A PROSPECTIVE COMPARATIVE STUDY OF POTENTIAL RISK FACTORS BETWEEN LUDWIG'S ANGINA AND LOCALISED ODONTOGENIC ABSCESSES

Thoganthiren Perumal Chettiar

A research report submitted to the faculty of Health Sciences, School of Oral Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Dentistry in the branch of Maxillo-facial and Oral surgery, 2006.

DECLARATION

I, Thoganthiren Perumal Chettiar, declare that this dissertation is my own work. It is being submitted for the degree of Master of Dentistry in the branch of Maxillo-facial and Oral surgery at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Ethics clearance by the Committee for Research on Human Subjects (Medical) has been granted as per protocol number M02-06-08.

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…….day of……2006

DEDICATION

To my wife Nalanie,

Our son Mayuran And daughter Pravani

ABSTRACT

Odontogenic abscesses and Ludwig's angina are infections commonly seen by maxillofacial surgeons. Both infections have periapical or periodontal origin and caused by oral bacteria. Ludwig's angina is an aggressive and fast spreading infection compared to odontogenic abscess. The origin and the responsible bacteria of these infections are similar but the development and response is different in patients. There is no comprehensive study that has investigated the bacterial and host factors involved in the development of there infections. The aim of this study was to compare the presence of bacteria and enzymes in to the pus samples collected from patients with odontogenic abscess and Ludwig's angina. Furthermore, various haematological and immunological tests were also compared between the two study groups. Forty two patients presenting with localized odontogenic abscesses and 15 with Ludwig's angina were selected. Patient was examined according to standard protocol and history was recorded. Bloods were collected for haematology and immunology tests and pus was collected for microbiology and enzymatic tests.

The results showed that highly virulent bacteria such as *Staphylococcus aureus* and black pigmented bacteroides were prevalent, increase in c-reactive protein, white blood cell count, IL6 and decrease in urea, circulating immune complexes and IgE in patients with Ludwig's angina.

Development of Ludwig's angina could be due to the aggressive bacteria, their byproducts and low immune response compared to the odontogenic abscesses.

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NOMENCLATURE AND ABBREVIATIONS

CIC	Circulating Immune Complexes
CRP	C-reactive proteins
˚C	Degree centigrade
FBC	Full Blood Count
Hb	Haemoglobin
Hrs	Hours
Ig	Immunoglobulins
IL6	Interleukin 6
LA	Lugwig's angina
LPS	Lipopolysaccharide
ml	Millilitre
n	Number of samples or patients
%	Percentage
PBS	Phosphate Buffered Saline
RF	Rheumatoid factor
RID	Radial immunodiffusion
SD	Standard deviation
TSA	Trypticase Soy Agar
TNF	Tumour Necrosis Factor
U & E	Urea and Electrolyte
WBC	White Blood Cells

PREFACE

Much has been published about odontogenic abscesses and the bacteria involved in the infection, but to date not much is known about similar infection, Ludwig's angina which is a fast spreading, severe form of cellulites. The purpose of this study was to compare the causative agents and their byproducts, and the host response between the two infections. The results will contribute to the understanding of the factors involved in the development and prevention of Ludwig's angina.

1. INTRODUCTION AND LITERATURE REVIEW

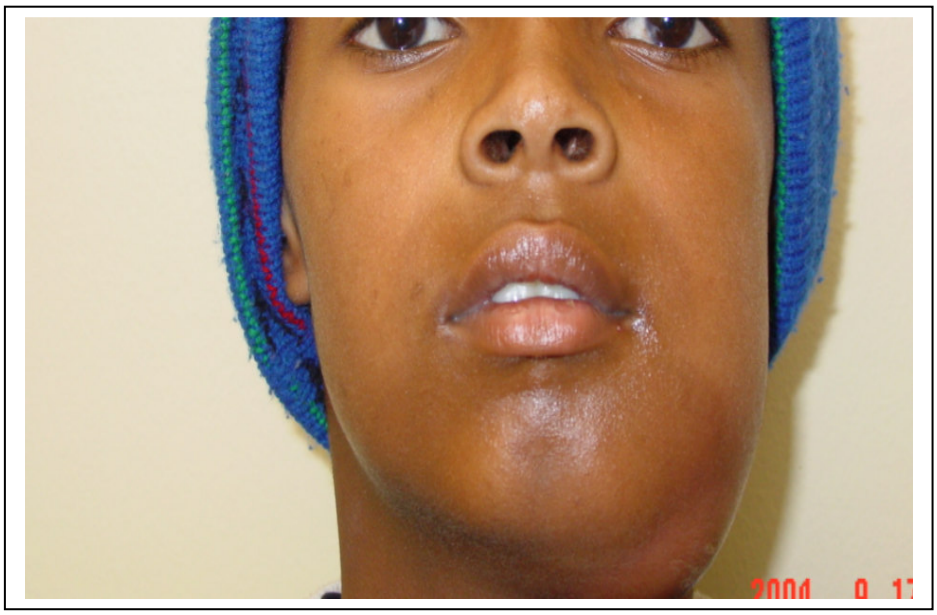
Odontogenic infections have two major origins: periapical, as a result of pulpal necrosis and subsequent bacterial invasion into the periapical tissue, and periodontal, as a result of deep periodontal pocket that allows inoculation of bacteria into the underlying soft tissues. The consequent infections can be either an odontogenic abscess or Ludwig's angina (LA). Odontogenic abscess is a slow spreading and less aggressive form of infection where as Ludwig's angina is the name given to an aggressive spreading cellulitis that affects the submandibular and sublingual tissue spaces bilaterally. Anecdotal evidence suggests that the spread of infection in LA is more frequent in patients with poor oral hygiene, intravenous drug abuse, trauma and tonsillitis (Fritsch and Klein, 1992). There is also reported increase in incidence of LA in diabetics and Human immunodeficiency virus positive patients (Har-El et al, 1994). Moreover our experience at the Division of Maxillo-facial and Oral Surgery, University of the Witwatersrand, has found that most patients with LA have no obvious risk factors as reported by other investigators (Polakow et al. –unpublished data). The factors involved in the development of the two resultant infections are unclear and therefore this study was undertaken.

1.1 Odontogenic abscesses

1.1.1 Origin

Abscesses are focal localized collections of purulent inflammatory tissue caused by suppuration buried in a tissue, an organ, or a confined space (Figure 1.1). They are produced by deep seeding of pyogenic bacteria into a tissue. Abscesses have a central region that appears as a mass of necrotic white cells and tissue cells. There is usually a zone of preserved neutrophils about this necrotic focus, and outside this region vascular dilation and parenchymal and fibroblastic proliferation occur, indicating the beginning of repair. In time, the abscess may become walled off by connective tissue that limits further spread. Odontogenic infections have two major origins: periapical, as a result of pulpal necrosis and subsequent bacterial invasion into the periapical tissue, and periodontal, as a result of deep periodontal pocket that allows inoculation of bacteria into the underlying soft tissues. Of these two, the periapical origin is the most common. Necrosis of the dental pulp as a result of deep caries allows a pathway for bacteria to enter the periapical tissues. Suppuration or purulent inflammation is characterized by the production of large amounts of pus or purulent exudates consisting of neutrophils, necrotic cells, and edema fluid. Certain bacteria (eg. *Staphylococci)* produce this localized suppuration and are therefore referred to as pyogenic or pus producing bacteria (Cotran et al., 1999)

Figure 1.1 Patient with odontogenic abscess



1.1.2 Host-organism interaction

1.1.2.1 Size of Inoculate

The size of the inoculate contributes to the occurrence of the infection. The larger the number of organisms per inoculate the greater the chance of an infection developing.

1.1.2.2 Virulence of organisms

Bacteria may cause disease by invasion of the tissues and the production of toxins. Invasion leads to damage of the host cells in the vicinity of the bacteria, while toxins are usually transported via the blood and lymphatics to remote sites in the body. Some species owe their pathogenicity to invasiveness alone, eg *Streptococcus pneumoniae* while others produce toxins eg. *Clostridium tetani.* Most depend upon both of these mechanisms for pathogenicity. In fact these two mechanisms are rarely separated.

The virulence properties are determined by a multitude of gene and gene products. Bacterial damage to host tissues depends on their ability to adhere to host cells, invade cells and tissues, and deliver toxic moieties. Bacterial adhesions that bind bacteria to host cells are limited in type but have a broad range of host cell specificity. The fibrillae covering the surface of gram-positive cocci such as *Streptococcus* are composed of M protein and lipoteichoic acids. Lipoteichoic acids are hydrophobic and bind to the surface of all eukaryotic cells, although with a higher affinity to particular receptors on blood cells and oral epithelial cells. In contrast to viruses, which can infect a broad range of host cells, facultative intracellular bacteria infect mainly epithelial cells, macrophages or both cell types. Bacterial endotoxin is a lipopolysaccharide that is a structural component in the outer cell wall of gram-negative bacteria. Most biological activities of lipopolysaccharide, including the induction of fever, macrophage activation, and B-cell mitogenicity, come from lipid A and the core sugars and are mediated by induction of host cytokines, including tumour necrosis factor (TNF) and IL-1 (Cotran et al. ,1999). Pathogenic bacteria sometimes produce enzymes capable of disorganizing host function by lysing host cells or the constituents of host tissue. Like exotoxins the enzymes are soluble products of bacterial cells. They are thermolabile proteins that are highly specific in their action. These enzymes can be divided into several categories according to their activity:

- Cytotoxic enzymes eg. Haemolysin, Streptolysin, Leucocidin
- Enzymes influencing clotting mechanisms eg. Coagulase, Nuclease, Fibrinolysin
- Enzymes attacking tissue constituents eg. Protease, Lecithinase, Collagenase

Once this tissue has become inoculated with bacteria and an active infection is established, the infection will spread preferentially along lines of least resistance. The infection will spread through the cancellous bone until it encounters a cortical plate. If this cortical plate is thin, the infection erodes through the bone and enters the soft tissues.

Spread of the infection from a specific tooth is determined by two major factors: the thickness of the bone overlying the apex of the tooth and the relationship of the site of perforation of the bone to muscle attachments of the maxilla and mandible. Once the infection erodes through the cortical plate of the alveolar process, it localizes in predictable anatomic locations. However there are occasions when the infection spreads rapidly to involve the submandibular spaces bilaterally, the submental space and the sublingual space resulting in Ludwig's Angina (Peterson et al, 1998).

1.1.2.3 Host defence

The pathogenicity of a micro-organism depends upon its ability to invade the tissues of a susceptible host. The progression of infection will depend largely on host defence mechanisms and properties exhibited by the micro-organisms itself. The host factors of importance are varied and include immunity, nutritional status and drug therapy. The first line of defence is formed by the intact integument (the skin and mucous membrane). This is followed by cellular and humoral immunity. Cellular immunity relates to the co-operation between lymphocytes and macrophages to eliminate micro-organisms. The cellular immune response occurs in the vascularized connective tissue, including plasma, circulating cells, blood vessels, and cellular and extracellular constituents of connective tissue. The circulating cells include neutrophils,

monocytes, eosinophils, lymphocytes, basophils and platelets. The connective tissue cells are the mast cells and fibroblasts. Two of the most important barriers that a micro-organism must overcome as it penetrates into the deeper tissues are phagocytosis and, in the case of gram-negative bacteria, the lytic action of serum (Janeway et al, 1994).

Humoral immunity involves antibodies which are proteins found in the fluid compartment of blood, or plasma, and in extracellular fluids. Antibodies are secreted by plasma cells which are in turn produced by B-lymphocytes. Antibodies are contained in the serum. Antibodies of all specifications are members of the same family of proteins termed immunoglobulins (Ig). There are five classes of immunoglobulins namely IgM, IgG, IgA, IgE and IgD with each class inducing a distinctive set of effector mechanisms for disposing of antigen once it is recognized (Charles A Janeway et al, 1994). Antibodies act by binding to pathogens and their products in extracellular spaces and ensuring protection of the host in one of three ways. First, antibodies may neutralize a pathogen or its toxic product simply by binding to it and preventing infection or toxic damage. Second, antibodies may opsonize a pathogen, enabling phagocytosis to ingest and destroy it. The third function of the antibodies is to activate complement, a system of plasma proteins that greatly enhance the ability of phagocytes to engulf and destroy certain bacteria. The complement system and the phagocytes that antibodies recruit are not themselves antigen specific; they depend upon antibody molecules to mark the particles as foreign (Janeway et al, 1994).

Not all pathogens grow in extracellular spaces where they are accessible to antibodies. All viruses and some bacterial pathogens and parasites enter cells where they multiply safely from attack. To rid the body of these invaders, a different system of recognition and response exists, the cell-mediated immune responses carried out by the T-lymphocytes. Cell mediated interactions depend on direct interactions between T-lymphocytes and cells of the host's body bearing the antigen they recognize (Janeway et al, 1994).

Patients that are not optimally nourished have a greater incidence of septic episodes. Patients with weight loss of more than 20% caused by illness such as cancer or intestinal disease have a 3-fold increase in infection rate (Way, 1991). Certain drugs may reduce the patient's resistance to infection by interfering with host defense mechanisms. Corticosteroids, immunosuppressive agents, cytotoxic drugs, and prolonged antibiotic therapy are associated with an increased incidence of invasion by fungi and other organisms not commonly encountered in infections (Way, 1991).

1.1.3 Microbiology

A microbiological investigation of a pus from an odontogenic infection may yield a single isolate, a mixture of 2 to 3 different bacterial species, or a complex mixture of microorganisms (Lewis et al., 1986; Brook et al., 1991). Well-controlled studies have shown that strict anaerobes are usually, the predominant organisms and isolated from all the infections. The common species isolated from orofacial odontogenic abscesses are shown in Table 1.1. Many investigations have demonstrated that *Viridans streptococci*, *Peptostreptococcus*, *Prevotella*, *Porphyromonas* and *Fusobacterium* are frequently isolated from orofacial odontogenic infections (Kuriyama et al, 2000a, 2000b; Gill and Scully, 1999; Baker and Fotos, 1994).

Table 1.1 Organisms commonly isolated from orofacial odontogenic abscesses

Aerobes	**Anaerobes**
Viridans streptococci	*Peptostreptococcus*
Staphylococcus	Pigmented *Prevotella*
Corynebacterium	*Fusobacterium*
Campylobacter	Nonpigmented *Prevotella*
Neisseria	*Gemella*
Actinomyces	*Prophyromonas*
Lactobacilli	*Bacteroides*
Enterobacter	*Eubacterium*
Haemophilus	*Veillonella*
Pseudomonas	*Propionibacterium*
Micrococcus	
Enterococcus	
Klebsiella	
Branhamella	

There is little information concerning the in vivo pathogenic potential of the bacteria commonly isolated from dental abscesses and therefore, until proven otherwise, all isolates should be regarded as equally important.

According to Finegold (Finegold SM, 1995) the organisms of greatest importance in mixed polymicrobial infections are those that are most virulent, those that are resistant to commonly employed antimicrobial agents, and those present in greatest numbers. Anaerobic bacteria appear to fulfill all these criteria in odontogenic infections. Anaerobic organisms are mainly streptococci of the Viridans variety, microaerophilic or facultative in growth requirements (Kolokotronis A, 1999; Von Konow et al, 1981; Lewis et al, 1986; Brook et al, 1991) whereas more fastidious pathogens such as *Eikenella corrodens*, may be involved in a majority of patients (Siqueira et al, 2001; Peterson and Thomson, 1999; Lewis et al, 1995).

There are a number of examples in medical microbiology where mixtures of strict anaerobes and aerobes or facultative bacteria are more pathogenic then either species alone. Such synergistic microbial interactions are likely to be important in the severity of dentoalveolar abscesses (Jenkins SG, 2001). Significant combinations associated with more severe clinical symptoms suggesting increased pathogenicity have been reported between *Fusobacterium* species and an unclassified *Eubacterium* taxon (Wade et al, 1994), between *Prevotella species* and *Peptostreptococcus micros* (Drucker et al, 1997) and also between various bacterial isolates and *Bacteroides forsythus* (Siqueira et al, 2001) which appears to be noninfective in monoculture (Takemoto et al, 1997). However, recent molecular methods have revealed the possibility that some members of the unculturable part of the oral flora may also be involved in dentoalveolar abscesses (Dymock et al, 1996, Siqueira et al, 2001).

Although the bacterial species encountered are highly regarded as members of the commensal oral flora (Chow et al, 1978, Rajasue et al, 1996), when these infections occur in hospitalized, postirradiated, or immunocompromised patients, *Staphylococcus aureus* as well as various

enteric gram-negative rods should also be considered in the etiologic diagnosis (Chow AW, 1992, Peterson and Thomson, 1999).

1.1.3.1 Microbiology at different stages of disease

A causative role of specific microorganisms in different stages of odontogenic infections has been revealed. Several bacteriologic studies have reported that in samples obtained during the initial 1-2 days of clinical symptoms, *Streptococcus milleri* group of organisms were frequently the predominant or sole isolates (Lewis et al, 1986, Fisher and Russell, 1993), strongly suggesting that at this stage of infection, which usually manifests itself as cellulitis, the causative organisms are facultative bacteria, mostly *streptococci*, which allow the infection to spread by elaborating spreading enzymes. Anaerobes are particularly important in the ensuing phase characterized by suppurative and abscess formation (Aderhold et al, 1981, Von konow et al, 1981, Heimdahl et al, 1985, Lewis et al, 1986), although they still depend on other, possibly less pathogenic, bacteria for nutritional requisites such as vitamin K and hemin (Flynn TR, 2000; Grenier and Mayrand, 1986)

1.1.3.2 Virulence

There are three most important virulence factors in anaerobic bacteria (Jenkins SG, 2001, Duerden BI, 1994): the ability to survive the oxygen tension of the living tissues, the presence of cell surface constituents such as capsular polysaccharide endotoxin, or in gram negative organisms lipopolysaccharide endotoxin, and the production of enzymes, toxins, or other substances associated with tissue damage (Table 1.2).

Table 1.2 Potential virulence factors of anaerobic bacteria isolated from orofacial odontogenic infections

Factor	Action
Superoxide dismutase	Aerotolerance
Capsular polysaccharide	Antiphagocytic, abscessogenic
Succinic acid	Antiphagocytic (leukotoxin)
Endotoxin	Cytotoxicity
Proteolytic enzymes	Tissue degradation, promotes bacterial invasion
Hydrogen sulphide	Cytotoxicity

Superoxide dismutase produced by moderate anaerobes renders them tolerant of oxygen levels of 2% to 8% (Loesche WJ, 1969, Tally et al, 1977) and is considered a prerequisite to their pathogenicity (Murdoch DA, 1998, Duerden BI, 1994, Hofstad T, 1984). Most of the organisms isolated from orofacial infections are moderate anaerobes. However, in a mixed infection, oxygen consumption by facultative organisms is also considered an important factor permitting the subsequent establishment of anaerobic flora (Murdoch DA, 1998, Styrt and Gorbach, 1989).

Capsular material, is believed to help bacteria avoid phagocytosis and killing by polymorphonuclear leukocytes (Sundqvist etal, 1982) by preventing the deposition of opsonins on the bacterial surface or by mimicking mammalian cell-surface determinants, thus sidestepping the host response (Klempner MS, 1984, Cross AS, 1994). In addition, capsule material induces abscess formation even in the absence of viable bacteria (Hofstad T, 1984, Cross AS, 1994).

Although most of the work has been done on *B. fragilis* (Cross AS, 1994), researchers have also demonstrated the pathogenicity of capsulate strains of pigmented and non-pigmented oral "*Bacteroides*" organisms (Takazoe et al, 1971,Brook et al, 1983) and gram positive anaerobic cocci (Brook and Walker, 1985) in experimental animals.

Lipopolysaccharide (LPS) endotoxins formed by *Prevotella* and *Porphyromonas* organisms are less potent than conventional endotoxins and are less likely to produce classic manifestations of endotoxic damage (Duerden BI, 1994). However, it has been shown that the LPS of *P. endodontalis* plays an important role in the initiation and magnification of maxillofacial abscess formation acting as a virulence factor by stimulating the production of inflammatory cytokines (Murakami et al, 2001). On the other hand, LPS from *Fusobacterium* has a structure similar to that of gram negative enteric rods (Bennett and Eley, 1993); the LPS of *F. necrophorum*, in particular, displays toxicity comparable to that of enterobacterial LPS, accounting for the high virulence of this organism (Duerden BI, 1994).

Anaerobic bacteria produce volatile sulfur compounds, such as methyl mercaptan and the highly cytotoxic hydrogen sulfide, which are responsible for the putrid smell of pus found in anaerobic infections and cytotoxicity (Bartlett JG, 1998, Persson et al, 1990).

However, certain bacterial species are regarded as legitimate pathogens irrespective of synergistic inter-relationships. In general, strictly anaerobic gram-negative rods are more pathogenic than facultative or strictly anaerobic gram-positive cocci in acute dentoalveolar abscesses (Lewis et al, 1988, Lorber B, 2000, Murakami et al, 2001; Siqueira et al, 1998) and *F. nucleatum* appears to be particularly associated with severity of orofacial odontogenic infections (Heimdahl et al, 1985). Another Fusobacterium species *Fusobacterium necrophorum*, which is considered the most virulent of the non-spore-forming anaerobes (Jenkins SG, 2001), is the major isolate from the blood in postanginal septicemia (Chow AW, 1992, Bennett and Eley, 1993), infected root canals (Drucker et al, 1997) and third-molar pericoronal pockets (Rajasuo et al, 1996).

1.1.4 Treatment

The primary therapeutic modality for orofacial odontogenic infections is surgical drainage of any discernible pus collection, followed by extraction or endodontic therapy of the responsible tooth (Aderhold et al, 1981, Lewis et al, 1990). Although antibiotics are generally considered adjunctive therapy (Flynn TR, 2000, Hupp JR, 1991), their value should not be underestimated, especially when drainage cannot be achieved or the infection shows signs of local extension or systemic involvement. In addition, antibiotic therapy becomes imperative in the case of immunocompromised patients, even with mild infection (Johnson BS, 1999, Chow AW, 2000).

When antibiotics are prescribed for the treatment of orofacial odontogenic infections, clinicians should choose them on a case-specific basis, and the choice should be based on several factors, such as laboratory data, patient health, age, allergies, drug absorption and distribution ability, plasma levels and occurrence of resistance. The laboratory data regarding bacteriology and antimicrobial susceptibility are crucial information for the clinician who is considering the administration of the antimicrobial therapy (Baker and Fotos, 1994, Kuriyama et al, 2000, NCCLS, 1998). However, it may take several days or even longer to obtain such data. Therefore antibiotics may be chosen empirically.

Beta lactam antibiotics, especially penicillin, have traditionally been recommended as a first-line antibiotic because penicillin have a low incidence of side effects (Gill and Scully, 1990; Sandor et al, 1998; Baker and Fotos, 1994; Heimdahl and Nord, 1985; Kuriyama et al, 2000) and relatively inexpensive (Baker and Fotos, 1994; Kuriyama et al, 2000). Some studies have suggested that the antimicrobial activity of penicillins has decreased against the causative bacteria related to orofacial odontogenic infections, such as streptococci and oral anaerobes (Gill and Scully, 1990; Heimdahl and Nord, 1985; Peterson LJ, 1991; Kuriyama et al, 2000). Among the predominant bacteria in orofacial odontogenic infections, beta lactamase activity has been demonstrated in anaerobic gram-negative rods (Peterson LJ, 1991; Nord et al, 1988) which is the most common mechanism of resistance in this drug. In penicillin hypersensitive patients, erythromycin and clindamycin have been recommended. The selected antibiotic should be effective against both streptococci and anaerobes (Kuriyama et al, 2000; Sobottka et al, 2002).

Therefore, clindamycin, cefoxitin, imipenem or beta lactam with beta lactamase inhibitor together with metronidazole should be administered (Brook et al, 1991; Lorber B, 2000; Hupp JR, 2002). Metronidazole is only effective against obligate anaerobes (Hupp JR, 2002).

1.2 Ludwig's Angina (LA)

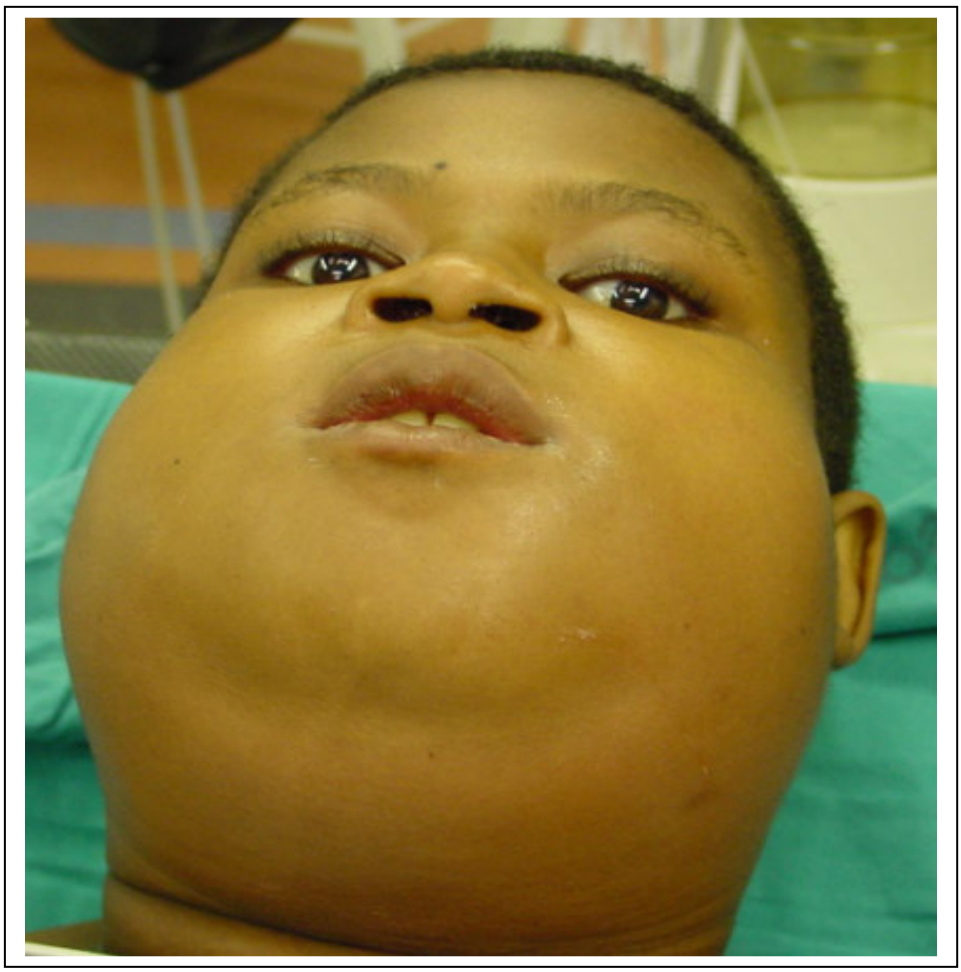
LA is the name given to an aggressive spreading cellulitis that affects the submandibular and sublingual tissue spaces bilaterally (Figure 1.2). The name was given in 1837 by Camerer when presenting a case similar to the five described the previous year by Wilhelm Frederick von Ludwig. An identical clinical condition has previously been called *Morbus strangulatorius, Angina maligne* and *Garotillo* from the Spanish word for the hangman's noose. All these names refer to the choking effect which the condition has on its victims (Johnson et al. , 1963). Most cases of LA follow dental infections, particularly periapical infections of the mandibular molars. Other conditions which have been reported to cause LA include trauma to the floor of the mouth and peritonsillar abscess while it is thought that mandibular fractures only rarely result in LA (Rosen at el, 1972).

The infection in LA is usually polymicrobial. The most common pathogen is *Viridans group Streptococcus* (40,9%) followed by *Staphylococcus aureus* (27,3%) and *Staphylococcus epidermis* (22,7%). Anaerobes, most commonly especially *Porphyromonas* and *Prevotella* species, *Fusobacterium* and anaerobic streptococci accounts for about 40% of positive cultures (Har-El G et al. , 1994). Occasionally coliform such as *Escherichia coli* and *Klebsiella species* have been reported. It is a mixed endogenous infection. Because of the severity of the condition, samples for microbiological assessment should always be obtained.

1.2.1 Management

The clinician needs to ensure that the airway remains open and maintenance of fluid balance is important. A sample of pus should be collected and high dosage empirical antibiotic therapy usually penicillin and metronidazole started immediately. Surgical drainage should be carried out as soon as possible and the primary source of infection should be eliminated.

Figure 1.2 Patient with Ludwig's angina



1.3 Aim

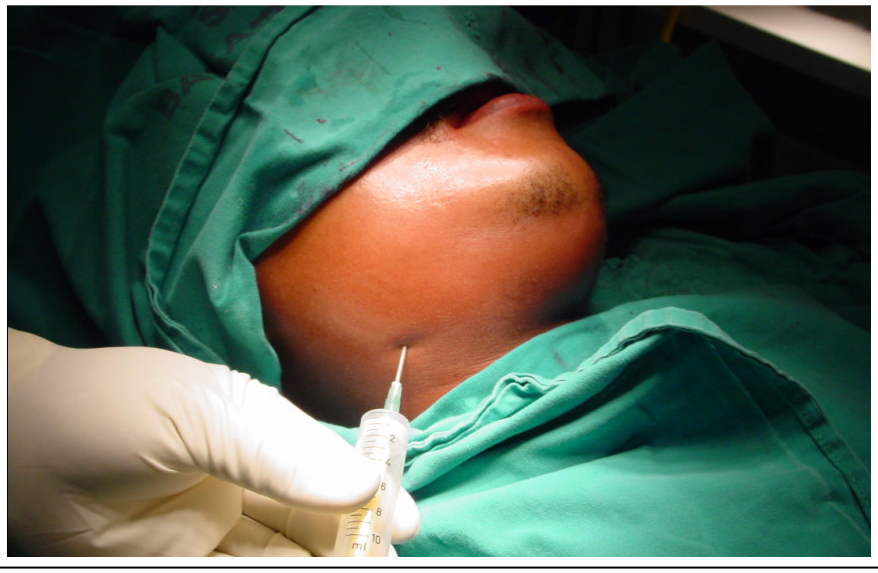
The aim of this project was to compare various factors that may influence the development of an odontogenic abscess and Ludwig's angina. The factors were delay in presentation, microorganisms involved in the infection and the enzymes they produce as well as virulence factors and the host response and immunity.

2. MATERIALS AND METHODS

2.1 Study population

Patients presenting with localized odontogenic abscesses and Ludwig's angina were selected from the Maxillo-facial surgery clinics and casualty departments of the Johannesburg General and Chris Hani Baragwanath hospitals. The sepsis trial was explained to these patients and those that agreed to be part of the trial signed the consent form (see appendix 1). 42 patients with localized odontogenic abscesses and 15 with Ludwig's angina were included in this study. Personal particulars of patients, date of symptoms, vital signs, location of infection, treatment rendered with type of airway management was recorded. Bloods were collected for haematology and immunology tests and pus was collected in a 5 ml syringe after the overlying skin was cleaned with hibident solution (Figure 2.1). One millilitre of pus was placed into Robertson's cooked meat broth and into phosphate buffered saline and transported to the oral microbiology laboratory within 2 hours of collection. In the event of this not being possible the sample was placed in an anaerobic jar and transported to the laboratory within 48 hours for microbiological and enzymatic tests.

Figure 2.1 Sample collection



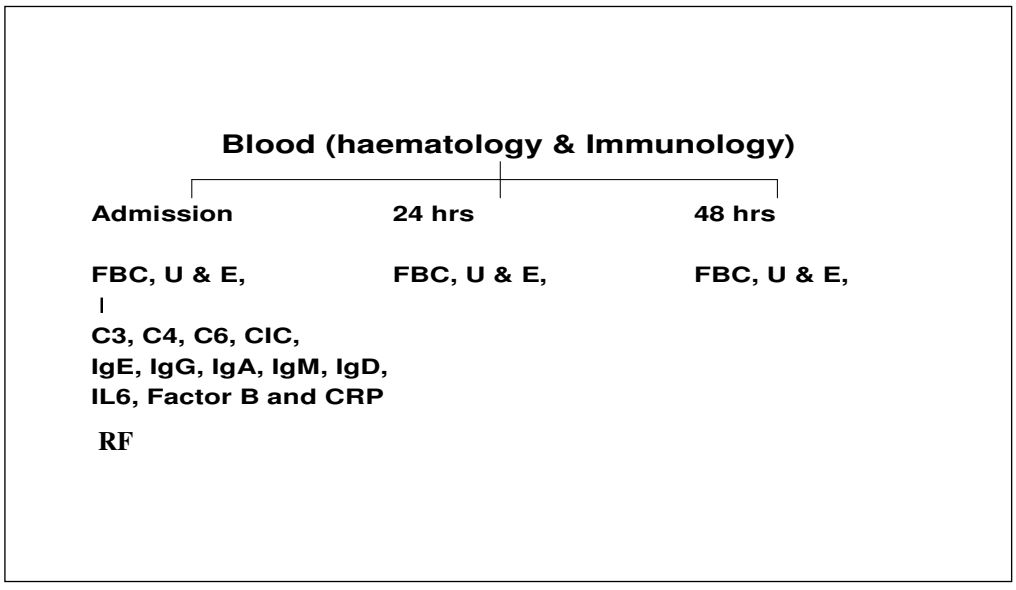
2.2 Haematology

Blood specimens for C-reactive proteins (CRP), full blood count, urea and electrolytes were collected on admission. Full blood count and urea and electrolytes were repeated at 24 hour and 48 hour intervals (Figure 2.2).

2.3 Immunology

4 x 5 ml specimen whole clotted blood were collected on admission and sent to the immunology laboratory where serum was routinely separated from blood samples by spinning in a centrifuge (Hettich; universal 16A, Tuttlingen, Germany) at a speed of 5000 revolutions per minute. The tests performed on separated serum were complements C3, C4, C6, C1 esterase inhibitor, C-reactive protein (CRP), circulating immune complexes (CIC), immunoglobulins G, M, D, A, E (IgG, IgM, IgD, IgA, IgE) including subclasses IgG1, IgG2, IgG3, IgG4, Cytokine IL6, Rheumatoid factor(RF), Factor B.

Figure 2.2 Tests performed in haematology and Immunology laboratory



2.3.1 C3, C4, C1 esterase inhibitor, CRP, CIC, IgG and subclasses and RF

The above were measured using the BN ProSpec nephelometer (Dade Behring, Marburg, Germany) and methods followed according to suppliers instructions. Nephelometry is the most commonly used and most accurate measurement principle for the immunochemical determination of protein in serum, urine and other body fluids. In an immunochemical reaction, the proteins contained in the human plasma or serum sample form immune complexes with specific antibodies. These complexes scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration.

2.3.2 IgE and IL6

These are measured using the principle of chemiluminescence. The IMMULITE 2000 Automated Immunoassay Analyzer (Diagnostic Products Corporation, Gwynedd, U.K.) is an instrument which performs chemiluminescent immunoassays. The IMMULITE 2000 system utilizes specific antibody-coated polystyrene beads as the solid phase. At the start of the first cycle the patients serum containing total IgE is added to a streptavidin coated bead in a Reaction Tube and incubated for 30 minutes at 37˚C. During this time the IgE in the patient's serum binds to Streptavidin on the bead. Upon completion of the first 30 minute incubation the Reaction Tube is washed to separate bound from unbound IgE and is ready for the second cycle.

At the start of the second cycle the alkaline phosphatase labeled anti-IgE enzyme is added to the washed bead. Another incubation follows for 30 minutes at 37˚C. During this time the alkaline phosphatase labeled anti-IgE attaches to the IgE from the patients sample. After the second cycle the bead is washed to remove any unbound enzyme labeled anti-IgE and a substrate (dioxetane) is added to produce light. The light is measured at the photometer tube after 5 minutes incubation at 37˚C. The intensity of the light is proportional to the amount of total IgE present in the patient's serum sample.

2.3.3 C6 and Factor B

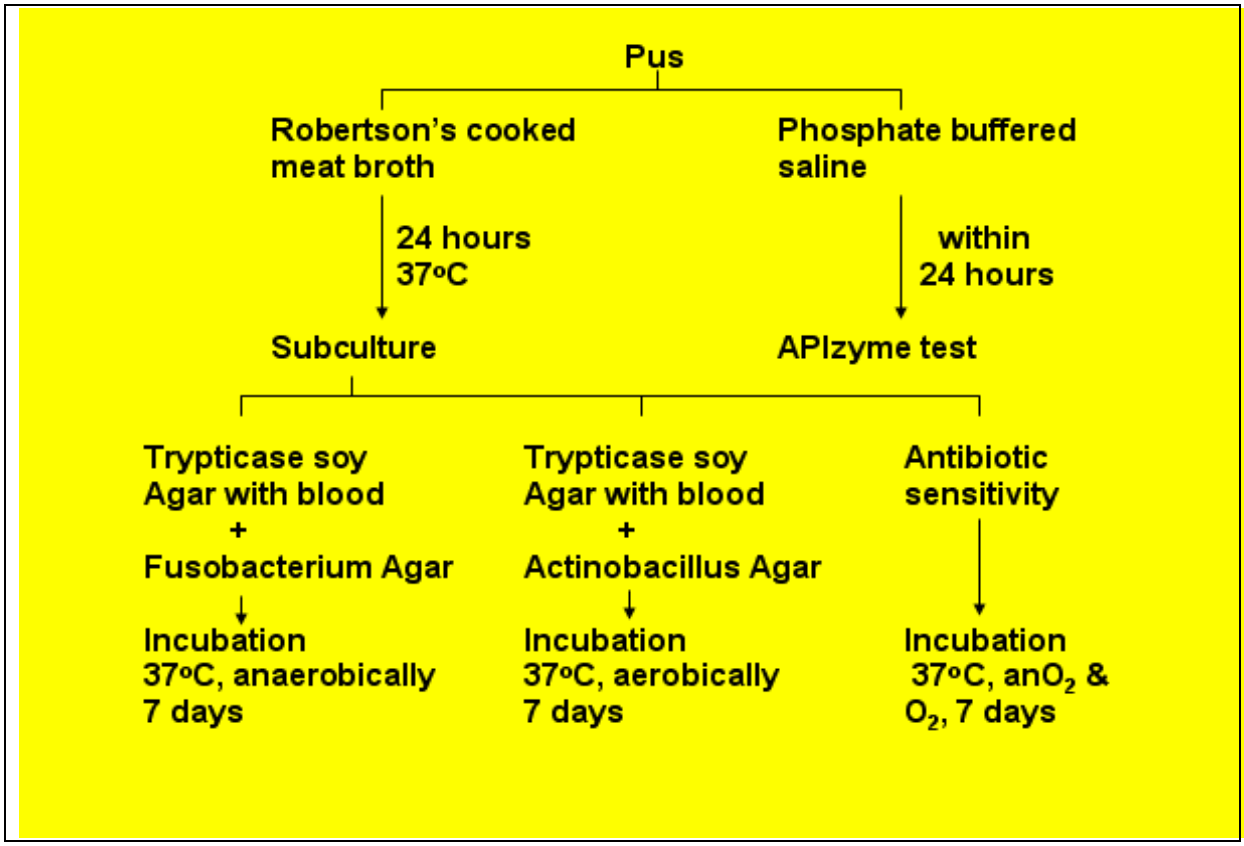
C6 and Factor B are measured using Radial immunodiffusion (RID) which is a technique that is routinely used for measuring the concentrations of various soluble antigens (usually protein) in biological fluids. The principle of the assay involves antigen diffusing radially
from a cylindrical well through an agarose gel containing an appropriate mono-specific antibody. Antigen-antibody complexes are formed which, under the right conditions, will form a precipitin ring. The ring size will increase until equilibrium is reached between the formation and breakdown of these complexes, this point being termed 'completion'. At this stage, a linear relationship exists between the square of the ring diameter and the antigen concentration. By measuring the ring diameters produced by a number of samples of known concentration, a calibration curve may be constructed. The concentration of the antigen in an unknown sample may then be determined by measuring the ring diameter produced by that sample and reading off the calibration curve. The minimum diffusion time used was 96 hours and 72 hours for C6 and Factor B respectively.

2.4 Microbiology

Approximately 1 ml of pus was placed in 10 ml of Robertson's cooked meat broth and transported to the oral microbiology laboratory within 2 hours of collection. In the event of this not being possible the sample was placed in an anaerobic jar and transported to the laboratory within 48 hours.

Robertson's cooked meat broth was incubated at 37^{o}C for 24 hours. After 24 hours the cultures were serially diluted. 0.1 ml of dilutions 1:100 and 1:1000 was spread onto two Trypticase Soy Agar (TSA) plates with blood. One plate was incubated aerobically and the other anaerobically at 37^{o}C for 1 week. 0.1 ml of a concentrated sample was cultured onto Actinobacillus medium, Fusobacterium agar, and 4 TSA plates. Antibiotic sensitivity tests (Table 2.1) were performed using the above 4 TSA plates. Antibiotic sensitivity discs were applied. Two TSA plates and a Fusobacterium plate were incubated anaerobically. Actinobacillus plate and two TSA plates with antibiotic discs were incubated aerobically (Figure 2.3). Cultures were identified using cultural characteristics, growth conditions, gram stain and morphological characteristics (Figure 2.4). Antibiotic sensitivity was recorded as resistant or sensitive according to the size of the zone of inhibition.

Figure 2.3 Tests performed in microbiology laboratory



2.5 Enzymatic tests

Approximately 1 ml of pus was transferred into 1ml of phosphate buffered saline (PBS). In the laboratory PBS was subjected to API ZYM (bioMérieux, Inc.) in order to obtain an enzymatic profile present in the pus or pathological material. The API ZYM strip is composed of 20 cupules, specially designed for the study of enzymatic reactions (Table 2.2). The base of the strip, containing synthetic substrates, is made of non-woven fibers. This base allows enzymatic reactions to take place, even if the substrates are insoluble. The enzymatic tests are inoculated with a dense suspension of organisms or pathological material which is used to rehydrate the enzymatic substrates.

The metabolic end products produced during the incubation period are detected through color reactions revealed by the addition of reagents (Figure 2.5).

Table 2.1 Antimicrobials used in this study

ANTIMICROBIALS	AEROBIC	ANAEROBIC
Streptomycin		
Gentamycin		
Cephalothin		
Trimethoprim		
Carbenicillin		
Penicillin		
Erythromycin		
Cloxacillin		
Oxytetracycline		
Clindamycin		
Amoxycillin		
Chloramphenicol		

Figure 2.4 Trypticase Soya Agar with blood showing growth of mix flora

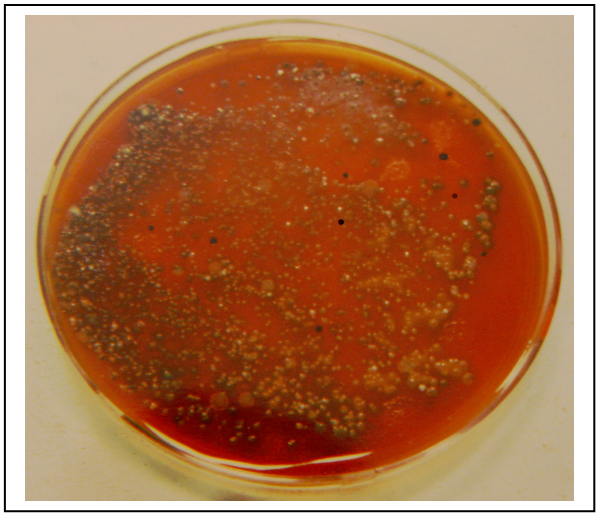


Figure 2.5 API ZYM kit with colour reactions

Table 2.2 Enzymatic profile investigated in this study

ENZYME	SUBSTRATE
CONTROL	
E2-Alkaline phosphatase	2-naphthyl phosphate
E3-Esterase (C4)	2-naphthyl butyrate
E4-Esterase Lipase (C8)	2-naphthyl caprylate
E5-Lipase (C14)	2-naphthyl myristate
E6-Leucine arylamidase	L-leucyl-2-naphthylamide
E7-Valine arylamidase	L-valyl-2-naphthylamide
E8-Cystine arylamidase	L-cystyl-2-naphthylamide
E9-Trypsin	N-benzoyl-DL-arginine-2-naphthylamide
E10-α-chymotrypsin	N-glutaryl-phenylalanine-2-naphthylamide
E11-Acid phosphatase	2-naphthyl phosphate
E12-Naphthol-AS-BI-phosphohydrolase	Naphthol-AS-BI-phosphate
E13-α-galactosidase	6-Br-2-naphthyl-αD-galactopyranoside
E14-β-galactosidase	2-naphthyl-βD-galactopyranoside
E15-β-glucuronidase	Naphthol-AS-BI-βD-glucuronide
E16-α-glucosidase	2-naphthyl-αD-glucopyranoside
E17-β-glucosidase	6-Br-2-naphthyl-βD-glucopyranoside
E18-N-acetyl-β-glucosaminidase	1-naphthyl-N-acetyl-βD-glucosaminide
E19-α-mannosidase	6-Br-2-naphthyl-αD-mannopyranoside
E20-α-fucosidase	2-naphthyl-αL-fucopyranoside

2.6 Statistical analysis of the data

Haematology and immunology results of blood samples, enzymatic test, microbiology and antimicrobial sensitivity test results from pus samples were compared between the two groups using two sample t-test for continuous variables and Fischer exact for proportions. P-values of $\leq$ 0.05 % was considered significant.

3 RESULTS

3.1 Study population

Comparison between genders showed a proportionately higher percentage (86.7%) distribution of males in patients with Ludwig's angina whereas in patients with Localized abscesses showed a higher percentage (28.6%) of female patients. There were mainly adult patients with an age range of 16 to 57 in patients with Localized abscesses and 21 to 51 in Ludwig's Angina. The time between first symptom noted by patient and presentation to hospital varied from 2 to 30 days in Localized abscesses and 3 to 9 days in Ludwig's Angina (Table 3.1). The results showed that Ludwig's angina is severe and spread faster than Localized abscess and therefore patients need hospitalization faster. None of these factors were statistically significant.

Table 3.1 Study population and delay in presentation

	Localized abscesses (n=42)	**Ludwig's Angina (n=15)**
Males	30 (71%)	13 (86.7%)
Females	12 (28.6%)	2 (13.3%)
Age(years) Mean (Range)	 32 (16-57)	 37 (21-51)
Duration(days) Mean (Range)	 6.93 (2-30)	 5.53 (3-9)

3.2 Haematology

The results of tests for haematology are shown in Table 3.2. Bloods for C-reactive proteins (CRP) were done on admission and that for white cell count, platelets, haemoglobin, creatinine, and urea were done on admission, 24 hours, and 48 hours. Results of both the study groups were compared using pooled values (admission, 24 hourly and 48 hourly). On admission, C-reactive protein (CRP) and pooled data of white blood cells and urea were significantly higher in patients with Ludwig's angina compared to the patients with localized abscess. Hemoglobin, platelet, creatinine did not differ significantly between the two groups. The results showed that the infection is severe and the immune response of patients in this group is very high compared to the patients with localized abscesses. High urea levels suggests that these patients with Ludwig's angina are dehydrated.

3.3 Immunology

The immunology tests results are shown in Table 3.3. Circulating Immune Complexes (CIC) and IgE were found to be significantly higher in patients with Ludwig's angina. Once again the results suggest that the immune response in patients with Ludwig's angina is high and infection is severe.

3.4 Microbiology

Culturable aerobic and anaerobic bacteria were investigated. The results are shown in Table 3.4 and 3.5. Streptococci were predominant bacteria in both the study groups and there was no statistical difference between the two groups. Most bacteria isolated from both the groups were normal oral flora except for the *Pseudomonas* and *Clostridium species.* *Pseudomonas* is a gut flora and occasionally isolated from mouth where personal hygiene is inadequate. Although the percentage of isolation of *Pseudomonas* was high in patients with Ludwig's angina, the difference between the two groups was not significant. *Propionibacterium* was isolated from 47% of the patients with ludwig's angina compared to 21% of the patients with localized abscesses which however was not significant (Table 3.4). *Staphylococci* and black pigmented bacteroides (*Porphyromonas and Prevotella species*) were found to be more prevalent in patients with Ludwig's angina. Both can be found in oral cavity and they are aggressive opportunistic pathogens (Table 3.4).

The ratio of aerobic and anaerobic isolates appeared to be the same in both the groups with aerobic isolates being slightly high (Table 3.5).

Table 3.2 Haematological analysis of blood obtained from patients with Localized abscesses and Ludwig's Angina

Tests	Localized abscesses (n=42)			Ludwig's Angina (n=15)			
	Admission	**24 hrs**	**48 hrs**	**Admission**	**24 hrs**	**48 hrs**	**p values**
	Mean ± SD (Range)	**Mean ± SD (Range)**	**Mean ± SD (Range)**	**Mean ± SD (Range)**	**Mean ± SD (Range)**	**Mean ± SD (Range)**	**Pooled**
C-reactive protein (CRP)	98.1 ± 95.8 (1.2-401)	Not done	Not done	213.0 ± 122.9 (61.9-455.0)	Not done	Not done	**0.0005***
White blood cell count (WBC)	10.64 ± 4.21 (3.70-22.50)	9.85 ± 4.51 (3.90-25.1)	7.41 ± 3.31 (2.67-22)	13.28 ± 3.85 (6.60-18.90)	11.8 ± 4.25 (5.5-19.3)	9.77 ± 4.57 (2.9-21.1)	**0.04***
Platelet	328.5 ± 112.1 (137-632)	328.8 ± 120) (146-694)	331.9 ± 97 (174-673)	320.4 ± 109.2 (144-542)	333.3 ± 92 (194-464)	347 ± 127.3 (90-525)	0.89
Haemoglobin (Hb.)	13.3 ± 2.19 (7.6-19.3)	12.19 ± 2.1) (5.7-16.0)	12.4 ± 1.82 (7.2-15.5)	12.78 ± 1.51 (9.6-15)	11.75 ± 1.37 (8.6-13.1)	11.32 ± 1.5 (8.3-13.6)	0.19
Creatinine	81.3 ± 20.2 (30-126)	75.6 ± 15.6 (44-102)	77.7 ± 13.9 (50-104)	107.9 ± 56.1 (60-243)	79.4 ± 19.95 (57-124)	74.9 ± 16.2 (51-96)	0.2
Urea	3.35 ± 1.58 (1.2-7.9)	3.29 ± 1.43 (1-8.2)	2.97 ± 1.18 (1-6.4)	8.16 ± 5.91 (2-21)	11.7 ± 19.6 (2.7-81)	8.6 ± 12.26 (1.6-51)	**0.04***

* indicates significant results

Table 3.3 Immunological analysis of blood obtained from patients with Localized abscesses and Ludwig's Angina

Immunoloy test	Localized abscesses (n=42)	Ludwig's Angina (n=15)	p-value
	Mean ± SD (Range)	Mean ± SD (Range)	
C3	1.6 ± 0.5 (0.4-2.9)	1.3 ± 0.5 (0.5-2.2)	0.0693
C4	0.3 ± 0.1 (0-0.6)	0.3 ± 0.2 (0-0.6)	0.8414
C1esterase	33.0 ± 12.3 (0.2-55.4)	29.9 ± 17.5 (0.2-50.8)	0.4585
CIC	8.9 ± 12.4 (0.5-50.8)	4.2 ± 1.8 (2.0-7.9)	**0.0209***
IgG	21.1 ± 11.8 (3.8-53.5)	15.8 ± 7.4 (5.8-29.6)	0.1065
IgM	1.5 ± 0.8 (0.2-3.4)	1.2 ± 0.6 (0.3-2.5)	0.1091
IgA	2.9 ± 1.4 (0.3-7.5)	2.6 ± 1.3 (1.1-4.9)	0.4675
IgD	0.1 ± 0.1 (0-0.5)	0.1 ± 0.1 (0-0.4)	0.4404
IgG1	14.4 ± 9.3 (2.7-42.8)	10.7 ± 5.8 (3.7-25.8)	0.1558
IgG2	3.0 ± 1.4 (0.8-7.2)	2.9 ± 1.9 (0.6-7.7)	0.9445
IgG3	1.0 ± 0.6 (0.1-2.9)	0.8 ± 0.3 (0.3-1.6)	0.0596
IgG4	0.5 ± 0.5 (0-2.1)	0.8 ± 1.8 (0.1-7.1)	0.572
IgE	218.9 ± 211.6 (5.0-770.0)	127.5 ± 100.4 (11.6-398.0)	**0.0331***
IL6	52.2 ± 157.4 (2.0-1000)	137.5 ± 255.3 (6.4-1000)	0.2405
RF	11.6 ± 2.6 (10.7-23.2)	12.5 ± 5.0 (10.7-30.0)	0.5459
C6	153.1 ± 42.5 (34.0-200.0)	130.2 ± 42.1 (62.0-200.0)	0.0789
Factor B	660.6 ± 135.5 (285.0-805.0)	648.8 ± 165.1 (420.0-800.0)	0.7866

* indicates significant results

Table 3.4 Microbiological analysis of pus obtained from patients with localized abscesses and Ludwig's Angina

Organisms Isolated	Localized abscesses (n=42)	Ludwig's Angina (n=15)	p-value
Genus	**No. of patients (%)**	**No. of patients (%)**	
Streptococci	39 (92.9%)	13 (86.7%)	0.599
Staphylococci	5 (11.9%)	6 (40%)	**0.0274 ***
Corynebacterium	6 (14.3%)	2 (13.3%)	1
Peptostreptococci	10 (23.8%)	4 (26.7%)	1
Propionibacterium	9 (21.4%)	7 (46.7%)	0.0937
Clostridium	1 (2.4%)	0 (0%)	1
Actinobacillus	3 (7.1%)	0 (0%)	0.5586
Neisseria	12 (28.6%)	3 (20%)	0.7351
Haemophillus	1 (2.38%)	0 (0%)	1
Pseudomonas	3 (7.1%)	3 (20%)	0.1799
Eikonella	1 (2.4%)	1 (6.7%)	Not done
Fusobacterium	16 (38.1%)	5 (33.3%)	1
Capnocytophaga	4 (9.5%)	0 (0%)	0.5641
Veilonella	2 (4.8%)	0 (0%)	Not done
Black Pigmented Bacteroides	11 (26.2%)	10 (66.7%)	**0.0109***
Bacteroides	5 (11.9%)	2 (13.3%)	Not done

*indicates significant results

Table 3.5 Distribution of aerobic and anaerobic microorganisms isolated from pus obtained from patients with Localized abscesses and Ludwig's Angina

Localized abscesses		Ludwig's Angina	
Aerobic (56%)	**Anaerobic (44%)**	**Aerobic (55%)**	**Anaerobic (45%)**
Streptococci	Peptostreptococci	Streptococci	Peptostreptococci
Staphylococci	Propionibacterium	Staphylococci	Propionibacterium
Corynebacterium	Clostridium	Corynebacterium	Fusobacterium
Actinobacillus	Fusobacterium	Neisseria	Black Pigmented Bacteroides
Neisseria	Veilonella	Pseudomonas	Bacteroides
Haemophillus	Black Pigmented Bacteroides	Eikonella	
Pseudomonas	Bacteroides		
Eikonella			
Capnocytophaga			

Table 3.6 Antibiotic sensitivity tests for Anaerobic organisms isolated from pus obtained from patients with Localized abscesses and Ludwig's Angina

Antibiotics	Localized abscesses (n=42)	Ludwig's Angina (n=15)	p-value
	No.of resistant isolates (%)	No.of resistant isolates %)	
Streptomycin	31 (73.8%)	8 (53.3%)	0.1975
Gentamycin	31 (73.8%)	11 (73.3%)	1
Cephalosporin	27 (64.3%)	8 (53.3%)	0.542
Trimethoprim	27 (64.3%)	7 (46.7%)	0.3581
Carbenicillin	19 (45.2%)	5 (33.3%)	0.5468
Penicillin	20 (47.6%)	5 (33.3%)	0.3805
Erythromycin	16 (38.1%)	11 (73.3%)	**0.0334 ***
Cloxacillin	29 (69%)	10 (66.7%)	1
Oxytetracyclin	26 (61.9%)	8 (53.3%)	0.76
Clindamycin	18 (42.9%)	8 (53.3%)	0.5543
Amoxycillin	29 (69%)	11 (73.3%)	1
Chloramphenicol	12 (28.6%)	6 (40%)	0.5205

*indicates significant results

Table 3.7 Antibiotic sensitivity tests for Aerobic organisms isolated from pus obtained from patients with Localized abscesses and Ludwig's Angina

Antibiotics	Group 1(n=42)	Group 2(n=15)	p-value
	Resistant isolates (%)	Resistant isolates (%)	
Streptomycin	24 (57.1%)	4 (26.7%)	0.07
Gentamycin	21 (50%)	8 (53.3%)	1
Cephalosporin	11 (26.2%)	3 (20%)	0.739
Trimethoprim	25 (59.5%)	9 (60%)	1
Carbenicillin	9 (21.4%)	8 (53.3%)	**0.0451 ***
Penicillin	11 (26.2%)	7 (46.7%)	0.1975
Erythromycin	14 (33.3%)	7 (46.7%)	0.3709
Cloxacillin	19 (45.2%)	11 (73.3%)	0.0768
Oxytetracyclin	15 (35.7%)	10 (66.7%)	0.0672
Clindamycin	14 (33.3%)	7 (46.7%)	0.3709
Amoxycillin	26 (61.9%)	7 (46.7%)	0.3685
Chloramphenicol	6 (14.3%)	2 13.3%)	1

*indicates significant results

3.5 Antibiotic sensitivity

Antibiotic sensitivity tests were not done on individual organisms but on mixed flora however aerobic and anaerobic bacteria were tested separately. The commonly used antibiotics (Tables 3.6 & 3.7) were tested for resistance. When the number of resistant isolates from both the groups was compared, high number of anaerobic isolates and aerobic isolates from patients with Ludwig's angina were resistance to erythromycin and carbenicillin respectively.

Table 3.8 Enzymatic analysis of pus obtained from patients with Localized abscesses and Ludwig's Angina

Enzyme	Localized abscesses (n=42)	Ludwig's Angina (n=15)	p-value
	No. of patients (%)	No.of patients (%)	
E2-Alkaline phosphatase	39 (92.9%)	15 (100%)	0.5586
E3-Esterase (C4)	31 (73.8%)	13 (86.7%)	0.4779
E4-Esterase Lipase (C8)	36 (85.7%)	14 (93.3%)	0.6622
E5-Lipase (C14)	1 (2.38%)	0 (0%)	1
E6-Leucine arylamidase	39 (92.9%)	15 (100%)	0.5586
E7-Valine arylamidase	40 (95.2%)	15 (100%)	1
E8-Cystine arylamidase	21 (50%)	6 (40%)	0.5595
E9-Trypsin	3 (7.14%)	3 (20%)	0.1799
E10-α-chymotrypsin	1 (2.4%)	0 (0%)	1
E11-Acid phosphatase	39 (92.9%)	15 (100%)	0.5586
E12-Naphthol-AS-BI-phosphohydrolase	42 (100%)	15 (100%)	
E13-α-galactosidase	6 (14.3%)	5 (33.3%)	0.1307
E14-β-galactosidase	30 (71.4%)	13 (86.7%)	0.3118
E15-β-glucuronidase	40 (95.2%)	15 (100%)	1
E16-α-glucosidase	34 (81%)	13 (86.7%)	1
E17-β-glucosidase	6 (14.3%)	5 (33.3%)	0.136
E18-N-acetyl-β-glucosaminidase	39 (92.9%)	13 (86.7%)	0.599
E19-α-mannosidase	4 (9.5%)	3 (20%)	0.3645
E20-α-fucosidase	3 (7.1%)	2 (13.3%)	0.599

3.6 Enzymatic analysis

Enzymatic analysis was done directly on pus samples. Results of enzymatic analysis as described in Table 3.8 showed no significance between the two study groups. Alkaline phosphatase, Esterase (C4), Esterase Lipase (C8), Leucine arylamidase, Valine arylamidase, Acid phosphatase, Naphthol-AS-Bl-phosphohydrolase, β-galactosidase, β-glucuronidase, α-glucosidase, and N-acetyl- β-glucosaminidase were commonly found in all the patients tested regardless of the type of infection.

4 DISCUSSION

4.1 Haematology

When an organism infects the body, the defense systems already in place may well be sufficient to prevent replication and spread of the infectious agent, thereby preventing development of disease. These establishment mechanisms are referred to as constituting the innate immune system. However, should innate immunity be insufficient to prevent the invasion by the infectious agent, so-called adaptive immune system then comes into action. The major elements that contribute to the innate immune system are soluble factors such as lysozyme, complement, C-reactive protein and interferon and cells such as phagocytes and natural killer cells. C-reactive protein is an acute phase protein and it is non-specific and raised in bacterial infection. Its role is to cover or coat bacteria so that it is ingested and eliminated by phagocytosis. The levels of C-reactive protein increase dramatically during inflammation (Mims et al, 2004).

In our results, CRP was significantly higher in patients with Ludwig's angina compared to the patients with odontogenic abscesses which suggests that the inflammatory response is very high amongst the Ludwig's angina group and the infection is severe and aggressive due to bacteremia.

White blood cells count (WBC) in patients blood measures polymorphonuclear neutrophils, monocytes, lymphocytes, eosinophils and monocytes. They are part of innate and adaptive immunity. In our results, WBC of both the groups was high suggesting presence of bacterial infection.

The WBC of patients with Ludwig's angina was significantly high suggesting that these patients had severe infection compared to the patients with odontogenic abscesses.

During severe infections, patients become dehydrated and as a consequence urea level in blood increases. In our results, urea levels in both the study groups went high, however, urea levels of Ludwig's angina was significantly higher. This shows that these patients were severely infected and dehydrated compared to the group with odontogenic abscesses.

The rest of the results showed no significant difference between the two study groups.

4.2 Immunology

Antibodies are part of adaptive immunity. They are immunoglobulin molecules which are synthesized by host B lymphocytes when they make contact with an infectious microbe, which acts as a foreign antigen. Each antibody has two identical recognition sites that are complementary in shape to the surface of the foreign antigen and which enable it to bind with varying degree of strength to that antigen. There are five major immunoglobulin classes in humans namely IgG, IgA, IgM, IgD and IgE. IgG is most abundant in the body whereas IgA protects external surfaces by existing in body secretions. IgM is very efficient against bacteremia and IgD is a lymphocyte receptor. IgE initiates inflammation raised in parasitic infections and causes allergy symptoms. IgE has a backbone site with a high affinity for specific receptors on the surface of mast cells. When microbial antigen attaches to these cell-bound antibodies, the surface receptors are cross-linked and transduces a signal to the interior of the cell.

This signal leads to the release of mediators capable of increasing vascular permeability and inducing polymorph chemotaxis (Mims et al, 2004).

In our results, IgE level was significantly lower in patients with Ludwig's angina compared to patients with odontogenic abscesses. It is uncertain why there is this reversal of pattern. It may suggest an immunological response to some organisms and one needs to exclude allergies and any correlation with asthmatics.

IL6 is a cytokine produced in response to infection and is a precursor to allowing the host to make more acute phase proteins (CRP). Both the study groups showed an increase in IL6 with patients with Ludwig's angina being higher. This is in keeping with non specific immunological response to bacterial infection and may explain the associated increase in CRP.

CIC (Circulating Immune Complexes) is a marker of increased antigen-antibody complexes not being removed from circulation. It is increased in both groups which indicates the host response to antigens is efficient. However patients with Ludwig's angina showed a significant decrease in CIC response and may give some clue to the severity and increased duration of these infections.

4.3 Microbiology

Odontogenic abscesses and Ludwig's angina are polymicrobial infections where both aerobic and anaerobic bacteria are involved. In my study, aerobic and anaerobic bacteria were found. Many studies have shown that majority of the isolates are streptococci, *Peptostreptococcus, Prevotella* and *Porphyromonas* (black pigmented bacteroides) and *Fusobacterium* (Gill and Scully, 1990, Sandor et al, 1998, Baker and Fotos, 1994, Peterson LJ, 1991, Kuriyama et al, 2000a, 2000b,Lewis et al, 1986). Majority of the patients in our study, 93% in odontogenic abscess group and 87% in Ludwig's angina group carried *streptococci. Streptococci* are generally known to be present at the initial stages of disease which manifest as cellulitis. The spread will occur due to the spreading enzymes such as fibrinolysin and haemolysins (Table 4.1) produced by these bacteria. It has also been stated that *streptococci* may also be important in the early phases of abscess formation as capable of providing a reduced environment for subsequent anaerobic invasion (Aderhold et al, 1981, Lewis et al, 1986, Kuriyama et al, 2000b). Anaerobes are particularly important in the ensuing phase characterized by suppuration and abscess formation. This model of microbial succession is consistent with the negative association found in clinical infections between facultative *streptococci* and strict anaerobes and also with the experimental observation that anaerobic gram-negative rods tend to produce necrotic abscesses, whereas facultative *streptococci* produce either diffuse inflammatory reaction or more frequently localized abscesses without peripheral necrosis (Lewis et al, 1988). However, the presence of *streptococci* in both the study groups was statistically non significant and therefore, the severity of Ludwig's angina is not due to the streptococci.

Table 4.1 Toxins and enzymes produced by *Streptococci*

Toxin/Enzyme	Activity
Hyaluronidase	Connective tissue breakdown
Haemolysin	Lysis of red blood cells
Streptokinase	Breakdown blood clots
Betaglucuronidase	Breakdown glucuronidic linkages
Proteases	Breakdown proteins
Capsule formation	Bypass phagocytosis
DPNase	Cardiotoxic and leucotoxic

The presence of *Staphylococci* and black pigmented bacteroides which consist of *Porphyromonas* and *Prevotella* was significantly different in both the groups. More patients with Ludwig's angina carried these organisms. *Staphylococci* are gram positive cocci in clusters. *Staphyloccus aureus* causes both common and uncommon infections. Higher proportion is found in the saliva of healthy people and on skin. A variety of virulence factors are produced by *Staphylococci* to facilitate entry, spread and damage to the host (Table 4.2). They are usually aggressive and in infection can be fatal.

Table 4.2 Toxins and enzymes produced by *Staphylococcus aureus*

Toxins/Enzyme	Activity
Toxin:	
Cytotoxins (α, ß, γ, δ)	Cell lysis
Leucocidin	Kills leucocytes
Epidermolytic toxin	Exfoliation and splitting of epidermis
Toxic shock syndrome toxin	Shock, rash, desquamation
Enterotoxin (A-E)	Induces vomiting and diarrhoea
Enzymes:	
Coagulase	Clots plasma
Catalase	Affects bactericidal activity of polymorphs
Hyaluronidase	Connective tissue breakdown
DNAase (nuclease)	DNA hydrolysis
Lipase	Breaks lipids of cell membrane
Penicillinase	Breakdown ß-lactam drugs
Protein A	Antiphagocytic

Black-pigmented bacteroides now called *Porphyromonas* and *Prevotella species* are gram negative rods and obligate anaerobes. They are generally found in subgingival plaque and sometimes in genitourinary tract. They are aggressive periodontal pathogens in humans and animals. They have many virulence factors that enables them to get established in the host, spread and cause severe damage (Table 4.3).

Table 4.3 Toxins and enzymes produced by *Porphyromonas* and *Prevotella species*

Toxins/Enzymes	Activity
Fimbriae	Mediate adhesion
Capsule	Defense against phagocytosis
Haemolysin	Lysis of red blood cells
Collagenase	Hydrolyse collagen fibres
Gelatinase	Breakdown gelatine
Protease	Breakdown protein
Lipopolysaccharides	Stimulate bone resorption
Phospholipase A	Mediate bone resorption
Alkaline and acid phosphatase	Alveolar bone breakdown
Fibroblast inhibitory factor	Impair healing
Volatile sulfur compounds	Increase the permeability of oral mucosa
Polyclonal B cell activator	Induce damaging host-mediated reactions

4.4 Antibiotic sensitivity

Antibiotic sensitivity was done directly from the pus sample instead of each isolate because the results can be obtained in one week. However, the sensitivity plates were incubated in aerobic and anaerobic conditions.

Anaerobic organisms were resistant in more than 50% of patients to streptomycin, gentamycin, cephalosporin, cloxacillin, oxytetracyclin and amoxicillin in both the groups (Table 3.6). However, more isolates from Ludwig's angina (73.3%) were resistant to erythromycin compared to the isolates from patients with odontogenic abscesses.

Aerobic isolates were generally sensitive to all the antibiotics in both the groups except for cloxacillin and oxytetracyclin in Ludwig's angina patients. There was a significant difference in resistances to carbanicillin between both the groups with Ludwig's angina being more resistant.

It is well established that the selected antibiotic against orofacial odontogenic infections should be effective against both streptococci and anaerobes (Kuriyama et al, 2000a, Sobottka et al., 2002). Erythromycin has been found to be a poor choice against streptococci and there is an emerging resistance to clindamycin. The lack of susceptibility may predict resistance to half-life macrolids such as azythromycin and clarithromycin. Penicillin remains still a drug of choice for aerobic bacteria (Kuriyama et al, 2000a). In our hospitals 26.2-46.7% of isolates from both the study groups were resistant to penicillin suggesting that it still can be used to treat patients (Table 3.6 & 3.7). Metronidazole is only effective against obligate anaerobes (Hupp J.R, 2002).

Metronidazole sensitivity was not performed because anaerobes are widely known to be sensitive to this drug. The combination of penicillin with metronidazole adequately covers the microbial flora of odontogenic abscesses (Flynn TR, 2000, Sbottka et al, 2002).

4.5 Enzymatic analysis of pus

Wide variety of enzymes which were studied in this report can be elaborated from oral gram-negative species and they are capable of acting on a variety of host macromolecules, such as constituents of connective tissue, complement components and immunoglobulins. Human polymorphonuclear granulocytes and other host cells furthermore produce many of the enzymes which can be detected in bacteria (Smolen and Weissmann, 1978), and the relative importance of bacterial enzymes and host-derived enzymes in the inflammatory process is unknown. In our study there was no significant difference between the two study groups in the presence of enzymes.

Alkaline phosphatase can be of bacterial or host origin (Poelstra et al, 1997). In bacteria, alkaline phosphatase is located in the periplasmic space, external to the cell membrane. Since this space is much more subjected to environmental variation than the actual interior of the cell, this enzyme is resistant to inactivation, denaturation and degradation and also has a higher rate of activity. In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, kidney, bone and the placenta. In our study majority of the pus samples from both the groups of patients showed the presence of this enzyme and it appears to have bacterial origin.

Esterase (C4) and Esterase Lipase (C8) are also found in some bacteria as well as in tissue generally produced by white blood cells. In our study (Table 3.8) most of the patients in both the study groups produced these enzymes which suggest that there was pus with white blood cells in the aspirated samples.

Leucine arylamidase and Valine arylamidase are produced by some bacteria such as *Pseudomonas* and fungi such as *Candida albicans*. Our results shows that many other bacteria produce these enzymes because 93-100% of the pus samples collected from patients in both the groups showed the presence. However there was no significant difference in the results from both the groups. Acid phosphatase is normally produced by many gram negative bacteria. The presence of this enzyme in the pus samples is probably due to the many species of gram negative bacterial isolates. Naphthol-AS-Bl-phosphohydrolase, β-galactosidase, β- glucuronidase, α-glucosidase and N-acetyl- β-glucosaminidase are produced by humans as well as bacteria. The origin of these enzymes, in pus samples cannot be established. However, they were present in both the groups of patients.

5 CONCLUSIONS

Patients with Ludwig's angina had significantly high c-reactive protein and white blood cells suggesting that it is a severe, aggressive bacterial infection and the inflammatory response is high compared to patients with odontogenic abscesses. Urea was significantly low suggesting that patients with Ludwig's angina were more dehydrated.

In our results, IgE level was significantly lower in patients with Ludwig's angina compared to patients with odontogenic abscesses. It is uncertain why there is this reversal of pattern. It may suggest an immunological response to some organisms and one needs to exclude allergies and any correlation with asthmatics.

IL6 is a cytokine produced in response to infection and is a precursor to allowing the host to make more acute phase proteins (CRP). Both the study groups showed an increase in IL6 with patients with Ludwig's angina being higher. This is in keeping with non specific immunological response to bacterial infection and may explain the associated increase in CRP.

CIC (Circulating Immune Complexes) is a marker of increased antigen-antibody complexes not being removed from circulation. It is increased in both groups which indicate the host response to antigens is efficient. However patients with Ludwig's angina showed a significant decrease in CIC response and may give some clue to the severity and increased duration of these infections.

Highly virulent bacteria such as *Staph aureus* and *Porphyromonas/Prevotella* are responsible for the infection that develops into Ludwig's angina. Anaerobic bacteria isolated from patients with Ludwig's angina were resistant to erythromycin and aerobic bacteria to carbanicillin.

There was no significant difference in the presence of enzymes in pus samples between the two study groups.

6 APPENDIX

CONSENT FORM ETHICAL APPROVAL

DEAR PATIENT,
My name is Theo Chettiar. I am a registrar working in the Division of Oral and Maxillofacial surgery at the Johannesburg and Baragwanath hospitals.
I am currently undertaking research on infections caused by teeth to better understand how they develop. The study involves the collection of pus and blood which are collected for microbiological and blood chemistry analysis as well as HIV testing. Please note that these are normal procedures for all septic patients. A total of 35 ml of blood will be drawn over a 48 hour period for all of above tests. The results of the above tests will not be disclosed to anyone. Confidentiality will be maintained at all times.

Participation in this study is voluntary and you are free to refuse to participate or to withdraw your consent and to discontinue participation at any time. Such refusal or discontinuance will not affect your regular treatments or medical care in any way. A signed copy of this consent form will be made available to you.

We appreciate your participation in this study in the hope that the results will improve management of these types of cases in the future.

I have fully explained the procedures, identifying those that are investigational, and have explained their purpose. I have asked if any questions have arisen regarding the procedures and have answered these questions to the best of my ability.

Date:---------- Doctor:----------
Contact number 0834919994 Dr.T.P. Chettiar

I have been fully informed of the procedures to be followed, including those that are investigational. I have been given a description of the attendant discomforts, risks and benefits to be expected and that refusal to participate in the study will not in any way compromise my treatment management. I understand also that if I have any questions at any time, they will be answered.

Date:---------- Patient(parent or guardian)----------

7 REFERENCES

Aderhold L., Knothe H., Frenkel G. The bacteriology of dentogenous pyogenic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1981; 52: 583-7.

Baker K.A.and Fotos P.G. The management of odontogenic infections. A rationale for appropriate chemotherapy. *Dent Clin North Am* 1994; 38: 689-706.

Bennett J.E., Dolin R., editors. Mandell, Douglas and Bennett's principles and practice of infectious diseases. 4th ed. Edenburgh: Churchill Livingstone; p. 2561-70.

Bennett K.W. and Eley A. Fusobacteria: new taxonomy and related diseases. *J Med Microbiol* 1993; 39: 246-54.

Bartlett J.G. Anaerobic bacteria. In: Gorbach S.L., Bartlett J.G., Blacklow N.R., editors. Infectious diseases. 2nd ed. Philadelphia Saunders. 1998; P. 1888-901.

Brook I., Frazier E.H., Gher M.E. Aerobic and anaerobic microbiology of periapical abscess. *Oral Microbiol Immunol* 1991; 6: 123-5.

Brook I., Gillmore J.D., Coolbaugh J.C., Walker R.I. Pathogenicity of encapsulated Bacetoides melaninogenicus group, B. oralis and B. ruminicola subsp. Brevis in abscesses in mice. *J Infect* 1983; 7: 218-26.

Brook I. and Walker R.I. The role of encapsulation in the pathogenesis of anaerobic gram-positive cocci. *Can J Microbiol* 1985; 31: 176-80.

Cotran R.S., Kumar V., Collins T. Robbins pathological basis of disease. 2nd edition. Philadelphia : Saunders, 1999: 50-86

Cross A.S. Inducing an abscess. *Lancet* 1994; 343: 248-9.

Chow A.W. Life-threatening infections of the head and neck. Clin Infect Dis 1992; 14: 991-1004.

Chow A.W. Infections of the oral cavity, neck and head. In: Mandell G.L., Bennett J.E., Dolin R. editors. Mandell, Douglas and Bennett's principles and practice of infectious diseases. 4th ed. Edinburgh: Churchill Livingstone 2000; p 689-702.

Drucker D.B., Gomes B.P.F.A., Lilley J.D. Role of anaerobic species in endodontic infection. *Clin Infect Dis* 1997; 25: (Suppl 2): S220-1.

Dymock D., Weightman A.J., Scully C., Wade W.G. Molecular analusis of microflora associated with dentoalveolar abscesses. *J Clin Microbiol* 1996; 34: 537-42.

Duerden B.I. Virulence factors in anaerobes. *Clin Infect Dis* 1994; 18(Suppl 4): S253-9.

Fritsch D.E. and Klein D.G. Ludwig's angina. *Heart and Lung* 1992, 21: 39-46.

Fisher L.E. and Russell R.R.B. The isolation and characterization of milleri group streptococci from dental periapical abscesses. *J Dent Res* 1993; 72: 1191-3.

Finegold S.M. Overview of clinically important anaerobes. *Clin Infect Dis* 1995; 20(Suppl 2): S205-7.

Flynn T.R. The swollen face: severe odontogenic infections. *Emerg Med Clin North Am* 2000; 18: 481-519.

Gill Y. and Scully C. Orofacial odontogenic infections: review of microbiology and current treatment. *Oral Surg Oral Med Oral Pathol* 1990; 70: 155-8.

Grenier D.and Mayrand D. Nutritional relationships between oral bacteria. *Infect Immun* 1986; 53: 616-20.

Hofstad T. Pathogenicity of anaerobic gram-negative rods: possible mechanisms. *Rev Infect Dis* 1984; 6: 189-99.

Heimdahl A., Von Konow L., Satoh T, Nord C.E. Clinical appearance of orofacial infections of odontogenic origin in relation to microbiological findings. *J Clin Microbio* 1985; 22: 299-302.

Hupp J.R. Antimicrobial, antiviral, and antifungal agents. Oral Maxillofac *Surg Clin North Am* 1991; 3: 273-85.

Hupp J.R. Antimicrobial pharmacology for maxillofacial infections. In: Topazian R.G., Goldberg M.H., Hupp J.R., editors. Oral and maxillofacial infections. 4th ed. Philadelphia: Saunders 2002; p. 112-25.

Har-el G., Aroesty J.H., Shaha A., et al. Changing trends in deep neck space abscess: A reptospective study of 110 patients. *Oral Surg Oral Med Oral Pathol* 1994; 77: 446-50.

Janeway C.A and Travers P. The Immune System in Health and Disease. Oxford : Garland Publishers 1994: 1:18-1:34

Jenkins S.G. Infections due to anaerobic bacteria and the role of antimicrobial susceptibility testing of anaerobes. *Rev Med Microbiol* 2001; 12: 1-12.

Johnson B.S. Principles and practice of antibiotic therapy. *Infect Dis Clin North Am* 1999; 13: 851-70.

Johnson W. S., Devine K.D., Wellman W.E., Fischback J.E. Ludwig's angina. Concept of therapy with report of a case. *Oral Surg Oral Med Oral Pethol* 1963; 16: 1023.

Kolokotronis A. β-lactamases producing anaerobic bacteria in dentoalveolar abscesses. *J Oral Dis* 1999; 41: 187-90.

Kuriyama T., Karasawa T., Saiki Y.,Yamamoto E., Nakamura S. Baceriologic features and antimicrobial susceptibility in isolates from orofacial odontogenic infections. *Oral surg Oral Med Oral Pathol Oral Radiol Endod* 2000a; 90: 600-8.

Kuriyama T., Nakagawa K., Karasawa T., Saiki Y., Yamamoto E., Nakamura S. Past administration of β-lactam antibiotics and increase in the emergence of β-lactamase-producing bacteria in patients with orofacial odontogenic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000b; 89: 186-92.

Klempner M.S. Interations of polymorphonuclear leucocytes with anaerobic bacteria. *Rev Infect Dis* 1984; 6(Suppl 1): S40-4.

Lewis M.A.O., Parkhurst C.L., Douglas C.W.I., Martin M.V., Absi E.G., Bishop P.A. et al. Prevalence of penicillin resistant bacteria in acute suppurative oral infection. *J Antimicrob Chemother* 1995; 35: 785-91.

Lewis M.A.O., MacFarlane T.W., McGowan D.A. Quantitative bacteriology of acute dento-alveolar abscesses. *J Med Microbiol* 1986; 21: 101-4.

Lewis M.A.O., MacFarlane T.W., McGowan D.A., MacDonald D.G. Assessment of the pathogenicity of bacterial species isolated from acute dentoalveolar abscesses. *J Med Microbiol* 1988; 27: 109-16.

Lewis M.A.O., MacFarlane T.W., McGowan D.A. A microbiological and clinical review of the acute dentoalveolar abscess. *Br J Oral Maxillofac Surg* 1990; 28: 359-66.

Lorber B. Bacteroides ,Prevotella, Porphyromonas, ans Fusobacterium species and other medically important anaerobic gram-negative bacilli). In: Mandell G.L., Bennett J.E.,

Dolin R., editors. Mandell, Douglas and Bennett's principles ans practice of infectious diseases. 4th ed. Edinburgh: Churchill Livingstone; 2000. p.2561-70.

Loesche W.J. Oxygen sensitivity of various anaerobic bacteria. *Appl Microbiol* 1969; 18: 723-7.

Murdoch D.A. Gram-positive anaerobic cocci. *Clin Microbiol Rev* 1998; 11: 81-120.

Mims C., Dockrell H.M., Goering R.V., Roitt I., Wakelin D, Zuckerman M. The innate defenses of the body. In Medical Microbiology 3rd ed. Mosby, Edinburgh, London, New York, Oxford, Philadelphia, St Louis, Sydney, Toronto 2004. p. 71-86.

Murakami Y., Hanazawa S., Tanaka S., Iwahashi H., Yamamoto Y., Fujisawa S. A possible mechanism of maxillofacial abscess formation: involvement of *Porphyromonas endodontalis* lipopolysaccharide via the expression of inflammatory cytokines. *Oral Microbio Immunol* 2001; 16: 321-5.

National Committee for Clinical Laboratory Standards. Methods for antimicrobial susceptibility tests for bacteria that grow aerobically. 4th edition. Approved standard. NCCLS document M7-A4. Villanova: National Committee for Clinical Laboratory Standards, 1998.

Nord C.E., Heimdahl A., Tunér K. Beta-lactamase producing anaerobic bacteria in the oropharynx and their clinical relevance. *Scand J Infect Dis* 1988; 57(Sppl): 50-4.

Poelstra K., Bakker W.W., Klok P.A. Hardonk M.J., Meijer D.K. *Lab Invest* 1997; 76: 319-27.

Peterson L. J., Ellis E. III, Hupp J.R., Tucker M.R. Contemporary oral and maxillofacial surgery. 3rd edition. St. Louis, Missouri : Mosby, 1998: 392-417

Peterson L.J. Microbiology of head and neck infections. In: Laskin D.M., Strauss R.A., editors. Oral and maxillofacial surgery clinics of North America. Vol 3. Philadelphia: W.B. Saunders. 1991. P. 247-57.

Peterson L.R. and Thomson R.B. Jr. Use of the clinical microbiology laboratory for the diagnosis and management of infectious diseases related to the oral cavity. *Infect Dis Clin North Am* 1999; 13: 775-95.

Persson S., Edlund M-B, Claesson R, Carlsson J. The formation of hydrogen sulfide and methyl mercaptan by oral bacteria. *Oral Microbiol Immunol* 1990; 5: 195-201.

Rajasuo A., Jousimies-Somer H., Savolainen S., Leppänen J., Murtomaa H., Meurman J.H. Bacteriologic findings in tonsillitis and perocoronitis. *Clin Infect Dis* 1996; 23: 51-60.

Rosen E.A., Shulman R.H., Shaw A.S. Ludwig's angina: a complication of a bilateral mandibular fracture: report of case. *J Oral Surg* 1972; 30: 196-200.

Smolen J.E.and Weissmann G. The granulocyte: metabolic properties and mechanisms of lysosimal enzyme release. In K. Havemann and A. Janoff (ed.), Neutral proteases of human polymorphonuvclear leukocytes. Biochemistry, physiology and clinical significance. Urban & Schwarzenberg, Baltimore. 1978 p. 56-76.

Sandor G.K., Low D.E., Judd P.L., Davidson R.J. Antimicrobial treatment options in the management of odontogenic infections. *J Can Dent Assoc* 1998; 64: 508-14.

Siqueira J.F. Jr, Rôças I.N., Souto R., de Uzeda M., Colombo A.P. Microbiological evaluation of acute periradicular abscesses by DNA-DNA hybridization. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; 92: 451-7.

Sobottka I, Cachovan G., Sturenburg E., Ahlers M. O., Laufs R., Plazer U. In vitro activity of moxifloxacin against bacteria isolated from odontogenic infections. *Antimicrob Agents Chemother* 2002; 46:4019-21.

Siqueira J.F. Jr, Magalhaes F.A.C., Lima K.C., de Uzeda M. Pathogenicity of facultative and obligate anaerobic bacteria in monoculture and combinded with either *Prevotella intermedia or Prevotella nigrescens*. *Oral Microbiol Immunol* 1998; 13: 368-72.

Styrt B. and Gorbach S.L. Recent developments in the understanding of the pathogenesis and treatment of anaerobic infections (first of 2 parts). *New Engl J Med* 1989; 321: 240-6.

Sundqvist G., Bloom G.D., Enberg K., Johansson E. Phagocytosis of Bacteroides melaninogenicus and Bacteroides gingivalis in vitro by human neutrophils. *J Periodont Res* 1982; 17: 113-21.

Takemoto T., Kurihara H., Dahlén G. Characterization of Bacteroides forsythus isolates. *J Clin Microbiol* 1997; 35: 1378-81.

Tally F.P., Goldin B.R., Jacobus N.V., Gorbach S.L. Superoxide dismutase in anaerobic bacteria of clinical significance. *Infect Immun* 1977; 16: 20-5.

Takazoe I., Tanaka M., Homma T. A pathogenic strain of Bacteroides melaninogenicus. *Arch Oral Biol* 1971; 16: 817-22.

Von konow L. Nord C.E., Nordenram A. Anaerobic bacteria in dentoalveolar infections. *Int J Oral Surg* 1981; 10: 313-22.

Wade W.G., Lewis M.A.O., Cheeseman S.L., Absi E.G., Bishop P.A. An unclassified Eubacterium taxon in acute dento-alveolar abscess. *J Med Microbiol* 1994; 40: 115-7.

Way W. Lawrence. Current Surgical Diagnosis and Treatment. 9th Edition. USA : Appleton & Lange. 1991: 6-9