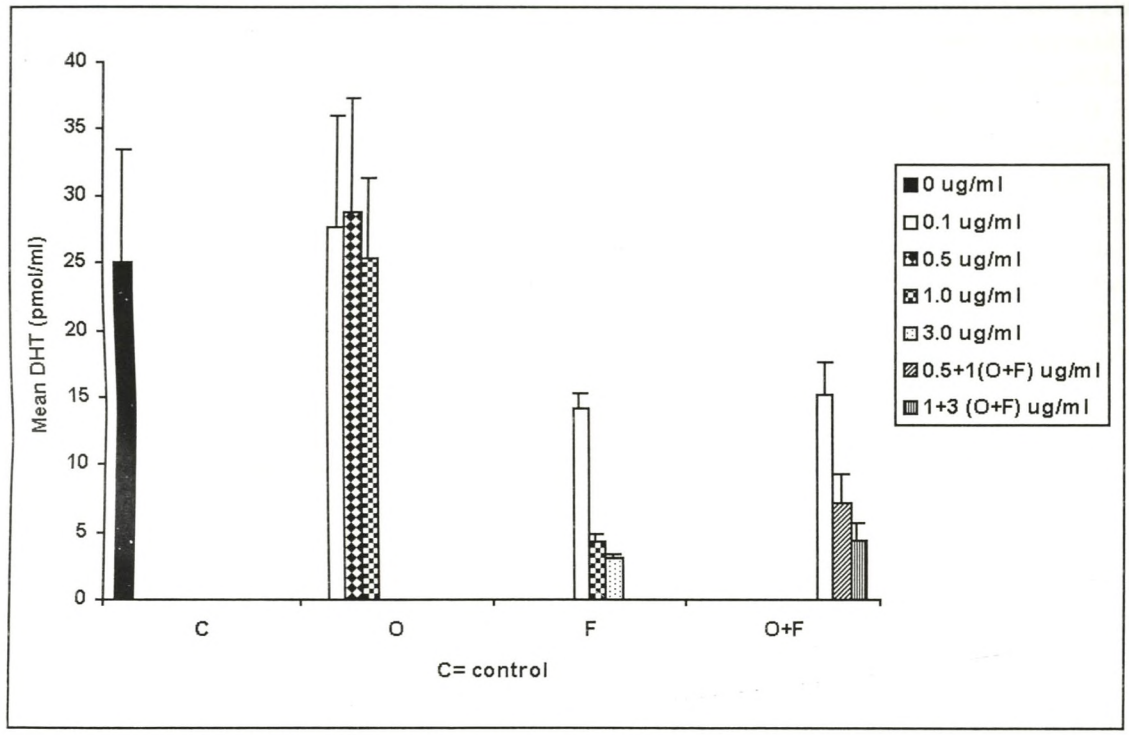

Figure 1. The metabolic conversion of 14C-T to DHT in the presence or absence of O/F/O+F in periosteal fibroblasts

Oestradiol on its own showed 12%, 16% and 2% increases in DHT formation over the control value, in response to its concentrations of 0.1, 0.5 and 1.0 μg/ml, respectively (n=4; $p<0.01$, Wilcoxon signed rank test for paired observations).

All three concentrations of finasteride (0.1, 1.0 and 3.0 μg/ml) caused 43%, 5.9-fold and 8-fold decreases in DHT formation respectively, compared to the control value (n=4; $p<0.01$).

O+F at concentrations of 0.1, (0.5_O+1_F) and (1_O+3_F) μg/ml too caused inhibitory effects on DHT synthesis from 14C-T compared to the control value. There were 39%, 3.4-fold and 5.6-fold decreases in DHT synthesis in response to the above concentrations respectively (n=4; $p<0.01$). This inhibition however was less obvious than that seen with finasteride alone. These results obtained for 5α-reductase activity in periosteal fibroblasts show a similar trend to that seen in gingival fibroblasts. [29]

Figure 2 shows the metabolic conversion of 14C-T to 4-A by periosteal fibroblasts in response to O/F and O+F. Oestradiol at its highest concentration studied (1.0 μg/ml) showed a 9% increase in 4-A synthesis from 14C-T compared to the control value (n=4; $p<0.01$, Wilcoxon signed rank test for paired observations). There were very

marked increases in 4-A synthesis from 14C-T in response to all concentrations of finasteride showing heightened 17β-hydroxy steroid dehydrogenase (HSD) activity. These increases were 55%, 83% and 82% in relation to 0.1, 1.0 and 3.0 μg/ml of finasteride concentrations respectively, when compared with the control value ($n=4$; $p<0.01$). The heightened 17β-HSD activity appeared to have been still maintained by all concentrations of finasteride, when combined with oestradiol (as seen in the increased yields of 4-A). Even the very low oestradiol concentrations which lowered 4-A synthesis very slightly when used alone, caused increased yields of 4-A when combined with finasteride at the same concentrations. This could be due to the extent of suppression of 5α-reductase activity, resulting in increased 17β-HSD activity, by finasteride. There were 28%, 48% and 45% increases in 4-A synthesis in response to 0.1, (0.5_O+1_F) and (1_O+3_F) concentrations of O+F compared to the control value ($n=4$; $p<0.01$).

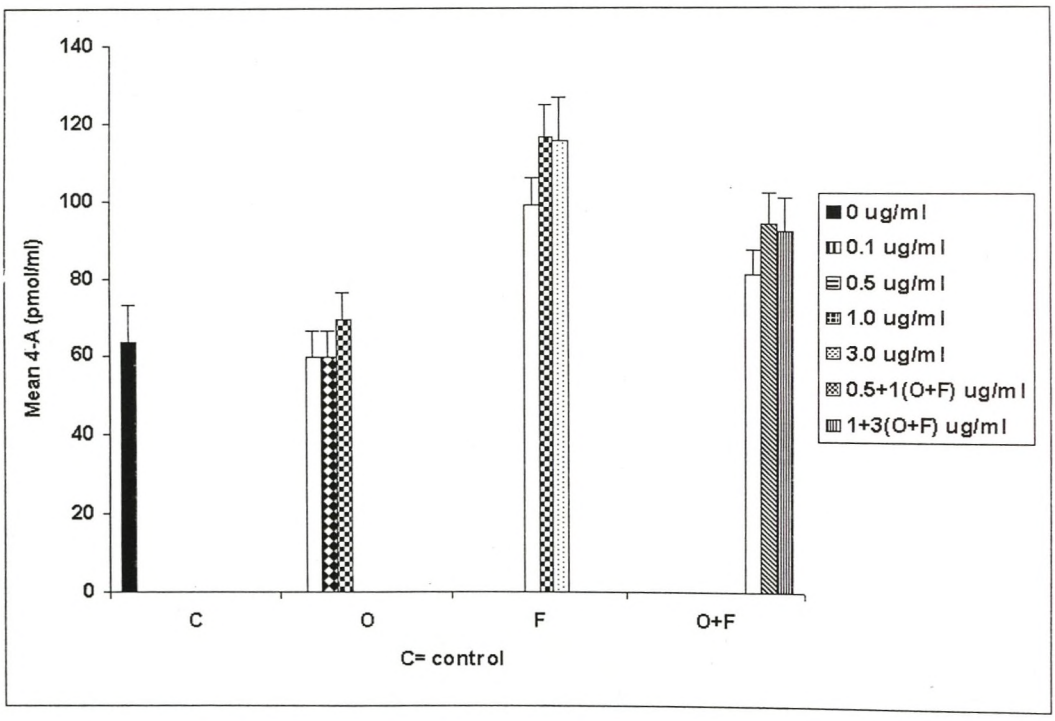

Figure 2. The metabolic conversion of 14C-T to 4-A in the presence or absence of O/F/O+F in periosteal fibroblasts

Effects of optimal concentrations of oestradiol / finasteride alone and in combination on the metabolism of 4-androstenedione by periosteal fibroblasts

Figure 3 shows the metabolic conversion of 14C-4-A to DHT by periosteal fibroblasts in response to optimal concentrations of O / F and O+F. The mean DHT values in response to different concentrations are compared with the mean value for the control incubations.

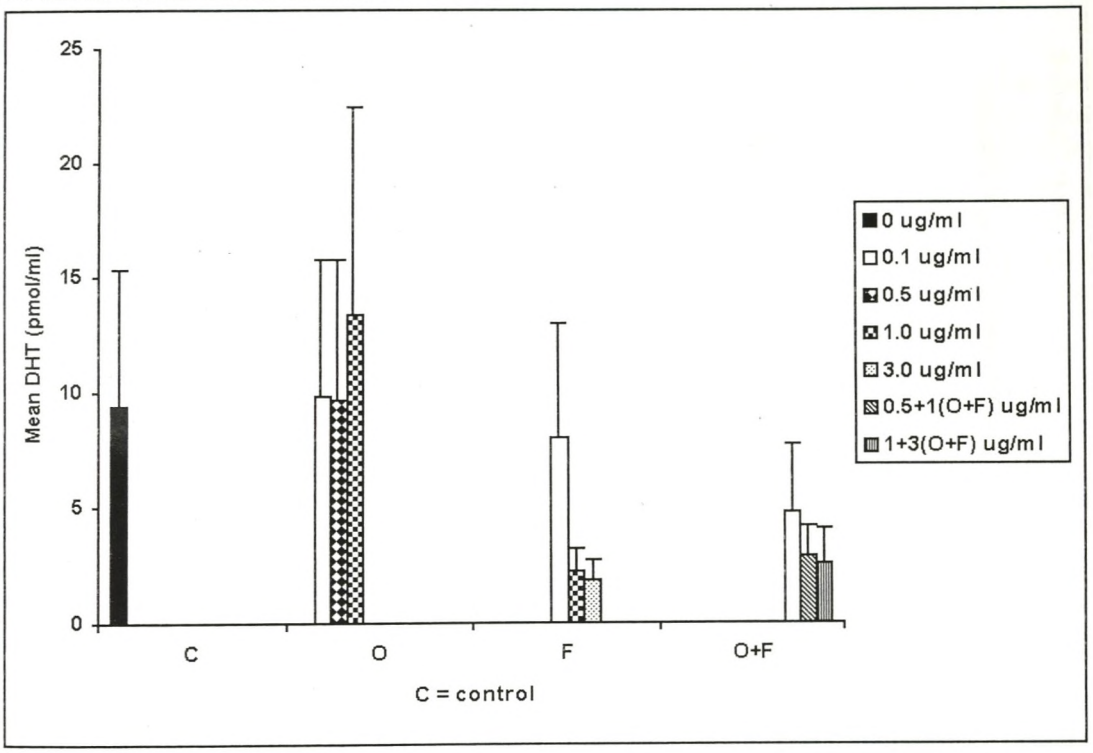

Figure 3. The metabolic conversion of 14C-4-A to DHT in the presence or absence of O/F/O+F in periosteal fibroblasts

All 3 concentrations of oestradiol on their own caused increases ranging from 2%-43% in DHT synthesis, over the control value. The maximum increase was 43%, compared to the controls in response to 1.0 μg/ml of oestradiol ($n=4$; $p<0.01$, Wilcoxon signed rank test for paired observations). Finasteride on its own caused 15%, 77% and 80% decreases in the yields of DHT in response to 0.1, 1.0 and 3.0 μg/ml concentrations respectively, compared to the control value ($n=4$; $p<0.01$).

O+F in combination, at all concentrations studied showed significant inhibitory effects on DHT synthesis. The maximum effect was seen in response to 1_O+3_F μg/ml concentration and there was a 72% decrease in the yield of DHT from the control value ($n=4$; $p<0.01$). This inhibitory effect of finasteride at 3 μg/ml on DHT synthesis was very marked in spite of the highest stimulatory effect of O at 1.0 μg/ml.

The metabolic conversion of 14C-4-A to testosterone in periosteal fibroblasts in response to O / F and O+F is given in Figure 4.

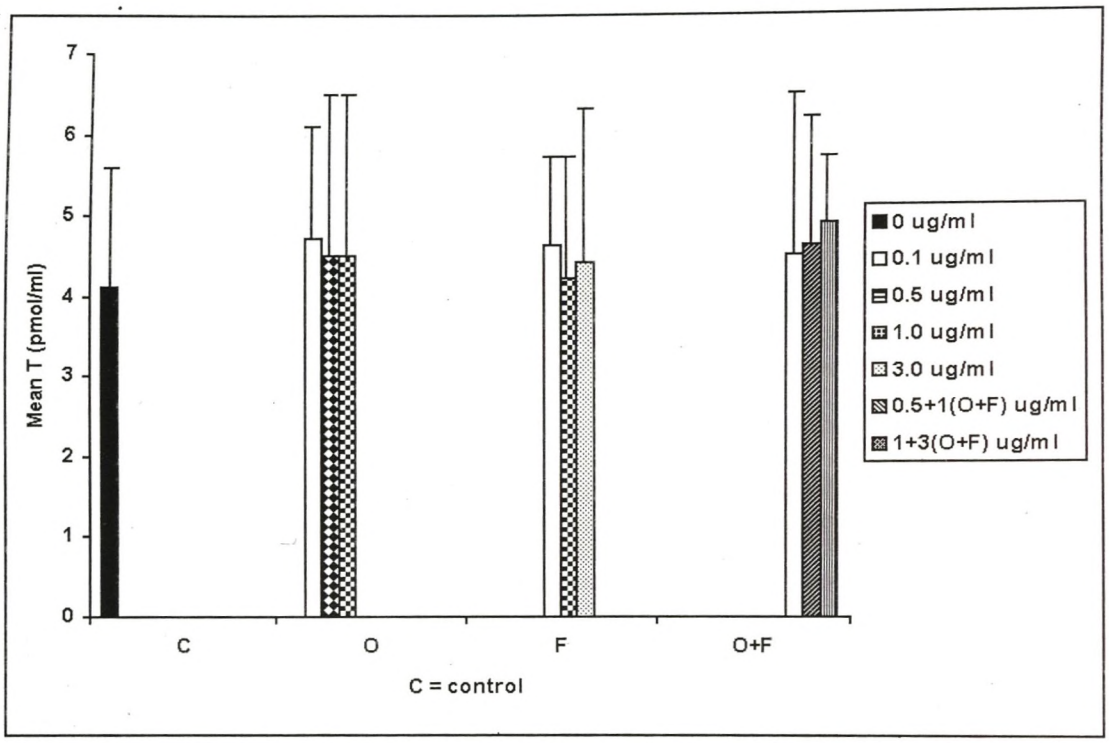

Figure 4. The metabolic conversion of 14C-4-A to T in the presence or absence of O/F/O+F in periosteal fibroblasts

The effect of oestradiol on testosterone synthesis was stimulatory in response to all 3 concentrations. There were 10%-15% increases in testosterone, compared to the controls, and the maximum increase was 15% in response to 0.1 μg/ml of oestradiol ($n=4$; $p<0.05$).

Finasteride also caused 2%-12% increases on the same synthetic activity, the maximum effect showing (12% increase, compared to the controls) at 0.1 μg/ml of finasteride ($n=4$; $p<0.05$).

The combinations of O+F at all concentrations studied caused stimulation of testosterone synthesis, with a maximum effect (20% increase) in response to 1_O+3_F concentration (significant at $p<0.01$). It was interesting to find that the magnitude of stimulation on testosterone synthesis at 1_O+3_F was higher than that of either O alone at 1.0 μg/ml or F alone at 3.0 μg/ml. Thus, the stimulatory effects of O or F alone at the above concentrations appeared to have been intensified when used in combination.

Discussion

Effects of the 5α-reductase inhibitor, finasteride on androgen metabolism by fibroblasts

The strong inhibitory effects of finasteride on 5α-DHT synthesis from androgen substrates in periosteal fibroblasts indicate that 5α-reductase activity in periosteal fibroblasts can effectively be

inhibited by finasteride. Since finasteride is a specific inhibitor of the 5α-reductase type 2 isozyme associated with metabolic activity in target tissues such as the prostate, this confirms the presence of type 2 isozyme in the periosteum. This may suggest target tissue activity in the periodontium, associated with anabolic functions. The effects of finasteride on 5α-reductase activity in gingival fibroblasts have also been shown by our previous studies and were found to be in agreement with the present findings.[23,26,29]

The specificity of the anti-androgen, finasteride as a 5α-reductase type 2 isozyme inhibitor is further confirmed by the fact that there was no inhibition, but stimulation in the metabolic conversion of 14C-testosterone / 14C-4-androstenedione to 4-androstenedione / testosterone for which the enzyme 17β-hydroxysteroid dehydrogenase is responsible. Soory & Virdi [23] also reported that testosterone as the androgen substrate did not show much change in the metabolic conversion to 4-A in response to finasteride confirming that 17β-hydroxysteroid dehydrogenase activity is not influenced by finasteride. The same effects on the above enzyme activities by finasteride have been shown in clinical applications such as benign prostatic hyperplasia (BPH). When, a group of men with BPH were treated with finasteride for 12 months, significant decreases in serum DHT have been observed after 6 and 12 months of its administration, but no effect was seen in serum testosterone levels.[30]

Effects of finasteride in combination with oestradiol (O+F) on androgen metabolism by fibroblasts

5α-reductase activity in relation to O+F at any of the concentrations studied did not appear to be stimulatory for both androgen substrates (figures 1 & 3). This is inspite of the significantly increased 5α-reductase activity by oestradiol alone at all concentrations in relation to both androgen substrates. Thus the stimulatory effects caused by oestradiol at all concentrations on 5α-reductase activity were nullified by finasteride at different concentrations, showing an intermediate response to that of O or F alone.

The optimal concentration of oestradiol giving rise to a maximum degree of stimulation in 5α-reductase activity appeared to be different in relation to the two androgen substrates used. Similarly, the expression of 5α-reductase receptors in periosteal fibroblasts could depend on the availability of optimal, critical concentrations of oestradiol.

The fact that all three concentrations of O+F gave rise to increased 5α-reductase activity, compared to that of finasteride alone at the same concentrations, has also confirmed the presence of oestradiol-induced 5α-reductase activity. This effect has been shown in relation to both androgen substrates, 14C-T and 14C-4-A in periosteal fibroblasts. In a similar study, minocycline-induced stimulation of 5α-reductase activity in gingival fibroblasts by 14C-testosterone and 14C-4-androstenedione has been reported to be nullified by the presence of finasteride at concentrations of 0.1 and 1.0 μg/ml.[23]

In this study, O+F stimulated 17β-hydroxysteroid dehydrogenase activity in periosteal fibroblasts with 14C-T and 14C-4-A as androgen substrates (Figures 2 & 4). This has been demonstrated by the significantly higher yields of testosterone / 4-androstenedione in response to all concentrations of O+F used, compared with controls.

As a result of 17β-HSD activity, remarkable anabolic effects in androgen target tissues have been observed in other studies. One such study reported that after administration of finasteride to the patients with benign prostatic hyperplasia,

serum DHT was significantly decreased but there was an increase in the mineral density of the vertebral bones. This effect has been attributed to the unaltered levels of serum testosterone. [30] Since testosterone could be an active hormone participating in bone metabolism, 17β-HSD activity would be very important in androgen target tissues.

In conclusion, the specific inhibition of 5α-reductase type 2 enzyme activity by finasteride in periosteal fibroblasts is suggestive of the presence of target tissue synthetic activity in human oral periosteum. Stimulatory effects of oestradiol on the above enzyme activities indicate the hormone-mediated tissue responses during repair which could be applicable to the diseased periodontium. This study shows how the in-vivo hormonal factors could modulate the action of other hormones through the enzyme systems available in the periodontium itself, and to bring about favourable effects towards clinically relevant issues such as periodontal wound healing and repair.

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Rate of apposition of dentine in the rat molar tooth

A.K.S. Arambawatta, C.D. Nanayakkara, M. Chandrasekera[1]

Division of Anatomy and Dental Anatomy, Department of Basic Sciences, Faculty of Dental Sciences, [1]Department of Anatomy, Faculty of Medicine, University of Peradeniya

Objective: A study was carried out to find out the apposition rate of dentine in various anatomical regions in the rat molar tooth. ***Materials and methods***: Five male and five female Sprague-Dawley rats receiving a normal diet were mated. From the day of mating, a bone marker, Calcein (fluorescein methylene iminodiacetic acid – 30 mg/kg body weight) was injected intraperitoneally to the female rats at weekly intervals. The injections were then continued on the new born rats up to seven weeks and 10 male rats were sacrificed. Ground sections were obtained from the molar teeth of rats. They were viewed under a fluorescent microscope and the distance between fluorescent lines in six different regions of the tooth were measured using a calibrated eyepiece micrometer graticule. ***Results***: The mean values of the rate of dentine formation in the cuspal region, cervical margin, level of the middle 1/3 of the root, level of the apical 1/3 of the root, apical region and the interradicular region were 8.43 ± 4.79 μm, 6.25 ± 3.17 μm, 4.85 ± 3.74 μm, 8.28 ± 3.46 μm, 10.57 ± 2.82 μm, and 4.50 ± 3.41 μm, respectively. The highest rate of dentine apposition was observed at the apical region (10.57 ± 2.82 μm) and the lowest rate was at the interradicular area (4.50 ± 3.41 μm). ***Conclusion***: According to the findings of this study it can be concluded that the rate of apposition of dentine varies in different regions in the rat molar tooth.

Key Words: Dentine apposition, rate, rat molar tooth, fluorscent labelling

Introduction

Much has been documented on the incremental nature of dentine apposition since this was first described by Andresen[1] and then by von Ebner.[2] The longer period incremental lines described by Andresen appeared to vary in periodicity and also to some extent between different species of animals and also from individual to individual.[3]

On the basis of experimental studies conducted by Schour and his co- workers [4,5,6,7], Okada[8] and Bowman [9] it is generally accepted that the average rate of dentine apposition is about 16 μm per day for small animals and 4 μm per day for the macaque monkeys and man. However, Kawasaki et al.[10] reported that dentine apposition varied from tooth to tooth and that the process of apposition may be affected by the position and the age of the odontoblast.

Growth rate characteristic of hard tissues are of considerable practical importance to zoologists and forensic scientists. Previous studies have documented the use of incremental lines in dentine to assess the age of seals and the incremental lines in cementum to assess the age of deer populations. [11]

The estimation of age at death helps in the establishment of identity of an individual. According to Boyde[12] each cross striation in

enamel rod represents one daily increment of growth and he reported how age at death of young human skeletal remains could be estimated using the incremental markings in the enamel. Cameron and Sims[13] who tried to establish the age of a girl of 11 years accurately reported the inability to do so using the published rates of dentine apposition.

The present study sets out to determine how regular dentine apposition is likely to be in different sites within a tooth and also the rate of dentine apposition at different sites in a tooth.

Materials and Methods

Five male and five female Sprague-Dawley rats were obtained at the age of 70 days. The males and females were kept separately in a light and temperature controlled environment. The rats were fed a normal diet. When aged 100 days the rats were mated. From the day of mating an intravital bone marker, Calcein (Fluorescein methylene iminodiacetic acid – 30 mg/kg body weight) was administered intraperitoneally to female rats at weekly intervals These intraperitoneal Calcein injections were then continued on the newborn rats up to seven weeks.

Ten male rats were anaesthetized by administering Pentobarbital sodium (50 mg/ml, 1-2 ml/kg body weight, and 5-10 times diluted solution) and intra- cardiac perfusion was carried out for 20 min using 10% formal saline.

Ground sections were prepared from the second molar teeth by sectioning them axially in the bucco-lingual plane using a hard tissue microtome (Leica SP 1600). They were then ground to a final thickness of 30-80 μm using carborundum stones (1000, 600,400) and polishing sheets. The sections were mounted on glass slides using canada balsam. The ground sections were viewed under a fluorescent microscope and the distance between the fluorescent lines were measured using a calibrated eyepiece micrometer graticule. The measurements were taken from 6 different anatomical regions in the molar tooth, namely the cuspal region and the cervical margin of the crown of the tooth, interradicular, middle 1/3, apical 1/3 and apical region of the root (Figure 1-7).The distance between the fluorescent lines indicated the weekly rate of apposition of dentine.

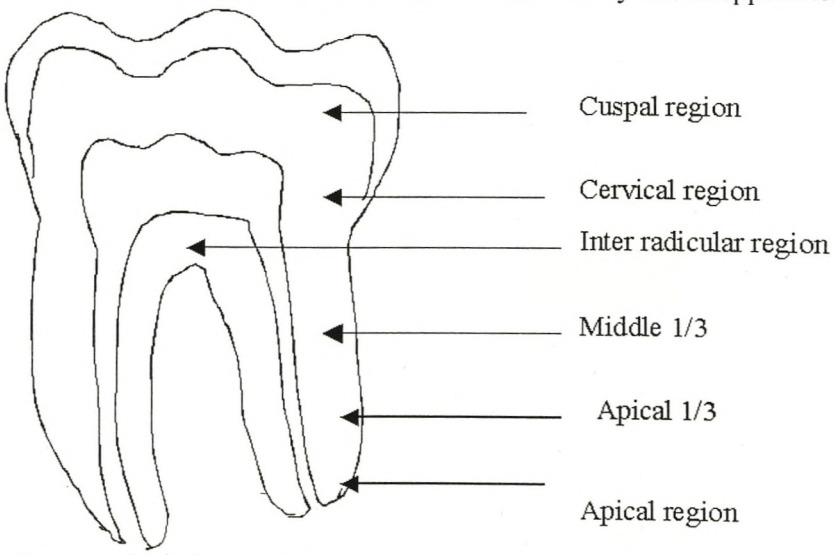


Figure 1. A diagram of a molar tooth showing the six regions in which dentine apposition was measured.

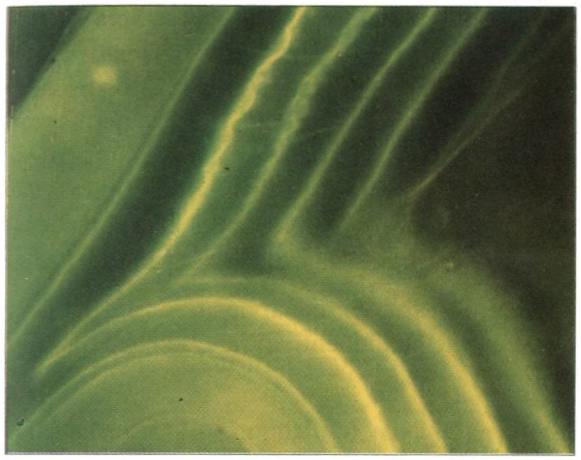


Figure 2

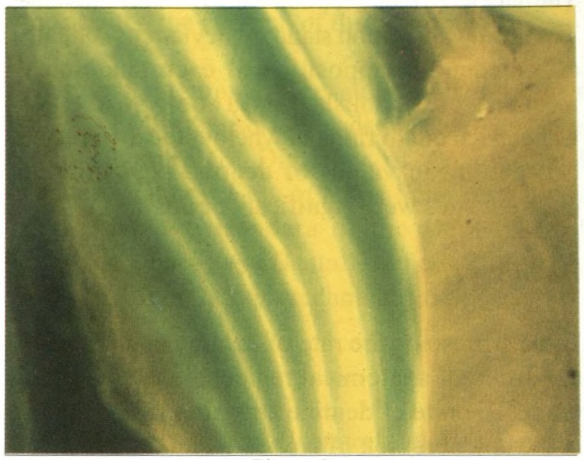


Figure 3

Figure 4

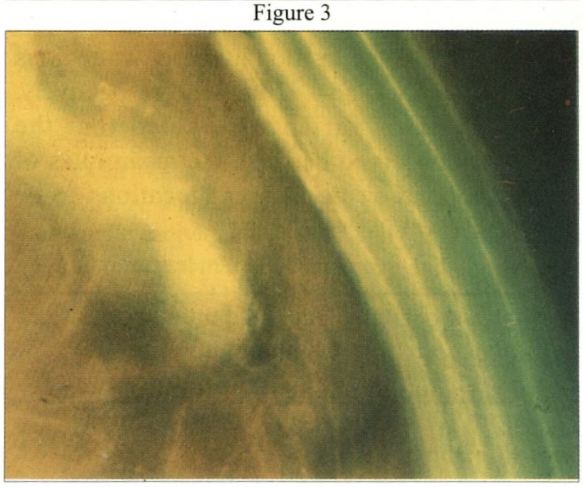
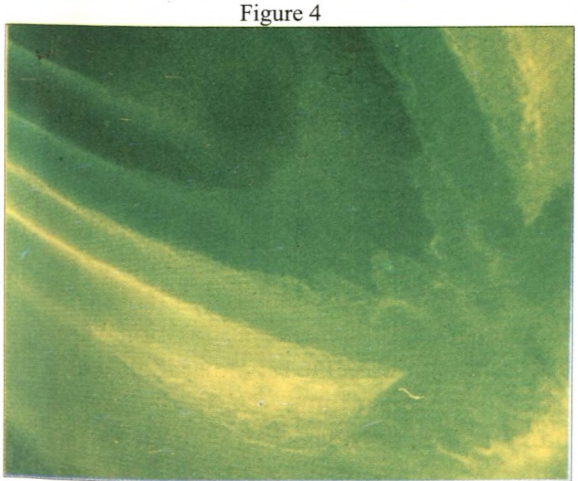


Figure 5



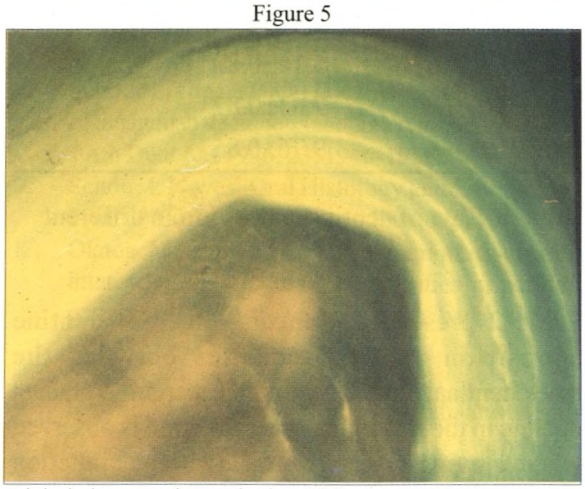


Figure 2,3,4,5,6and 7.Photomicrographs showing the fluorescent labels in cuspal, cervical, middle 1/3 of root, apical 1/3 of root, apical and interadicular regions

The data were analyzed using the SAS programme. The mean values (and standard deviations) of daily rate of dentine apposition at each anatomical location were established and a one-way ANOVA was carried out to find out whether the differences between mean values of dentine apposition at different sites were significant or not.

Results

Table 1 presents the rate of dentine apposition in the different anatomical regions of the rat molar tooth. The rate of dentine apposition was highest in the apical region of the tooth and lowest in the interradicular region of the root, and the mean values of dentine apposition in these two regions were 9.78 μm/day and 4.42 μm/day , respectively. The analysis of variance revealed that the differences observed between the mean rates of dentine apposition at different locations to be significant ($P<0.05$).

Anatomical region in the tooth	Rate of dentine apposition (μm per day) Mean±SD
cuspal	8.99± 1.42
cervical	6.94±1.42
inter. Rad.	4.42±0.75
cer 1/3	5.14±0.85
mid 1/3	7.63±0.87
apical 2	9.76±0.85

Table 1. Rate of dentine apposition in different regions in the rat molar tooth

Table 2 presents the mean values of dentine apposition at different sites between the amelodentinal junction and pulpal margin. A gradual decline in the rate of dentine formation is observed when moving from the amelodentinal junction towards the pulpal margin.

The time period	Rate of dentine apposition (μm per day) Mean±SD
1st week	12.85±1.07
2nd week	9.43±0.87
3rd week	7.71±0.57
4th week	8.57±0.81
5th week	7.37±0.62

Table 2. Rate of dentine apposition (μm per day) between the amelodentinal junction and the pulp margin

Discussion

On the basis of previous experimental studies, it is generally accepted that dentine apposition occurs at an average rate of 16 μm/day in small animals and 4 μm/day in human and monkey.[4,5]

The rate of dentine apposition has been described as very regular and of constant magnitude.[14] However the findings of Ahlgren[15] and Miller[16] are contradictory to the findings of Schour and Massler.[14] Ahlgren[15] and Miller[16] reported that the rate of dentine apposition varies at different sites in a tooth. The studies on dentine apposition by Schour and Hoffman[4,5], Ahlgren[15] and Miller[16] were conducted on the incisor tooth of the rat. The incisor tooth of the rat is a continuously erupting tooth and therefore dentine apposition occurs throughout life.

In the present investigation the studies on the pattern and rate of dentine apposition were carried out on the second molar tooth of the rat. The results of the present study (Table 1) reveal that the rate of dentine apposition varies in different regions in the rat molar tooth and is not of constant magnitude. These findings of the present study are consistent with the findings of Ahlgren[15] and Miller[16] on dentine apposition of the rat incisor tooth.

According to the findings of the present study on the rat molar tooth and the studies by Ahlgren[15] and Miller[16] on the rat incisor tooth, it can be concluded that in both molars and incisors the apposition of dentine is not regular and differs in different regions in a tooth. Therefore it is important to bear this fact in mind if age estimation at death is carried by means of dentine appositional rates. Cameron and Sims[13] who attempted to estimate the age of a girl of 11 years accurately reported the inability to do so using the published rates of dentine apposition.

The rate of dentine apposition was highest in the apical region of the tooth. Rapid dentine formation in this region may be necessary to bring about an elongation of the root and to increase the tooth height and thus enabling the tooth to erupt and reach its functional position in the oral cavity. A similar finding has been reported by Dean et al.[3] who studied the rate of tooth height increase. They showed a higher rate of tooth height increase during the period of root completion (13 and 26 μm/day) than in the period of early root formation(4 and 6 μm/day).

Another finding of this study was the decline in the rate of the dentine apposition as the odontoblasts move away from the amelo-dental junction towards the pulpal region. Perusal of literature reveals that such a finding with regard to dentine apposition in the region of the crown of a tooth has not been documented so far. As reported Ahlgren[15] and Kawasaki et al. [10] the secretory activity and the rate of migration of an odontoblast cell varies considerably during its life cycle. Odontoblast secretion and migration as a general rule start slowly, reach a peak and then slow up as dentine formation nears completion. It is also reported that the activity of an odontoblast varies with the type of tooth and its position of the tooth. The decline in the rate of dentine apposition from the amelodentinal junction towards the pulpal margin in the present study could be attributed to the age and the position of the odontoblast.

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Knowledge and attitudes of Sri Lankan dental students towards HIV infection and AIDS

A.N.B. Ellepola[1,2], R.L. Wijeyeweera[3], A.P. Weerakoon[4], S. Anil[1,5], L. P.Samaranayake[1]

[1]Oral Bioscience, Faculty of Dentistry, University of Hong Kong, Hong Kong [2]Department of Oral Medicine and [3]Department of Community Dental Health, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka, [4]Office of the Divisional Director of Community Health Services, Kandy, Sri Lanka, [5]Faculty of Dentistry, University of Kerala, Kerala, India

Abstract

Objective:The objective of the present study was to ascertain the knowledge and attitudes of dental students in Sri Lanka towards HIV infection and AIDS. ***Design of the study***: A questionnaire-based survey was conducted among final year dental students at the Faculty of Dental Sciences, University of Peradeniya, Sri Lanka to determine their knowledge and attitudes towards HIV infection and the issues it raises for them. ***Results***: Although the majority of the dental students were aware of the facts related to AIDS, the spread and oral manifestations of HIV infection, there were considerable gaps in their knowledge with regard to the virology and dental management of infected patient. ***Conclusion***: Further educational efforts on HIV infection / AIDS and its implication in routine dental practice should thus be directed towards dental students in Sri Lanka.

Key words: HIV infection, attitudes, knowledge, Sri Lankan dental students

Introduction

The pandemic of Human Immunodeficiency Virus (HIV) infection is now firmly entrenched in the South East Asian region. The World Health Organization has forecast that the Indian sub continent epidemic will overtake that of the African scenario in a few years.[1] This implies that as health care professionals, clinical dental students should be knowledgeable of the disease process, oral manifestations and modes of transmission appertaining to HIV infection. Furthermore, they should be familiar with preventive strategies and educating the general public.

Though there is a slim possibility of contracting / transmitting this infection through procedures adopted in routine dental practice[2], dental professionals show a disturbing reluctance to treat HIV-infected or AIDS patients.[3] Such attitudes seem to prevail globally despite explicit guidelines from various health care organizations[4,5], which stress the importance of nondiscrimination in the delivery of oral health care to these individuals.

There have been a few studies on the attitudes and knowledge of dental students towards HIV infection and AIDS in the West.[6-9] In contrast, there is no such information with regard to dental

students in Sri Lanka, a South Asian island. The Faculty of Dental Sciences of the University of Peradeniya is the only institution in Sri Lanka which train dental surgeons. The Final BDS (Bachelor of Dental Surgery) students of this faculty do treat patients under specialized supervision as part of their clinical training and may come across HIV-infected or AIDS patients during such procedures. Hence the main aim of the study was to assess the knowledge and attitude towards HIV infection and AIDS among Final BDS dental students.

Material and methods

A total of 150 clinical final BDS dental students from the Faculty of Dental Sciences, University of Peradeniya, Sri Lanka participated in the study. A structured pre-tested questionnaire [2,10], comprising 18 questions (single and multiple response), was issued to each participant without prior notice soon after a common gathering of dental students in a lecture theatre. The respondents were first assessed on their knowledge of HIV, its transmission, the risk groups, common oral manifestations, and source of information on HIV-infection and AIDS. Further, they were questioned on the seriousness of the disease, attitudes towards HIV-infected patients and cross infection protection implemented during routine patient treatment and management. The final group of questions was an attempt to obtain information pertaining to the experience with HIV-infected patients and its impact on staff and other patients.

Results

The response rate to the questionnaire was 100%. The likelihood of HIV transmission in the dental clinic was rated as very likely or likely by 44% and 39% respectively, whereas 8% and 9% thought this to be an unlikely or extremely unlikely occurrence.

A majority of the participants believed the following three diseases as the most common oral manifestations of AIDS from a list of eight possible options; namely Kaposis sarcoma (82%), oral candidosis (79%) and hairy leukoplakia (58%). The other diseases selected were herpes infection (52%), acute ulcerative gingivitis (49%), and aphthous stomatitis (29%). Only a few believed that, lichen planus (7%) and xerostomia (6%) to be of important manifestations in the mouth.

The majority of dental students were aware of the modes of transmission of HIV infection (Table 1). However a few ambivalent responses were received with regard to transmission via breast feeding, saliva, social kissing and insect bites.

With regard to the infectivity and virology of HIV, only 22% were aware that the simple disinfectants such as household bleach could inactivate contaminated spills whilst 48% were unaware of this fact. However, almost all respondents (98%) believed that the procedures used for preventing hepatitis B infection are appropriate for avoiding HIV infection. Similarly the majority of the dental students (78%) correctly affirmed that it might take a 2 month period for the appearance of HIV antibodies in blood whereas the rest either disagreed (17%) or did not know the answer (5%) to this statement. Further, the risk of HIV transmission via a contaminated needle stick injury was thought to be more than 1% by the majority (71%) of the respondents.

When the dental students were assessed on their awareness / knowledge of HIV infection, 93% believed they had adequate knowledge. The majority (88%) believed that scientific journals and text books based on AIDS were the most useful source of information on HIV infection and AIDS, while others believed that the latter could be achieved by attending seminars (53%), popular press (51%), television (33%), speaking to

Table 1: Dental students' knowledge about transmission of HIV infection expressed as percentage values based on the question " In your opinion the following transmits HIV infection"

Methods of HIV transmission	Yes	No	Don't know
Breast feeding	32	60	8
Insect bites	12	72	16
Saliva	33	35	32
Oral sex	95	3	2
Sharing cutlery	2	93	5
Sharing crockery	2	93	5
Sharing razors	85	3	12
Anal intercourse	100	-	-
Vaginal intercourse	100	-	-
Blood	100	-	-
Hairdressers	12	79	9
Acupuncture	91	1	8
Social kissing	7	91	2
Swimming pool	2	97	1

specialists (26%) and speaking to colleagues (21%).

On the seriousness of the AIDS pandemic, the majority of the students (89%) believed this to be a serious threat to public health of the country while the rest surmised the problem to be either real but limited, or exaggerated out of proportion.

With regard to routine clinical practice all students (100%) as part of the general regulations of the faculty used a standard health history form for their patients. The majority of the students used gloves (93%). The other barrier devices used by the respondents included masks (68%) and eye protection (33%). The majority of the students were aware of the high-risk population groups where extra precautions have to be taken. These include migrant workers (97%), homosexual males (98%), drug addicts (97%) and haemophiliacs (96%). Further a few (33%) believed that family and close friends of AIDS patients should also be treated similarly (Table 2).

In relation to treatment of HIV-infected patients none of the students believed that they had treated HIV-infected or AIDS patients. A few respondents (23%) have discussed the problem of AIDS with the academic staff and the ancillary staff of the Faculty. A minority of the students (41 %) said their patients have expressed concern about sterilization procedures and contracting AIDS during routine dental procedures. An overwhelming majority (93%) contended that all HIV-infected patients should be reported to a government body and should carry an identity card (71%) detailing their health status.

Finally, the majority of the dental students (68%) felt that there should be an obligation by the dental

Table 2: Risk groups and use of extra precautionary measures in dentistry expressed as percentage values based on the question " For whom of the following would you use extra precautionary measures in dental practice "

Risk groups	Yes	No	Don't know
Migrant workers	97		3
Homosexual males	98	1	1
Haemophiliacs	96	3	1
Drug addicts	-	97	3
Family and close friends of AIDS patients	33	13	54

student/ surgeon and supporting staff to disclose their HIV status and 97% believed that being HIV positive is not an acceptable reason for dismissal. Furthermore, in the case of an HIV-positive dental student / surgeon, 52% felt that he/she should be allowed to practice while 43% were opposed and 5% undecided on the issue.

Discusion

The overwhelming majority of the dental students (83%) believed that the likelihood of HIV transmission in clinical dentistry is likely/very likely. However in reality the likelihood of HIV transmission is extremely low as shown by a single dental practitioner who is reported to have acquired the infection in a clinical setting[5] and another report where a dental practitioner has been implicated in its transmission to a patient.[11] On the other hand 17% of the students still considered it as an unlikely scenario. As the dental students must know the true picture and not have a vague knowledge (i.e. very likely, likely, unlikely), this is rather disturbing considering the unabated global spread of the disease With regard to the general clinical knowledge on oral manifestations of HIV infection it is comforting to note that a majority of dental students appear to be aware of these manifestations. Similar results have been observed in previous studies involving dental practitioners in South Africa and India.[2,10]

With regard to the infectivity and virology a number of dental students were uncertain as to the risk of HIV transmission via different routes/ sources. For instance, although the majority surmised that the risk of HIV transmission via a single, contaminated needle stick injury is more than 1%, a figure of 0.4% is widely regarded as the likely estimate.[12] Further the fact that some of the respondents thought that insect bites, breast feeding, saliva and social kissing may act as an HIV source and that family and close friends of HIV infected AIDS patients should be treated with extra precautions reflect the wrong information and knowledge that the dental students have acquired. Thus, the current policy of universal infection control[13,14,15] should be made available to all dental health care workers to ally fears and minimize confusion and discriminatory treatment during dental care of HIV-infected individuals.

With regard to routine clinical practice it is heartening to note that all students as part of the general regulations of the faculty maintained a standard history form of their patients. Further, it

is comforting to note that 93% of the students used gloves in routine dental practice. However, the use of other barrier devices was relatively less. This approach is not encouraging. Universal cross infection protections should be routinely used and all patients considered infectious.[13-16] It should also be noted that a substantial proportion (41%) of dental students were questioned by patients on sterilization procedures and contracting AIDS during dental treatment. This is encouraging and agrees with studies in other parts of the world with regard to public perception of cross infection prevention in dentistry.[17]

Dental students in the survey population had a liberal attitude towards supporting staff that could be HIV positive as the majority (97%) felt that HIV carriage is not an acceptable reason for dismissal. Almost half of the students concurred that HIV seropositive dental surgeons may continue to practice while the rest disagreed or were undecided on this issue. While a number of authorities agree that the ethical and moral responsibility of HIV-infected dental surgeons should be to obtain medical advice frequently and to change the practice accordingly for the benefit of their patients[18,19], others have suggested that HIV-infected health care workers should refrain from undertaking invasive procedures or should make patients aware of their seropositive status.[20] However, a consensus on this subject does not appear to be emerging as yet and until then it would be salutary to adhere to local guidelines if and when such situations are encountered.

In conclusion, the current results are in many respects similar to those of other studies among dental health care workers[2,10] wherein a number of gaps in the attitude and knowledge on HIV infection have been demonstrated. This is the first survey attempting to ascertain the attitudes and knowledge of dental students in Sri Lanka towards HIV infection and AIDS. However, the survey results cannot be extrapolated to the dental community in Sri Lanka at large. Despite this caveat it is hoped that our survey has stimulated dental students to seek answers to the gaps in their knowledge and to further their educational efforts towards HIV infection in general and infection control in particular.

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REVIEW ARTICLE

Modifications to the original method of Kimura and Pearsall in adherence of Candida species to human buccal epithelial cells

[1] J.G . Panagoda and [2] L.P.Samaranayake

[1]Division of Microbiology, Faculty of Dentistry, University of Peradeniya, Sri Lanka

[2]Oral Bio-Science Laboratories, Faculty of Dentistry, University of Hong Kong, Hong Kong.

Abstract

Candida species cause diseases such as oral thrush, fungaemia, endocarditis, dermatitis and ocular infections. Adherence of these species to biological surfaces such as buccal epithelial cells (BECs) of the host is an important step in colonization and pathogenesis. The method of Kimura and Pearsall[19] has been widely used in most of the adherence studies involving *Candida* species. However, there has been a number of modifications to the original method of Kimura and Pearsall[19] thus resulting in erratic adherence results. Therefore, a review was carried out using the Medline survey (1978-1998) to evaluate these modifications. The information has revealed a wide changes to the original method of Kimura and Pearsall[19] with respect to the quality of BECs, growth conditions and *Candida* species / strain variations.

Key words: adherence, buccal epithelial cells, *Candida*, modifications

Introduction

Candida albicans is the most important unicellular fungal pathogen that causes human infections.[1] However, the clinical importance of *Candida* species other than *C. albicans* has also been demonstrated by many research workers. Kiehn et al.[2] have shown that 32 % of *Candida* species isolated from cancer patients constituted species other than *C. albicans.* Investigations of Horn et al.[3], Cooper et al.[4], Beck-Sauge et al.[5] involving immunocompromised hosts, diabetics, neonates and surgical patients have indicated that about 20 - 40 % fungal infections are due to emerging *Candida* species. *C. parapsilosis* is such an emerging fungal pathogen and has been frequently isolated from a number of superficial and systemic infections of man.[6-7]

There has been a growing interest in studying the virulent factors of *Candida* species.[8-9] Thus a large number of investigations have focused on the adhesion of *Candida* species to human buccal epithelial cells.[10-18], Interestingly, the method of Kimura and Pearsall [19] has been used to evaluate the adherence of *Candida* species to human BECs. However, in most of these studies there have been a number of modifications to the original method of Kimura and Pearsall.[19] Therefore, the main aim of this review is to evaluate the *in vitro* modifications to the method of Kimura and Pearsall[19] in adherence of *Candida* species to human BECs.

Modifications

The degree of adherence of *Candida* species to biological surfaces has been studied using eukaryotic cells such as BECs, cultured cell lines (e.g. HeLa cells and keratinocytes).[20] Of these the BECs adherence assay first described by Kimura and Pearsall [19] has been the most popular and a large number of investigators have used this *in vitro* adherence method (Table 1). However, there have been many modifications to the original method of Kimura and Pearsall [19] (Table 1). This has resulted in reports with wide variations in reported adherence values of *Candida* species (especially of *C. albicans*) to human BECs (Table 2). The reasons for such variations could also be attributed to differences in the *Candida* strain or phenotype, the quality of BECs, the assay medium and the variations in other conditions used in the study (e.g. temperature). Furthermore, modifications in the technical details of the assay method such as the volume of the washing buffer and the rate of washing are thought to contribute to erratic results. A review of these in the context of the current investigation is given below.

Table 1 Experimental conditions used in the *in vitro* BEC adherence assays of *Candida* species

Author	*Candida* Species	Year	BECs	Yeast Concentration /ml	Assay medium (PBS)	Growth medium	Incubation period (hrs)	Washing frequency	PBS (ml)
Kimura and Pearsall[19]	CA	1978	A/P	1×10^8	pH7.2/0.01M	SDA/SDB	1-3	once	70
Kimura and Pearsall[21]	CA	1980	A/P	1×10^8	pH7.0/0.01M	-	1-3	once	70
Samaranayake and MacFarlane[22]	CA	1982	A/P	4.3×10^7	pH7.2/0.1M	L/G/X/M	1	twice	70
Critchley and Douglas[23]	CA/CP	1985	-	1×10^7	pH7.2/0.15M	Gl	45 min	-	-
Barrett-Bee *et al.* [24]	CA/CP	1985	A/SD	1×10^7	pH7.2/0.15M	-	50 min	-	-
Brenciaglia *et al.* [25]	CA	1986	A/P	1×10^7	-	-	1	once	15
Ghannoum *et al*[1]	CA	1986	A/P	1×10^7	*pH7.0	-	2	twice	5
Tobgi *et al.*[14]	CA	1987	C/P	1×10^7	pH7.2/0.1M	-	1	once	60
Tobgi *et al.*[14]	CA	1987	A/SD	1×10^7	pH7.2/0.1M	-	1	once	60
Tobgi[26]	CA/CP	1989	A/SD	$4.0\text{-}4.5 \times 10^7$	pH7.2/0.15M	S	1	once	50
Darwazeh *et al.* [15]	CA	1991	A/P	1×10^7	-	S	1	five	6
Darwazeh *et al.* [27]	CA	1994	A/P	-	pH7.2/0.1M	-	1	five	6
Samaranayake[28]	CA	1995	A/P	1×10^7	pH7.2/0.1M	-	1	five	6
Darwazeh *et al.* [27]	CA	1997	A/P	1×10^7	pH7.2/0.1M	RPMI	1	five	6
Tsang [29]	CA	1998	A/P	1×10^7	pH7.2/0.1M	-	1	once	70

CA - *C. albicans*, CP - *C. parapsilosis*, A - adult, BHI - brain heart infusion, C - children, - Not known, PBS - Phosphate buffered saline, * Hanks balanced solution (HBSS), P - pooled, SD - single donor, SDA - Sabouraud's dextrose agar, SDB - Sabouraud's dextrose broth, RPMI - an enriched medium, L - lactose, X - xylitol, S - sucrose, Gl - glucose, G-galactose. A constant temperature of 37 ^{0}C has been used in all the experiments.

Table 2 Adherence of *C. parapsilosis* and *C. albicans* isolates to BECs of various human donors.

Author	Year	Source	Number of isolates		*C. parapsilosis* per 100 BECs	*C. albicans* per 100 BECs
			C. parapsilosis	*C. albicans*		
Kimura and Pearsall[19]	1978	Oral	ND	3	-	134.60
Kimura and Pearsall[21]	1980	Oral	ND	2	-	288.00
Samaranayake and MacFarlane[22]	1982	Laboratory	ND	2	-	147.00
McCourtie and Douglas [17]	1984	Laboratory	ND	9	-	111.00
Critchley and Douglas[23]	1985	Laboratory	1	1	1088.00	184.00
Barrett-Bee *et al.* [24]	1985	Laboratory	1	4	117.00	854.00
Brenciaglia *et al.* [25]	1986	Septicaemic	ND	2	-	268.30
Tobgi *et al.* [14]	1987	Oral	ND	1	-	12.70
Tobgi *et al.* [14]	1987	Oral	ND	1	-	42.70
Ghannoum *et al.* [1]	1988	Laboratory	ND	2	-	328.00
Tobgi[26]	1989	⊗	5	22	262.00	988.00
Darwazeh *et al*[15].	1991	Oral	ND	1	-	119.00
Samaranayake *et al.* [28]	1994	OralOral	ND	1	-	110.00
Darwazeh *et al.* [27]	1994	Oral	ND	1	-	215.00
Samaranayake *et al.* [28]	1995	Oral	ND	5	-	155.00
Darwazeh *et al.* [27]	1997	Oral	ND	1	-	313.00

ND- not done, ⊗ - *C. albicans* from oral / vaginal sources and *C. parapsilosis* origin not known, θ - *C. albicans* from oral sources and *C. parapsilosis* from oral / aural / wound / skin sources

Human BECs have been chosen for *in vitro* adherence experiments of *Candida* species by the vast majority of investigators as they are the natural surface for yeast attachment in the oral cavity. On reviewing these adherence experiments conducted from 1978 - 1998, a significant variation in the quality of BECs used was observed. Kimura and Pearsall[19,21], used BECs of both adults and children, whereas others have used adult BECs only.[14, 24, 26] Pooled BECs of adults have been used in the *Candida* adherence studies of Kimura and Pearsall.[19,21] Further, studies have demonstrated that adhesive properties of BECs usually differ from donor to donor.[30] Kennedy [20] in an extensive review showed that the variation in adherence of *Candida* species to BECs could be reduced by pooling BECs of different donors, thus substantiating the work of Kimura and Pearsall.[19,21] However, Barrett-Bee et al.[24], Tobgi et al.[14], Tobgi[26] have used BECs of a single donor in their adherence studies contrasting the conditions of Kimura and Pearsall.[19,21]

Kennedy and Sandin[30] suggested that the adherence of *Candida* to BECs could be influenced by the assay medium used. Phosphate buffered saline (PBS) has been used in most of the experiments for this purpose.[19,21,25] However, sterile Hanks balanced solution (HBSS) has been used in the study of Ghannoum et al.[1]

It has been reported that the growth conditions of the culture modulate the *in vitro* adherence of *Candida*.[10,19,22,23,30,31] Thus Kimura and Pearsall[19] and King et al. [10] demonstrated greater adherence of *Candida albicans* to BECs at 37 ^{0}C than at 25 ^{0}C and most investigators have used the former temperature.

On evaluating the influence of different growth media on the adherence of *C. albicans* to BECs Kennedy and Sandin[30] demonstrated optimum adherence of *C. albicans* when cells were grown in chemically defined media rather than in complex media further confirming the growth media used by Kimura and Pearsall.[19,21]

There is no information on the effect of the volume of the buffer and the number of steps involved in washing of filters on the final count of adherent yeasts. Hence, various researchers have used different volumes of washing fluid, and repetitive washing to remove the non-adherent yeasts from the BECs surfaces. However, Kimura and Pearsall[19,21] used a single step washing technique which yielded reproducible results.

Another important feature which affects results of adherence studies is the origin of the *Candida* isolates used in the investigations.[13, 17, 26] Table 2 presents the details of the origin of the *C. parapsilosis* and *C. albicans* isolates used in previous adherence studies reported in the literature. These strains (in the reviewed studies) have been isolated from different regions of the world and it is possible that variation in BEC adherence of these isolates could be due to this geographic diversity. Interestingly, clinical isolates of *Candida* have been utilized in most of these investigations and it has been demonstrated that they adhere better than the laboratory grown isolates.[23]

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CASE REPORTS

Oral manifestations of cyclic neutropenia

S.P.A.G Ariyawardana
Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya

Introduction

Cyclic neutropenia is a rare haematological disorder of unknown etiology.[1-5] Human cyclic neutropenia was originally described by Leale, in 1910. The disease is characterized by a severe but transient neutropenia that recur approximately every 21 days[5] and during the period of remission the neutrophil count remains slightly lower than normal.[3] Compensatory monocytosis commonly occurs during the neutropenic phase.[2] Further, cyclic reduction of platelet and reticulocyte numbers also may occur.[4]

Cyclic neutropenia occurs both as an adult and a childhood onset disease with equal gender distribution.[4] Adult onset disease is an acquired condition characterised by increased numbers of large granular leucocytes (LGL) whereas the childhood onset disease has normal LGL counts.[6] Regulatory abnormality affecting blood cell formation at the pleuripotent stem cell level has been regarded as the underlying mechanism for congenital or childhood onset cyclic neutropenia.[7,8,9] During the neutropenic episodes patients experience malaise, anorexia, fever, lymphadenopathy, oral ulceration and pharyngitis.[4,7,8]

Oral ulceration and periodontal disease are common clinical manifestations and therefore the dentist may be the first person to whom a patient presents with cyclic neutropenia. Gingivitis and periodontitis are commonly seen in cyclic neutropenia.[10] The clinical course is usually benign compared with other forms of neutropenia.[4] However, there have been some serious consequences and Palmer and colleagues[5] in 1996 reported the death of 4 children due to clostridial or *E. coli* colitis in his study of 9 families. The diagnosis is not obvious at the time of presentation with the manifestations of cyclic neutropenia. However, the recurrent nature of the disease with characteristic periodicity and concomitant neutropenia will make the diagnosis easier.

A case of cyclic neutropenia with oral manifetations is presented in this case report.

Case Report

A 15-year-old Italian male was referred to the Oral Health Unit at Auckland Hospital, Auckland, New Zealand by a general practitioner. The medical records available with him revealed that he was having a neutropenia, which appeared to be congenital and cyclical in nature. About a decade ago he had been investigated at the Royal Alexandra Hospital for Children in Sydney, Australia, and the presence of cyclic neutropenia had been confirmed. None of his two elder sisters suffered from the disease. His father had maturity onset diabetes mellitus and was on oral hypoglycaemic agents. His mother was in good health. His complaint was the presence of recurrent mouth ulcers and the reddened gums. There was also a description of white plaque like areas in the mouth. His problem had recurred

several times. The ulcers were found to be painful and there was bleeding from the gums on brushing teeth. Every two to three weeks he has had a week-long course of acyclovir for herpetic infection in the mouth. He had otherwise been generally well with no other problems. He was 73 kilograms in weight and had a good appetite.

At the time of examination there were extensive oral ulcers involving the left lateral border and the dorsum of the tongue. The ulcers were round or ovoid ranging in size from 2 to 8 mm in diameter with irregular margins and erythematous halo (Figure 1). The attached gingivae were erythematous with some ulceration at the margins (Figure 2). There was substantial amount of plaque which had added to the inflammatory process in the gingiva. In general, his oral hygiene was not satisfactory and halitosis was present. The tonsils were enlarged and erythematous (Figure 3). The soft palate and the pharyngeal wall were also erythematous but there was no evidence of any candidal infection. There was non-tender submandibular lymphadenopathy. He was otherwise healthy looking and afebrile. There was no hepatosplenomegaly.

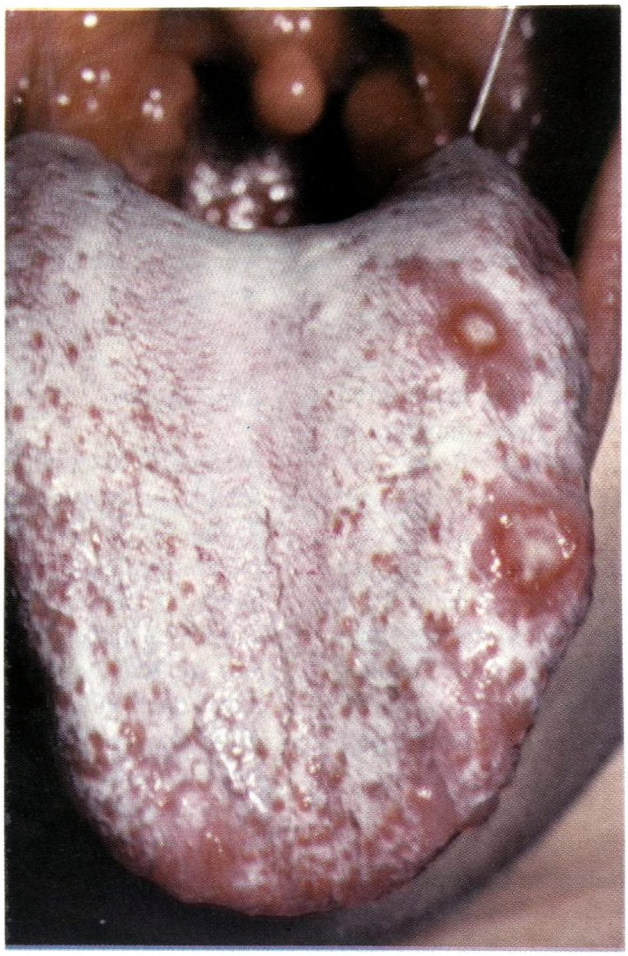

Fig 1. This picture shows ulceration in the dorsum of the tongue with sloughy floor and erythematous margin.

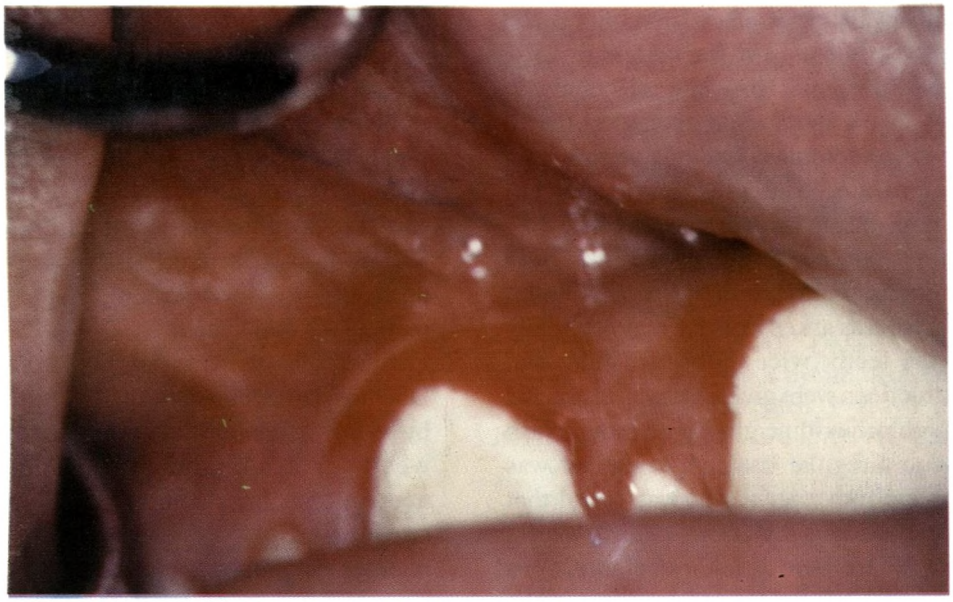

Fig 2. This picture shows erythematous attached gingiva with ulceration at the margin.

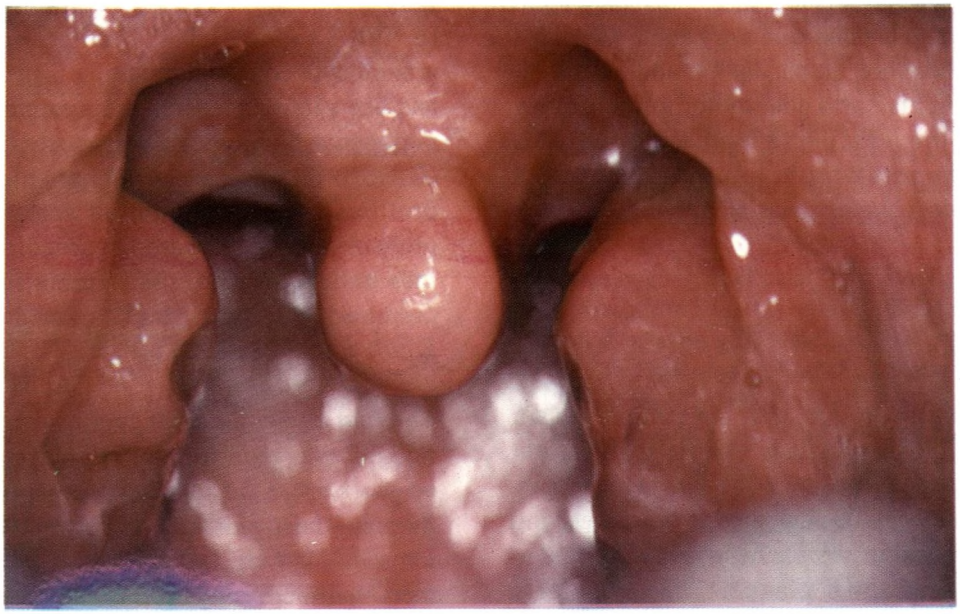

Fig 3. This picture shows enlarged tonsils and inflamed mucosa

The patient was referred to a haematologist concomitantly by the same general practitioner and was seen at the Diagnostic Haematology Unit, Auckland, New Zealand. His white cell count was $3.4x10^9$/L with a moderate neutropenia at $1.09x10^9$/L. Other haematological findings were within the normal range. The oral features were consistent with those known to occur in cyclic neutropenia. An oral hygiene program was instituted commencing with thorough dental prophylaxis. He was asked to use dental floss daily and brush the teeth twice daily. Brushing and flossing instructions were given. He was instructed to use 0.2% chlorhexidine gluconate mouthwash twice daily on a regular basis. The patient was reviewed one month later and it was found that the oral hygiene was satisfactory and the ulcers were healing. There were no additional infections seen in the mouth. The enlarged tonsils and the submandibular lymph nodes did not show any remarkable change.

Further follow up was impossible since the patient had to return to his home country at the end of his holidays. Therefore, the patient was advised to visit his dentist in his home country for further follow up. He was provided with a letter addressed to his dentist to ensure that his dental condition remained stable and oral hygiene was maintained to a high standard.

Discussion

Painful oral ulceration was the patient's main concern. The patient was unable to maintain adequate oral hygiene due to the pain in the mouth. His gingivitis may be attributed to the underlying neutropenia and it was further aggravated due to the accumulation of plaque. The oral ulcers resembled recurrent aphthous ulceration. However, it is unusual to find aphthous ulcers on the dorsum of the tongue.[11] Although recurrent oral ulcers are seen in primary and secondary immunodeficiency disorders, it is considerably rare to find immunodeficiency in most patients with oral ulcers.[12] Very extensive oral ulceration also has been reported as a manifestation of cyclic neutropenia.[1,13] Periodontal destruction could be so rapid in patients with cyclic neutropenia that it can lead to premature exfoliation of teeth.[10,14] Therefore, it is mandatory to prevent rapid destruction of periodontal tissues by ensuring meticulous oral hygiene.

Diagnosis of cyclic neutropenia can be made only by serial differential counts at least three times per week for a minimum of 6 weeks.[9] Cyclic neutropenia has been treated with low dose corticosteroids[15,16] or cyclosporin.[17] These regimes were found to be successful in adult onset type. However, childhood onset type has not been found to respond to these medications or other interventions including splenectomy, androgens, lithium and plasmapheresis.[4,18]

In the recent times recombinant human granulocyte colony stimulating factor (rhG-CSF) has been introduced for the treatment of cyclic nutropenia. Granulocyte colony stimulating factor (G-CSF) is a human hormone which stimulate proliferation, differentiation and activation of progenitor cells of neutrophil granulocyte lineage into mature neutrophils. Human rhG-CSF is produced by recombinant DNA technology using *E.coli* and mammalian cells.[19]

The first attempt to treat cyclic neutropenia with rG-CSF was reported in 1989 by Hammond and co-workers.[8] During the course of treatment, there has been a dramatic improvement in symptoms and signs of clinical illness.[7,8] Subsequently there was increasing evidence to support the success of the treatment with rG-CSF.[20,21,22,23]

From a dental point of view, careful dental and

periodontal care and timely antibiotic treatment are the mainstays of management. There should be particular care in managing dental problems in patients with cyclic neutropenia. Dental treatment, which involves invasive procedures, should be avoided during the episodes of severe neutropenia. Prophylactic antibiotic cover must be given prior to such a procedure.[24] It is utmost important to maintain high standards of oral hygiene to prevent the onset and perpetuation of periodontal disease and to prevent infection of oral ulcers. Thereby, the discomfort experienced by the patients can be minimized.

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Cutaneous leishmaniasis: A newly emerging endemic disease in Sri Lanka

MAM Sitheeque, NN Baheerathan[1], AK Suraweera[2]
Departments of Oral Medicine and [1,2]Oral Pathology, Faculty of Dental Sciences, University of Peradeniya.

Introduction

Leishmaniasis is a group of tropical and subtropical diseases caused by protozoan parasites belonging to the genus *Leishmania*. These parasites have two stages in their life cycle, a non-flagellate *amastigote* stage in vertebrate hosts such as humans, dogs and rodents and a flagellate *promastigote* stage in a female sandfly belonging to the genus *Phlebotomus* or *Lutzomyia*. The two stages are linked when the sandfly vector feeds on the blood of the vertebrate host. In humans leishmaniasis occurs in three forms: the systemic and often fatal visceral leishmaniasis, the destructive mucocutaneous leishmaniasis (espundia) prevalent mainly in Central and South America and cutaneous leishmaniasis (CL). CL itself is subdivided into 'cutaneous leishmaniasis of the old world' and the 'new world leishmaniasis'. The form of CL depends on the species of parasite involved, the geographical area of the world, and the host response. None of the three forms of leishmaniasis had been encountered in Sri Lanka until reports of cases of CL appeared in the very recent past, although visceral and cutaneous leishmaniasis are endemic in neighbouring India.

CL of the old world, also known as 'oriental sore', is a well-known skin infection endemic in the Middle East, Mediterranean litoral states, Sub-Saharan Africa, Central Asia, Pakistan and India. The infection is caused by one of three species of *Leishmania*, viz *L.tropica*, *L.major* and *L.aethiopica*. The species predominantly associated with the disease in the Middle East, Pakistan and India is *L.tropica*. The species of *Phlebotomus* that are associated with the spread of CL are *P.papatasii* and *P.sergenti*. However, these species of sandfly are not known to exist in Sri Lanka although *P. argentipes*, the species linked to visceral leishmaniasis is known to exist in the country. Sandflies are poor flyers inhabiting small crevices and found mostly in shrub jungle areas.

A skin lesion of CL appears at the site of the sandfly's bite following an incubation period of about 2 or 3 months. Initially an indurated, indolent and painless papule appears and progresses slowly to a nodule and then to a plaque. The site then becomes ulcerated and if untreated will slowly heal under a scab leaving a depressed scar in the end.

The disease was virtually unheard of in Sri Lanka until 1990 when two cases of CL were reported as imported disease in Sri Lankans who acquired the disease during their employment in Africa and the Middle East.[1] However, Atukorale et al .[2] reported the first case of indigenous CL from the arid zone of Southwestern Sri Lanka. A second case of a locally acquired infection was then documented from Central Sri Lanka by Seneviratne et al. [3] Seneviratne[4] has recently reported few more cases from the Uva province of Sri Lanka in an oral communication. We now report another locally acquired case of CL encountered in 1998 from the Polonnaruwa district of the North-central province of Sri Lanka raising the

possibility that the disease is probably more widespread within the country than it would seem from the above two reports.

Case Report

A 13- year old schoolgirl from the North-Central provincial town of Polonnaruwa presented at the Oral Medicine Clinic of the University Dental Hospital, Peradeniya, in September 1998 with a non healing and partially ulcerated nodular swelling on her upper lip that was present for about six months. The lesion was totally asymptomatic and she appeared healthy otherwise. She had never left Sri Lanka and had always lived in Polonnaruwa. Before she presented at our clinic the patient had undergone various investigations including a VDRL test and had received a variety of treatment. The treatment she had received included topical steroids and intralesional methyl prednisolone with no improvement. The patient and her accompanying relative were not aware of any occurrence of similar lesions in people in their neighbourhood.

Examination revealed an ulcerated nodular lesion that measured approximately 2cm x 1.2cm on the left upper lip vermilion just crossing the mucocutaneous margin. (Figure 1) The surface had a dark red crust covering the ulcerated area for most part with scaly keratosis on the edges. No exudate was observed and palpation revealed mild induration but no tenderness. No regional lymph node enlargement was found.

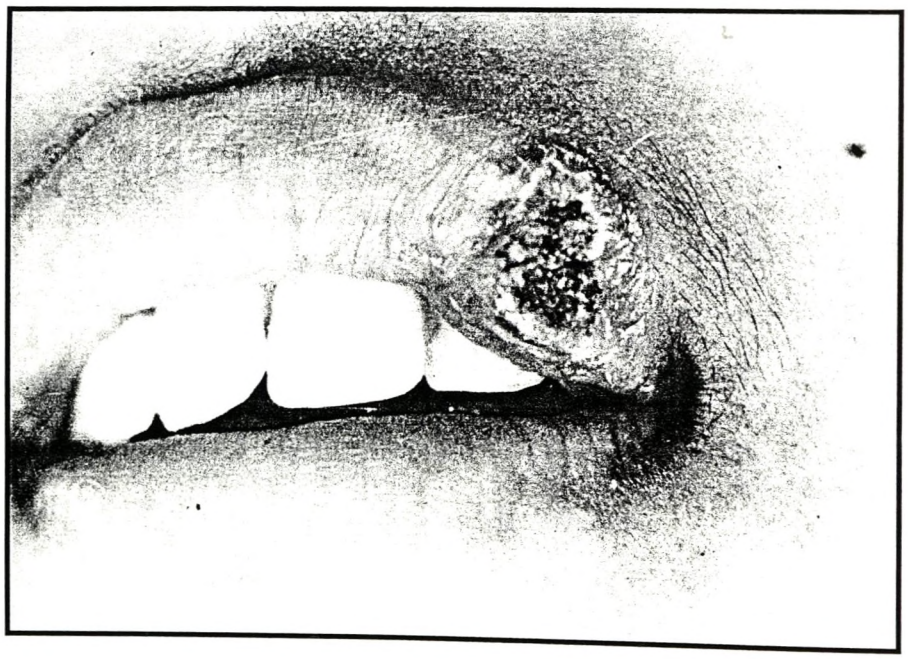

Figure 1. This picture shows the ulcer on the left upper lip

Investigations and diagnosis

CL was suspected from the long history and the characteristic clinical features. Microscopy of a smear treated with Giemsa stain showed many macrophages heavily laden with the typical intracellular *amastigote* forms of the leishmania organism confirming the clinical diagnosis (Figure 2). Histopathological study of a biopsy sample revealed identical findings. Attempts to culture the organism on Novy-Nicole-McNeal medium at the Department of Parasitology, Faculty of Medicine, Peradeniya,[5] proved unsuccessful. A full blood count and erythrocyte sedimentation rate were found to be within normal limits. When the lesion occurs on the face, clinically it may resemble a basal cell carcinoma, or a lesion of lupus erythematosus, lupus vulgaris etc. A lip lesion may mimic squamous cell carcinoma, keratoacanthoma or syphilitic chancre.

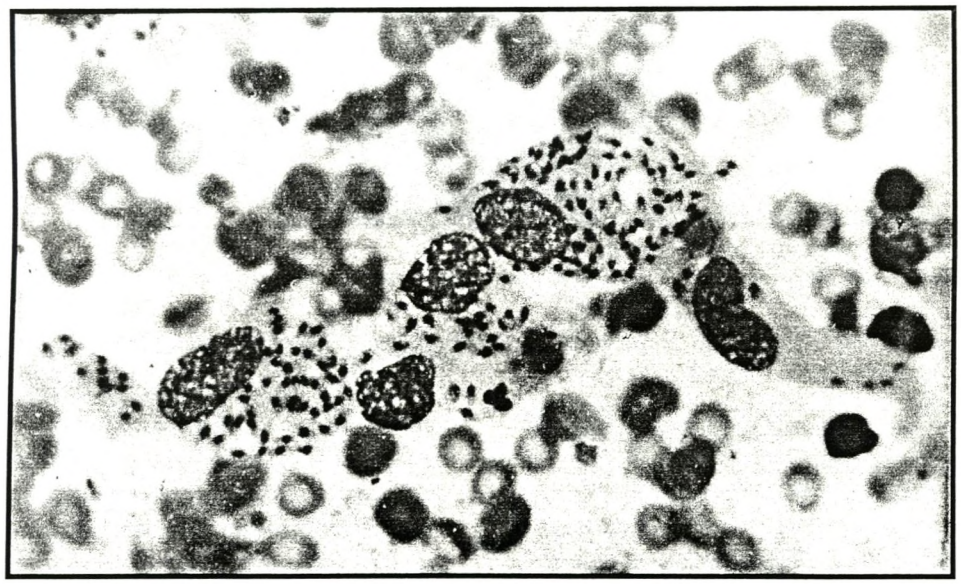

Figure 2. This picture shows the smear treated with Giemsa stain showing many macrophages heavily laden with the typical intracellular *amastigote* forms of the leishmania organism

Treatment

Although most cases of cutaneous leishmaniasis can be expected to heal spontaneously, aided by the patient's cell mediated immunity, this patient was treated with cryotherapy in view of the long duration and the intralesional and topical steroid treatment she had received. The lesion was subjected to two applications of a nitrous oxide cryoprobe (at -70 °C), each of 45 seconds duration, with a complete thaw between the two applications. After two weeks, only a small nodular area at the periphery remained and it was subjected to another 45 seconds application of the cryoprobe. When reviewed a further two weeks later the lesion had completely healed. The patient was thereafter followed at monthly and two monthly intervals. The lesion had not recurred and remained healed at the end of twenty four months. A mildly depressed scar was found at the site of the healed lesion (Figure 3).

Figure 3. The picture showing the site of the lesion after treatment

Discussion

This report is one of the few cases of autochthonous cutaneous leishmaniasis in Sri Lanka reported within a period of six years. It was only as recently as in 1990, that Naotunne et al[1] had asserted that CL is an imported disease in Sri Lanka while reporting the disease in two locals returning from employment abroad in endemic countries. However the present case together with the other cases of locally acquired CL referred to above show that the disease could no longer be considered an imported disease, although it must be conceded that none of the species of *Phlebotomus* responsible for the spread of CL is known to exist in this country.[3]

Our case comes from a province that is geographically remote from the localities of the previous reports of the infection in the country. Moreover, no case of cutaneous leishmaniasis has been reported from this location previously. Thus our report heightens the need to intensify epidemiological and parasitological study of CL in Sri Lanka. This would entail the identification of the species of leishmania responsible for the local CL infection, the sandfly species associated with the transmission of the infection and possible zoonotic reservoirs of the parasite. Seneviratne et al.[3] suggest that the locally acquired cases could probably be the result of the recently imported parasite becoming established in local cycles or alternatively species of *Phlebotomus* previously not linked to CL may be acting as vectors. The same authors further suggest that the nutritive requirements of the local strains of the parasite could be different and the failure to culture the organism could probably be due to these requirements being not met. From an epidemiological standpoint this case and the other recently reported cases of indigenously acquired cases of CL could alter the global map of endemic

regions of the disease.

From the point of view of the dental profession it is interesting to note that almost all reported cases of locally acquired CL have occurred on the face of the affected individuals. Several reports indicate that perioral and facial lesions of CL constitute a larger proportion of lesions seen in patients [6,7]. Therefore, it is important that dental practitioners even in non-endemic countries become familiar with the clinical features of the disease and with the diagnostic and treatment methods so that unnecessary and probably harmful treatment regimes such as topical or intralesional steroids are avoided. The conventional leishmanicide, sodium stibogluconate (*Pentostam*[R]), a pentavalent antimony compound is the drug of choice for extensive disease or unsightly lesions. A life-long immunity usually develops in the affected individual with the slow healing of the lesion [8,9].

Cryotherapy appears to have healed the lesion of cutaneous leishmaniasis in our patient contrary to some reports where this method had been unsuccessful[10]. In our case no relapse was observed on review even after twenty months.

Acknowledgements: We wish to thank Prof (Mrs) M de S Wijesundara, Professor of Parasitology, Faculty of Medicine, University of Peradeniya, for her kind contribution in the confirmation of the diagnosis of this case.

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AN APPRECIATION

Dr. David Barmes

Dr. David Barmes, the former Chief, Oral Health Unit Geneva has passed away peacefully in his sleep on the 13th January 2001, in New South Wales Australia. Apparently he was spending a holiday with his family at a beach town there. I have the privilege of knowing him very closely since 1984.

His first visit to Sri Lanka was in 1985, as a W.H.O. Short Term Consultant where he assisted us in drawing up a National Oral Health Preventive Programme based on the analysis of the data gathered from the first National Oral Health Survey implemented in Sri Lanka. He participated in a Workshop for Regional Dental Surgeons and Senior Medical Officers of Health held at that time and later was the Guest of Honour at the first Inaugural Scientific Sessions of the College of Community Dentistry of Sri Lanka.

Subsequently he made several visits to Sri Lanka accompanied by the Scientist attached to the Oral Health Unit Geneva, Jennifer Sardo Infirri and assisted us in a number of ways in drawing up strategies and approaches for the improvement of Oral Health of the community.

Several changes recommended by him were implemented in the School Dental Service for greater productivity and efficiency of the service. I had the opportunity to work closely with him in the Oral Health Advisory and Research Group meeting of the W.H.O. for a number of years.

He was responsible for the close collaboration that the W.H.O. built up with the F.D.I., I.A.D.R., and the A.P.D.C. and was a familiar figure that was seen at these meetings. His presentations at these meetings were interesting, lucid, incisive and humorous and full of anecdotes and kept the audience spell-bound.

After many decades of service at the W.H.O. Head Quarters in Geneva, he retired and joined the National Institute of Dental and Cranio Facial Research at the National Institute of Health in Bethesda.

In spite of his multifarious activities as the Chief, Oral Health Unit, he found time to play cricket and always enjoyed a game of cricket with his friends.

He led a full life and did yeoman service to improve the Oral Health of the community in the development as well as the developing world.

The Dental Profession is entering a period of significant transition, with technology, public policy, economics as the major drivers of this transition. David's loss at this juncture will be felt by the entire Oral Health Community. He had the vision that in the near future we would witness the metamorphosis of the Dental Surgeon into an Oral Physician.

David was a family man. He had five children, and his wife Rosemary lives in Switzerland.

He has a unique place in the field of Oral Health for his commitment and dedication to the profession. His death is an irreparable loss to the entire Oral Health sector in the world and we deeply sympathise with his wife Rosemary for the great loss she has sustained personally.

A Global Health Fund had been established and designated as David E. Barmes Health Fund. The address is : Friends of the N.I.D.C.R., Connecticut Avenue, N.W. Suite 200, Washington D.C. 20036, U.S.A.

Dr. K.D.G. Saparamadu.

Instructions for Authors

The Sri Lanka Dental Journal publishes the following categories of articles which have relevance to Dentistry and allied sciences.

1. Leading articles – One article per issue. It may be solicited by the Editor. Authors are welcome to submit leading articles on current topics of interest, one's expertise or commentaries on general practice etc. They should be approximately 1500 words in length. References should be 20 or less.

2. Reviews – Reviews are detailed surveys of published research pertinent to dentistry and associated sciences. They should be critical in nature and should not normally exceed 3000 words and 30 references.

3. Research articles – Articles resulting from research work belong to this group. Results from routine clinical examinations or laboratory investigations will not be considered under this category. Subjects may vary from clinical trials to basic science research, historical analysis to dental economics. They should not exceed 3000 words and 30 references. A reasonable number of tables and illustrations will be accepted.

4. Short reports – These include reports on current topics, modified techniques, new materials, practice management etc. Interesting results from routine clinical work or laboratory investigations also may be accepted.

5. Case reports – Reports such as of rare diseases or conditions, modifications to accepted treatment procedures, new management methods etc. may be included in this category.

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The manuscripts should be typed, double-spaced, on A4 (212x297 mm) paper and submitted in correct English; both English and American spelling are acceptable, provided this is consistent throughout the manuscript. Manuscripts not submitted in proper format or in poor English may be returned without review. The format of a manuscript should be as

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Abstract page - Original and review articles must contain an abstract of approximately 250 words with four specified subtitles:

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2) **Material and methods:** A description of experimental procedure including applicable statistical evaluation.
3) **Results:** A summary of the new, previous unpublished data and results.
4) **Conclusion:** A statement of the study's conclusion 3-5 key words according to Index Medicus should be provided.

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The authors should consider the ethical aspects of their research and ensure that the work has been approved by an appropriate Ethical Committee. Where applicable, a copy of the ethical clearance certificate should be attached. In human experimentation, informed consent from individuals should be obtained and this should preferably be stated.

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Tables - The tables should be numbered in the order of appearance in Arabic numerals. Each table should have a brief explanatory title. Each table should be typed on a separate sheet, with due regard to the proportion of the printed column/page.

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Journals

Standard journal article

Bartlett JG, O'Keefe P. The bacteriology of the perimandibular space infections. J Oral Surg 1979; 37: 407-409.

Corporate (collective) author

WHO COLLABORATING CENTRE FOR ORAL PRECANCEROUS LESIONS. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol 1978; 46: 518-539.

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Barker DS, Lucas RB. Localised fibrous growth of the oral mucosa. J Dent Res 1965; in press.

Books and other monographs

Pindborg JJ. Atlas of diseases of the oral mucosa. 5th edition. Copenhagen: Munksgaard, 1992; 50-66.

Chapter in book

Boyde A. Amelogenesis and the structure of enamel. In: Cohen B, Kramer KH (eds). Scientific Foundations of Dentistry. William Heinemann Medical Books Ltd. London, 1976; 335-352.

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International statistical classification of diseases and related health problems, 10th revision, vol 1. Geneva: World Health Organisation, 1992; 550-564

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