CHAPTER-I

1. INTRODUCTION

Infective endocarditis (IE) is a serious life-threatening infection of endocardium and heart valves caused mostly by bacteria. Other cardiac structures like chordae tendineae, mural endocardium, myocardium and pericardium may be primarily or secondarily infected.

In comparison to other heart diseases, IE is relatively an uncommon disease. Although many developments have taken place with respect to antimicrobial drug therapy in the treatment of disease, its incidence is high, with 3.3 cases per 100,000 population per year in the United Kingdom, with similar figures for the United States and 1.4–4.0 cases per 100,000 population per year in Europe (CDC, 2004). In 2006, American Heart Association reported 29,000 hospital discharges and 2,370 deaths with IE as a primary or secondary diagnosis. So far no national data is available for the incidence rate of IE in Nepal. Shahid Gangalal National Heart Centre (SGNHC), a tertiary care referral centre for heart patients in Nepal, has reported the admission of 27 patients for IE in the year 2007 which constituted 1.31% of the total hospital admissions in that year.

The range of microbial species that cause IE is extraordinarily wide ranging from bacteria, fungi, Mycobacteria, Mycoplasma and Ricketssia. Gram positive cocci have always dominated the scene as major etiological agents (Khanal *et al*., 2004). More commonly encountered organisms include Streptococci, Enterococci, Staphylococci and the HACEK group of organisms (*Haemophilus parainfluenzae, Haemophilus aphrophilus, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella* spp. and *Kingella* spp.).

Despite medical preventive strategies and advances in technology, IE is still associated with high morbidity and mortality rates (Niwa *et al.,* 2005). The overall mortality rate for both native valve and prosthetic valve endocarditis remains as high as 20 to 25% with death resulting primarily from hemodynamic deterioration and central nervous system embolic events (Mylonakis and Calderwood, 2001).

The diagnosis of IE is based on clinical, laboratory and echocardiography findings (Giessel *et al.,* 2000). Positive blood culture is a major diagnostic criterion for IE and is a key in identifying the etiological agent and its antimicrobial susceptibility (Bayer *et al*., 1998). However, blood cultures are persistently negative in 5-10% of patients who satisfy diagnostic criteria for IE often delaying diagnosis and start of treatment with profound impact on clinical outcome (Beynon *et al.,* 2006). New diagnostic approach includes culture and microbiological assessment of vegetations and infected valvular tissue in order to isolate the microorganisms involved in infection and to establish an appropriate postoperative antibiotic treatment which has yielded a better understanding of blood culture-negative endocarditis (Mylonakis and Calderwood, 2001; Renzulli *et al.,* 2000). Further, the guidelines of European Society of Cardiology (ESC) recommend culturing and examining all infected or possibly infected cardiac tissue excised during cardiac surgery from patients with active or suspected infective endocarditis irrespective of preoperative cultural results.

Early prosthetic valve endocarditis (Early PVE) is an infrequent complication but is serious and possibly lethal (Lepidi *et al.,* 2005). Such infections are caused mainly because of contaminations during surgery with antimicrobial-resistant bacteria. Further, a positive post-surgery valve culture is a significant risk factor for post-operative colonization of newly implanted prostheses and hence subsequent relapse in the form of early PVE (Murashita *et al.,* 2004). This is always a medical emergency that must be managed immediately which warrants early diagnosis and aggressive medical and surgical therapy to prevent mortality and long term morbidity.

Hence the present study of microbiological and histopathological investigation of the resected valves and vegetation from the treated and suspected IE patients is utmost important at the earliest possible moment in guiding an optimal post-operative antimicrobial therapy. Further, the study also helps in diagnosing concealed IE in intra-operatively suspected cases (Chuard *et al.,* 1998).

CHAPTER-II

2. OBJECTIVES

2.1 GENERAL OBJECTIVES

To carry out bacteriological and histopathological study of the heart valves resected from the treated and suspected cases of Infective Endocarditis (IE).

2.2 SPECIFIC OBJECTIVES

- To isolate and identify bacteria from resected heart valve samples.
- To determine the antibiotic susceptibility pattern of the bacterial isolates.
- To examine Hematoxylin-Eosin and Tissue-Gram stained resected heart valve sections histopathologically
- To correlate cultural findings with histological findings.
- To identify the risk factors of infective endocarditis.

CHAPTER-III

3. LITERATURE REVIEW

3.1 HISTORY

The history of infective endocarditis spans more than 350 years when the disease was first described by a French Renaissance physician, Jean François Fernel in 1554 and its effective therapy started approximately 50 years ago. Yet, IE continues to become a disease of diagnostic and therapeutic challenge (Brusch, 2007).

The history of IE can be divided into several eras. Lazaire Riviere first described gross autopsy findings of the disease in 1723. In 1885, William Osler presented the first comprehensive description of endocarditis in English. Lerner and Weinstein presented a thorough discussion of this disease in modern times in their landmark series of articles, 'Infective Endocarditis in the Antibiotic Era' published in 1966 in the New England Journal of Medicine. Since 1980s, IE could be described as the disease of the era of intravascular devices.

3.2 INFECTIVE ENDOCARDITIS AS A DISEASE

The term Infective Endocarditis has been variously defined. Infective endocarditis is a serious life-threatening infection of endocardium and heart valves caused mostly by bacteria.

Infective endocarditis refers to an active intracardiac infection that resides on one or more heart valves. Other cardiac structures like chordae tendineae, mural endocardium, myocardium and pericardium can be primarily or secondarily involved. Endovascular infection can also occur at more remote sites, in association with aortic coarctation, patent ductus arteriosus or surgically constructed vascular shunts (Patrick, 2007).

Center for Disease Control and Prevention (CDC) has defined endocarditis as a non-contagious chronic infection of the valves or lining of the heart that is mainly caused by bacteria, although fungi may also be associated.

European Society of Cardiology (ESC) defines IE as an endovascular microbial infection of intracardiac structures facing the bloodstream including infections of large intrathoracic vessels and of intracardiac foreign bodies. Formerly known as bacterial endocarditis, endocardial infections are currently named infective endocarditis in order to include both bacteria and fungi (Horstkotte *et al.*, 2004).

Bacterial endocarditis is an infection of inner surface of heart or heart valves caused by bacteria usually found in mouth, intestinal tract or urinary tract. It can occur with many congenital heart defects but is most common in aortic valve lesions, patent ductus arteriosus (unrepaired), Tetralogy of Fallot, ventricular septal defects, coarctation of aorta and mitral valve prolapse with mitral regurgitation (Brookfield, 2005).

3.3 ANATOMY AND PHYSIOLOGY

Heart is a pumping organ of the cardiovascular system. Its function is to maintain a constant circulation of blood throughout the body.

3.3.1 Endocardium

The endocardium is the innermost layer of tissue that lines the chambers of the heart, myocardium and heart valves. It is a thin, smooth, glistening membrane which permits smooth flow of blood inside the heart (Waugh and Grant, 2001). It is a fibrous layer lined with simple squamous epithelial tissue (endothelium) and some connective tissue. Its cells are embryologically and biologically similar to the endothelial cells that line blood vessels.

3.3.2 Heart Valves

Heart valves are thin flexible flaps of connective tissue being located on each end of the two ventricles through which blood passes before leaving each chamber of the heart. The valves prevent the backward flow of blood.

Structure of heart valves

A heart valve consists of flap-like folds called cusps or leaflets of endocardium reinforced by dense connective tissue which is continuous with that of fibrous rings of skeleton of heart. These rings completely surround the outlet through which blood flows and prevent an outlet from becoming dilated when the heart contracts and forces blood through it.

Types of heart valves

There are four valves in the heart: two atrioventricular (AV) valves and two semilunar valves. The right AV valve is known as tricuspid valve and the left AV valve is called bicuspid valve or mitral valve. Free ends of their cusps are attached to strong yet delicate tendinous cords called chordae tendineae which are continuous with nipple-like papillary muscles in the wall of the ventricle.

The right semilunar valve is called pulmonary valve and the left semilunar valve is an aortic valve. Each semilunar valve contains three cusps which lack chordae tendineae.

3.4 PATHOGENESIS

The rarity of endocarditis despite frequent transient bacteraemia indicates that the normal healthy endocardium is resistant to infection when challenged transiently by small numbers of organisms in the circulation of an immunocompetent host (Collee *et al.*, 2006; Thiene, 2006). It is probably because microorganisms do not easily adhere to the endocardial surface and constant blood flow helps prevent them from settling on endocardial structures (Pelletier, 2006).

The most accepted mechanism for the development of IE is ‘Injury-Thrombus-Infection Theory’ (Thiene, 2006). According to this theory, the development of IE requires an interaction among the following factors: (a) host factors that predispose the endothelium to infection, (b) circumstances that lead to transient bacteraemia and (c) the tissue tropism and virulence of the circulating bacteria (Mornos and Ionar, 2006).

The endocardium may be damaged congenitally, nosocomially (e.g., during surgery), immunologically (e.g., in Rheumatic heart disease), as a result of previous valvular heart

diseases (e.g., past-IE), or simply as a consequence of old age (like in degenerative heart disease). Prosthetic valves pose a particular risk (Pelletier, 2006).
Any damage or alteration on endothelium stimulates hemostasis by triggering coagulation which leads to the deposition of platelets and fibrin. The resulting platelet-fibrin complex builds up a sterile vegetation called non-bacterial thrombotic endocarditis (NBTE) which is more receptive to colonization by blood-borne bacteria than is the intact endothelium (Collee *et al.,* 2006; Hardley, 2004; Mornos and Ionar, 2006).

The second factor is bacteraemia which results in the colonization of NBTE converting sterile vegetation into an infective one (Thiene, 2006). Most of the transient bacteraemia are short-lived, often not preventable and are of without any consequence. Bacteria rarely adhere to an endocardial nidus before which they get removed from the circulation by various host defenses (Brusch, 2007). However heavy challenges that accompany intravenous drug abuse, the use of indwelling intravascular catheters in a debilitated host and the tissue injuries associated with cardiac surgery or the use of indwelling intra-cardiac devices may allow acute infective endocarditis to occur (Collee *et al.,* 2006).

Once microorganisms establish themselves on the surface of vegetation, platelet aggregation and fibrin deposition accelerate at the site. As the bacteria multiply, they get covered by ever-thickening layers of platelets and thrombin, which protect them from neutrophils, immunoglobulin, complement and other host defenses. Organisms deep in the vegetation hibernate because of paucity of available nutrients and are therefore less susceptible to bactericidal antimicrobials that interfere with bacterial cell wall synthesis (Brusch, 2007). This web of platelets, fibrin, inflammatory cells and entrapped organisms is called infective vegetation. The resulting vegetation ultimately seed bacteria into the blood at a slow but constant rate (Forbes *et al.*, 1998).As the valves of the heart do not actually receive any blood supply of their own, once an organism establishes a hold on the valves forming vegetation, the body cannot easily get rid of them.

The coincidence of bacteraemia and NBTE does not uniformly result in IE. To cause IE, the organism must be able to persist and propagate on the endothelium. This requires tissue tropism, virulence of the circulating bacteria and their resistance to host defenses (Mornos and Ionar, 2006). Gram positive cocci (mostly, a few species of Streptococci, Staphylococci

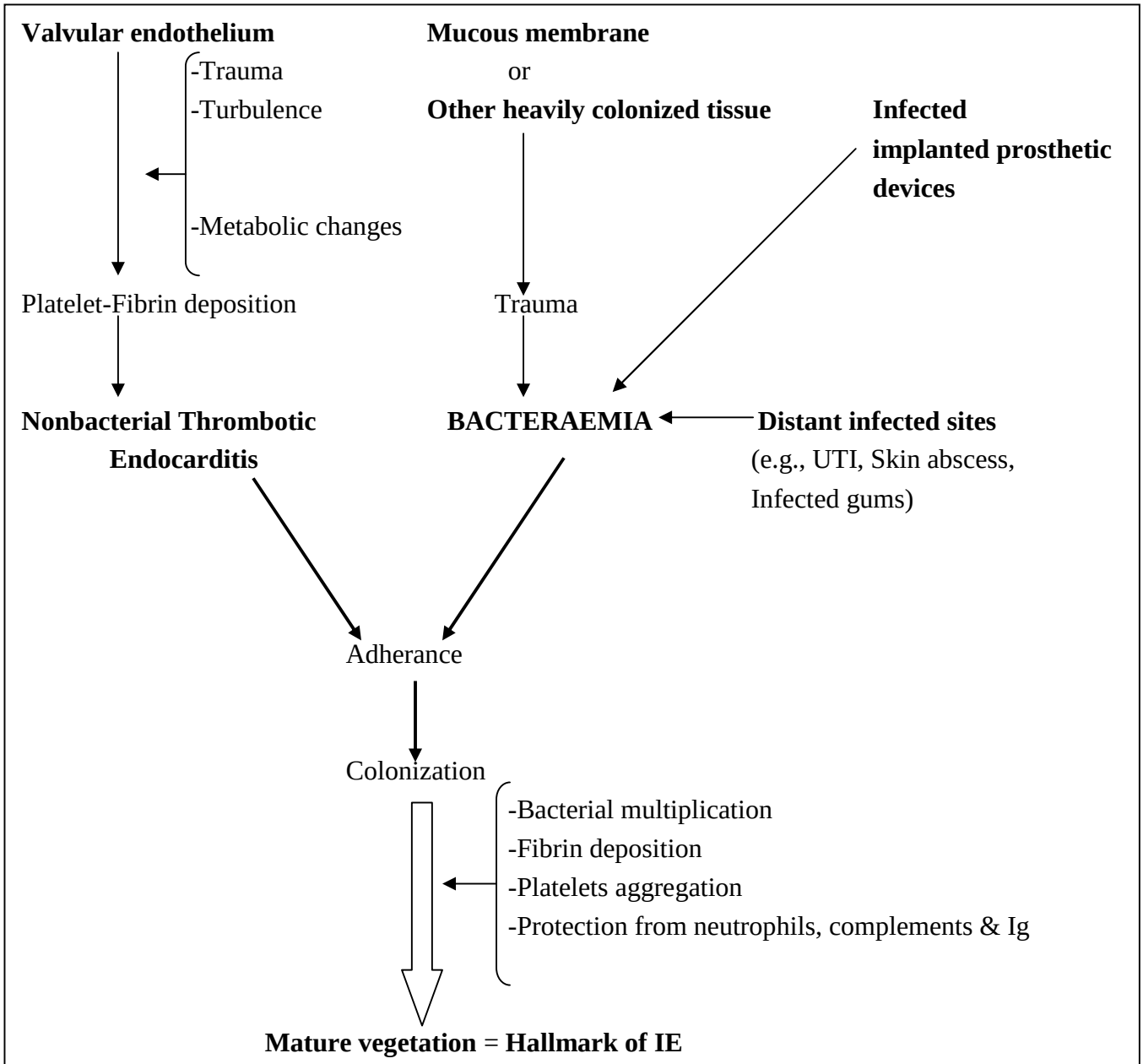


Figure 1: Proposed mechanism for the Pathogenesis of IE.

(Source: Arnold and Scheld, 1997)

and Enterococci) have always dominated the scene as major etiological agents (Khanal *et al.*, 2004) because they resist bactericidal action of complement and possess fibronectin receptors for the surface of fibrin-platelet thrombus. Among many other characteristics of IE-producing bacteria demonstrated in vitro and in vivo, some features include the following (Brusch, 2007):

- Glucan production by certain strains of Viridans streptococci,
- Mucoid production by few strains of *Staphylococcus aureus*
- Platelet aggregation as shown by *S. aureus* and *Streptococcus sanguis*
- Resistance to platelet microbicidal proteins as shown by *S. aureus*
- Increased adherence to heart valves by Enterococci, Viridans streptococci, *S. aureus*
- Possession of FimA surface adhesin by Viridans streptococci and Enterococci

Gram negative bacilli other than HACEK group are regarded as less frequent cause of IE. Low adhesiveness of gram negative enteric bacilli to heart valve and fibrin and susceptibility of many strains to complement mediated bacteriolysis probably are the causes of this disparity (Khanal *et al.,* 2004).

3.5 MICROBIOLOGY OF INFECTIVE ENDOCARDITIS

The range of microorganisms that causes IE is extraordinarily wide including bacteria, fungi, Mycoplasmas, Rickettsias, Chlamydia and protozoan parasites. The frequency of the occurrence of particular microbial species in endocarditis partly reflects the frequency of their occurrence in the bloodstream and partly indicates their relative tendency to adhere to fibrin, their relative virulence and their relative resistance to host defenses. Thus gram positive cocci particularly Viridans streptococci and Staphylococci are high in the league, whereas gram negative aerobic bacilli like coliform organisms, which adhere poorly to fibrin, are low (Collee *et al.,* 2006).

POSSIBLE PATHOGENS OF INFECTIVE ENDOCARDITIS

Infective endocarditis may be caused by a wide range of organisms though some special associations are well recognized. They are as follows:

Viridans streptococci

Viridans streptococci form a mixed group of microorganisms found primarily in oral cavity, which include *Streptococcus sanguis*, *S. mitis*, *S. salivarius*, *S. mutans* and *Gemella morbillorum,* formerly called *S. morbillorum* (Hoen, 2006). Many bacteria can cause endocarditis in patients with underlying valve problems, but Viridans streptococci are responsible for approximately half of all bacterial endocarditis (Gandhi, 2006) and

approximately 40% cases of subacute bacterial endocarditis (Giessel *et al.*, 2000). They tend to localize in the damaged and weakened valve cusps. *S. sanguis, S. mutans and S. mitis* are most frequently isolated species in streptococcal endocarditis (Forbes *et al.*, 1998). In the Dutch study (1991), 66% cases of native valve streptococcal IE were caused by oral streptococci (Eykyn, 1998).

Table 1: Potential etiological agents of Infective endocarditis

Clinical impression	Potential etiological agents
Native valve endocarditis (NVE)	Viridans streptococci (*Streptococcus sanguis*, *S. salivarius*, *S. mutans*, *S. mitis* etc.), *Enterococcus faecalis*, *E. faecium*, *E. durans*, *Streptococcus bovis*, *S. equines*, *S. pyogenes*, *S. agalactiae*, *Staphylcoccus aureus*, Coagulase-negative Staphylococci, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, HACEK group of bacilli, *Pseudomonas* spp., *Listeria* spp, *Corynebacterium* spp., (fungi like *Candida* spp., *Torulopsis glabrata*, and *Aspergillus* spp, in debilitated condition)
Prosthetic valve endocarditis (PVE)	
1. Early PVE	*Staphylococcus epidermidis*, *S. aureus*, aerobic gram-negative rods, fungi (usually *Candida* spp. and/or *Aspergillus* spp.), *Streptococcus* spp., *Enterococcus* spp., *Corynebacterium* spp.
2. Late PVE	Viridans streptococci, *Staphylococcus* spp., *Enterococcus* spp. (Staphylococci, gram-negative rods, fungi, and Corynebacteria are isolated in earlier infections occurring at less than 18 months)
Endocarditis in intravenous drug abusers	*Staphylococcus aureus*, *Streptococcus* spp., *Enterococcus* spp., gram-negative rods (mostly *Pseudomonas* spp., *Serratia* spp.), *Candida* spp., *Plasmodium* spp., *Leishmania* spp., anaerobic oral bacteria

(Source: Isenberg, 2004)

Enterococci

Enterococci are frequently implicated in nosocomial bacteraemia and in IE that is resistant to medical therapy. However, enterococcal endocarditis is much less common than enterococcal bacteraemia; the frequency being less than 10% among patients with enterococcal bacteraemia (Mylonakis and Calderwood, 2001). The incidence of enterococcal endocarditis is about 7% of all cases of native valve IE; in the Dutch series they accounted for 7.4% of 326 cases and at STH for 6.5% of 277 cases. Stepanovi *et al.* (2004) has reported the incidence of enterococcal IE to be 7.9–13.1 % of all reported episodes of IE. Most cases are caused by *E. faecalis* but there are occasional cases of other species such as *E. faecium*, *E. durans* and *E. avium.* It has been estimated that *E. durans* is responsible for less than 1 % of all enterococcal IE episodes (Stepanovi *et al.*, 2004).

Although uncommon, enterococcal endocarditis is more difficult to treat than streptococcal endocarditis because Enterococci are intrinsically more resistant to antibiotics than Streptococci. The recent emergence of high-level gentamicin resistance and vancomycin resistance in Enterococci has rendered IE with such strains virtually untreatable with antibiotics alone. Some 40% of patients with enterococcal IE have no previous history of heart disease or murmurs (Scheld and Sande 1995); it is thus presumed that Enterococci can attack normal heart valves but this remains unproven. Enterococcal endocarditis is most commonly seen in elderly men, many of whom have had genitourinary infection, urinary tract infection or other manipulation or trauma in the preceding 3 months (Eykyn, 1998).

Staphylococci

Staphylococci now account for nearly 30% of community-acquired native valve IE and they are also important cause of hospital-acquired IE. Most of these staphylococci are *S. aureus*, but now an increasing proportion is Coagulase-negative Staphylococci (CONS) (Eykyn, 1998).

Staphylococcus aureus : Some 80% of Staphylococci isolated from the cases of community-acquired native valve IE are *S. aureus* (Eykyn, 1998). Overall, *S. aureus* is the most common cause of IE, including PVE, acute IE and that in IVDA (Brusch, 2007). In recent series, it has surpassed Viridans streptococci as the most common cause of IE (Mylonakis

and Calderwood, 2001). *S. aureus* attacks normal or abnormal valves producing severe fulminating infection, valvular destruction with abscess formation occurring within few days (Eykyn, 1998). *S. aureus* IE is more severe than the IE produced by other pathogens (excluding fungi) with a high in-hospital mortality of 30% to 46% (Remandi *et al.*, 2007). Traditionally, *S. aureus* was mainly implicated in native valve endocarditis. However in a recent study, it is also the main cause of prosthetic valve endocarditis (Hoen, 2006).

Coagulase-negative Staphylococci (CONS) : With the ever-increasing use of intravenous catheters, intra-arterial lines and vascular prostheses, organisms found as normal or hospital-acquired inhabitants of human skin are able to gain access to the bloodstream and find a surface on which to grow, including heart valves and vascular endothelium. In such a setting, CONS have been increasingly implicated as causes of IE (Forbes *et al.*, 1998). They account for approximately 30% of PVE cases and less than 5% of NVE cases (Brusch, 2007). The infecting species is most often *S. epidermidis*. Many other species have been reported, including *S. hominis, S. lugdunensis, S. simulans, S. warneri, S. capitis, S. caprae and S. sciuri* (Eykyn, 1998).

HACEK group of bacteria

HACEK is an all-inclusive term for endocarditis due to *Haemophilus parainfluenzae, Haemophilus aphrophilus, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella kingae* (Giessel *et al.*, 2000). They are gram negative fastidious bacilli having a predilection for heart valve and rarely cause infections other than IE, and thus their isolation from blood cultures is virtually diagnostic of IE (Eykyn, 1998). HACEK organisms are the most common gram-negative bacilli isolated from IE patients (Brusch, 2007). Clinically, these cases are characterized by a subacute or chronic course, and often present with embolic lesions from large vegetations (Keys, 2008). They account for approximately 5 to 10% of cases who are not injection drug users (Giessel *et al.*, 2000).

Non-HACEK gram negative bacilli

Gram negative bacilli other than HACEK are regarded as less frequent cause of endocarditis though these species frequently cause gram negative sepsis. They are associated with certain

percentage of endocarditis in IVDU and PVE (Khanal *et al.,* 2004). Morpeth *et al.* reported the most common pathogens in patients with non-HACEK gram-negative bacilli endocarditis as *Escherichia coli* (29%) and *Pseudomonas aeruginosa* (22%). Other causative pathogens were *Klebsiella* spp. (10%), *Serratia* spp. (8%), *Proteus mirabilis* (6%), *Stenotrophomonas maltophilia* (6%), *Enterobacter cloacae* (4%) and *Salmonella enterica* serotype Enteritidis, *Morganella morganii, Yersinia enterocolitica, Burkholderia cepacia, Oligella urethralis, Achromobacter xylosoxidans,* and *Acinetobacter calcoaceticus* (2%).

Unusual etiological agents yielding blood-culture negative IE

Small proportion of IE is caused by unusual etiological agents which fail to be diagnosed by standard microbiologic techniques. Blood cultures are often negative when IE is caused by fastidious organisms such as *Abiotrophia* spp., HACEK organisms, *Brucella* spp., and fungi. While blood cultures are always negative when etiological agents are intracellular pathogens like *Coxiella burnetii, Bartonella* spp., *Chlamydia*, *Legionella* and as recently demonstrated, *Tropheryma whipple* (Hoen, 2006; Prendergast, 2004).

Fungal IE

Candida and *Aspergillus* species cause the majority of fungal IE. The most common species is *Candida albicans*, followed by *Candida parapsilosis*. Endocarditis due to *Histoplasma capsulatum* and *Cryptococcus neoformans* is rare (Keys, 2008). Intravenous drug abusers, prosthetic-valve recipients, patients with long-term central venous catheters and immuno-compromised patients are at the highest risk for fungal IE. It should be suspected in the presence of negative blood cultures, bulky vegetations, metastatic infection, perivalvular invasion, or embolization to large blood vessels (Nayar *et al.,* 2006).

3.6 CLASSIFICATION AND TERMINOLOGY

Traditionally, infective endocarditis has been clinically classified as Subacute and Acute on the basis of severity of clinical presentation and rate of progression of untreated disease (Mornos and Ionac, 2006).

SUBACUTE BACTERIAL ENDOCARDITIS (SBE)

Subacute endocarditis is a more common and chronic disease and comprises of almost 70% cases of bacterial endocarditis. It has more indolent course and usually develops insidiously and progresses slowly over weeks to months (Pelletier, 2006). SBE often develops on abnormal, damaged or defective valve cusps after asymptomatic bacteraemia due to periodontal, gastrointestinal, or genitourinary infections (Mornos and Ionac, 2006). It develops most commonly when an organism of low virulence infects already damaged or defective heart valves. Viridans streptococci are the most common cause being responsible for approximately 40% of SBE. Other possible pathogens include *Enterococcus* spp., *Streptococcus bovis, S. milleri* group*, Staphylococcus epidermidis, Abiotrophia defectiva, Gemella morbillorum, Granulicatella* spp., *Haemophilus* spp., *Coxiella burnetii* (Pelletier, 2006). SBE has only modest toxicity and rarely causes metastatic infection and if untreated, takes more than 6 weeks and even a year to be fatal (Mornos and Ionar, 2006). It usually produces large firm vegetations comprising of dense fibrin and platelet aggregates with bacterial colonies.

ACUTE BACTERIAL ENDOCARDITIS (ABE)

Acute endocarditis has a fulminant course with greater potential for rapid hemodynamic decompensation. When bacteria are virulent or bacterial exposure is massive, ABE can affect even normal valves (Brusch, 2007; Pelletier, 2006). It is usually caused by highly virulent pyogenic bacteria such as *S. aureus, S. pyogenes, S. agalactiae,* Pneumococci and Gonococci. It usually develops abruptly and has marked toxicity and progresses over days to several weeks to valvular destruction and metastatic infection with a very bad prognosis (Mornos and Ionar, 2006; Pelletier, 2006). Vegetations are large and crumbling and valve destruction is greater than SBE.

Although general concept of acute and subacute endocarditis can sometimes be helpful, it is important to realize that many organisms do not fit neatly into one or other category. An increasing number of patients with subacute disease do not have known heart disease, and patients with an acute clinical presentation may have valvular disease. Furthermore endocarditis caused by viridans streptococci can present acutely usually when the diagnosis

had been delayed or missed (Eykyn, 1998). Hence this mode of classification has been discouraged now.

In contrast to older classification, the basis of present classification scheme refers to a) activity of the disease and recurrence; b) diagnostic status; c) pathogenesis; d) anatomical site and e) microbiology (Horstkotte et al., 2004).

With respect to the activity of the disease, IE has been classified as Active and Healed. This differentiation is especially important for patients undergoing surgery. Active IE is present if positive blood cultures and fever are present at the time of surgery or positive cultures of excised tissue are obtained at surgery, or active inflammatory morphology is found intra-operatively, or surgery has been performed before completion of a full course of antibiotic therapy. Healed endocarditis is considered to be present when, at operation, there are macroscopic signs of endocarditis in the absence of positive tissue cultures or when blood cultures are negative within 4 weeks of surgery (Yoshida *et al.,* 1991) or when surgery is performed after completion of antibiotic treatment in the absence of signs of active infection (fever, leukocytosis). Infective endocarditis is Recurrent if it develops after an eradication of previous episode of IE. It is a dreaded complication with high mortality. While Persistent IE is the one in which the infection has never been truly eradicated (Horstkotte *et al.,* 2004).

On the basis of the diagnostic status, IE can be classified as Definite, Suspected and Possible. The diagnosis of IE is Definite if during septicaemia, involvement of endocardium can be demonstrated by echocardiography. If IE is strongly suspected clinically but involvement of the endocardium has not been proven so far, then endocarditis should be classified as Suspected to express a more or less high suspicion of IE. If IE is only a potential differential diagnosis in febrile patients, one should describe this as Possible IE (Horstkotte *et al.,* 2004).

On the basis of the pathogenesis, IE can be classified into at least four categories: Native valve endocarditis (NVE), Prosthetic valve endocarditis (PVE), endocarditis in intravenous drug abusers (IVDAs) and Nosocomial infective endocarditis (NIE) (Hoen, 2006).

Native Valve Endocarditis is an infection occurring in normal or abnormal native heart valves. Depending on the etiological agent, it may be either acute progressing fulminatingly or it may be subacute having mild and chronic course.

Prosthetic Valve Endocarditis is an infection of implanted prosthetic valve. It develops in 2 to 3% of patients within a year after valve replacement surgery and in 0.5% of patients per year thereafter (Pelletier, 2006). It accounts for 10-15% of all cases of IE (Beynon *et al.,* 2006). Prosthetic valve endocarditis may be classified as Early or Late in onset according to the timing of symptoms in relation to the original valve surgery. A distinct shift in the pattern of infecting organisms is seen one year after surgery. Staphylococci predominate in early onset PVE, whereas the microbiological spectrum in late onset PVE mirrors that of native valve endocarditis (Beynon *et al.,* 2006).
Cases with onset occurring within two months after surgery are called Early-onset PVE. It develops as a complication of valve surgery and is caused mainly by antimicrobial-resistant contaminants at valve replacement surgery. The most common etiologies are *S. epidermidis, S. aureus,* gram-negative bacilli, diphtheroids, *Candida* spp. and *Aspergillus* spp. (Mornos and Ionar, 2006). It is acquired perioperatively and thus can be considered nosocomial (Horstkotte *et al.,* 2004). Cases that occur more than 12 months after surgery are called Late-onset PVE. It is caused mainly by transient asymptomatic bacteraemia with community acquired microbes, most often *Streptococcus* spp., *S. epidermidis,* gram-negative bacilli and HACEK (Brusch, 2007).

Injection of contaminated materials into bloodstream such as with self-administered intravenous drugs is a significant risk factor for IE (Brusch, 2007). In IVDAs, the prevalence of IE is approximately 60 times higher than in an age-matched population (Horstkotte *et al.,* 2004). The incidence of IE in IVDAs is 1-5% a year and seems to be rising steadily in the population of developed countries (Beynon *et al.,* 2006). Right sided heart valves, particularly, tricuspid valve is more often infected. Most of the cases are presumed to have normal tricuspid valves. Skin is the commonest source of infection and *S. aureus* is the most common pathogen accounting for nearly 50% of cases. Other organisms include non-HACEK gram-negative bacilli particularly *Pseudomonas* spp., Viridans streptococci, fungi (Morpeth *et al.,* 2007).

Nosocomial Infective Endocarditis is defined as occurring more than 72 hours after admission to a hospital or as directly related to the procedures performed in hospital within the preceding six months of admission (Horstkotte *et al.,* 2004). It is usually secondary to central venous line-related bacteraemia, TPN lines, pacemakers, etc. NIE comprises 5-29% of all cases of IE and may carry mortality up to 40-56% (Horstkotte *et al.,* 2004). The most frequent pathogen is *S. aureus.* .

On the basis of the anatomic sites involved, IE can be broadly categorized as Right-sided IE and Left-sided IE. Due to the differences in clinical manifestation, pathogenesis and prognosis, IE involving structures of the left and the right heart should be distinguished (Horstkotte *et al.,* 2004). Right-sided endocarditis involving the tricuspid valve and less often the pulmonary valve may result from intravenous use of illicit drugs or from central vascular lines. Organisms may originate from the skin (Mornos and Ionar, 2006). In other patients without a history of intravenous exposure, endocarditis is more frequently left-sided.

Regarding microbiology of IE, when the causative organism has been identified, it should always be included in the terminology as it provides crucial information regarding clinical presentation, treatment and prognosis (Horstkotte *et al.,* 2004).

Blood Culture Negative Endocarditis is an important category of IE. Continuous bacteraemia and a high frequency of positive blood cultures are typical of IE (Bayer *et al.,* 1998). However negative blood cultures occur in 2.5–31% of all cases of IE, often delaying diagnosis and the onset of treatment with profound impact on clinical outcome (CDC, 2004; Prendergast, 2004). The most common reasons for blood culture-negative IE are (Kandel, 2004; Prendergast, 2002): inadequate microbiological techniques, infection with fastidious bacteria, infection with non-bacterial organisms, in the cases of NBTE with sterile vegetation, and most importantly, prior antibiotic administration. The administration of antimicrobial agents before blood cultures are obtained reduces the recovery rate of bacteria by 35% to 40%. The antimicrobial susceptibility of the organism and the duration and nature of prior antimicrobial therapy together determine the length of time that blood cultures will remain negative (Bayer *et al.,* 1998).

3.7 EPIDEMIOLOGY

3.7.1 INCIDENCE

As infective endocarditis is not subjected to registration and prospective studies on its incidence are rare and contradictory, there is considerable uncertainty about the present incidence of the disease (Horstkotte *et al.,* 2004).

The incidence of IE varies from country to country (Hoen, 2006). In a general population, the incidence of IE has been estimated between 1.5 - 6 cases per100,000 people/year but it is clearly high in at risk cohorts. The incidence of IE in IVDA group of cases is estimated to be 150 to 2000 per100,000 people/year (Mornos and Ionar, 2006).

In 2006, American Heart Association (AHA) reported 29,000 hospital discharges and 2,370 deaths with IE as a primary or secondary diagnosis (Patrick, 2007).According to Beynon *et al.* (2006), the incidence of IE is approximately 1.7-6.2 cases per100,000 people/year.

In 2004, CDC reported that although many developments have taken place with respect to the medical and surgical treatment of the disease, its incidence is continuing to rise, with 3.3 cases per 100,000 people per year in the United Kingdom, with similar figures for the United States and 1.4–4.0 cases per 100,000 people per year in Europe.

No national data regarding the incidence of IE in Nepal is available. Shahid Gangalal National Heart Centre (SGNHC) has reported the annual attribution of IE in total hospital admissions to be 1.9% in the year 2002, 1.2% in 2003, 0.55% in 2004, 2.58% in 2005, 1.43% in 2006 and 1.31% in 2007 (Manandhar *et al.,* 2007).

3.7.2 AGE / GENDER

Depending on an exposure to risk factors and presence of underlying predisposing factors, IE can occur at any age (Brusch, 2007; Pelletier, 2006). Fifty years ago, in developed countries and still in many of the developing countries, native valve endocarditis was mainly a disease of young adults with previously well identified valve disease, particularly, rheumatic heart disease (Eykyn, 1998; Hoen, 2006). In Cates and Christie's study (1951) of 442 cases of subacute bacterial endocarditis seen between 1945 and 1948, 62% of patients

were aged between 15 and 35 years (Eykyn., 1998). Since then, epidemiological and demographical profile of IE has significantly changed (Cabell *et al.,* 2002). In countries with a low incidence of rheumatic fever, IE in the pediatric group is rare (Eykyn, 1998). As increased longevity has given rise to degenerative valvular disease, placement of prosthetic valves and increased exposure to nosocomial bacteremia, the median age of patients has now gradually increased (Mylonakis and Calderwood, 2001). It has become more prevalent among the elderly with 25-50% of cases occurring in those older than 60 years (Brusch, 2007; Pelletier, 2006).

According to CDC (2004), an average age group affected is in the fifth decade. Renzulli *et al.* reported the mean age of the patients to be 44.95±1.03 years. In the study conducted by Cabell *et al.* in 2002, the mean age of the cases was 57 years. Feringa *et al.* quoted the mean age of patients to be 53±16 years. Similar data of 59 years was presented by Zegdi *et al.* in their study in 2008. Kandel in 2004 reported the mean (± S.D) age of the patients with definite IE to be 34.15(±15.45) years.

Overall, IE is more common in males than in females (Eykyn., 1998). Men are affected about twice as often as women (Beynon *et al.,* 2006; CDC, 2004; Pelletier, 2006). Mylonakis and Calderwood quoted the mean male-to-female ratio to be 1.7:1. In the study conducted by Kandel in 2004, male is to female ratio was found to be 2.4: 1.

3.8 PREDISPOSING FACTORS

Damage to heart valves is the most important identifiable risk factor for the development of IE (Baum, 2001). All forms of valvular heart diseases increase the risk of endocarditis. The heart valves may be damaged as a result of congenital heart defects, acquired valvular heart diseases, previous episode of endocarditis, damage resulting from a heart attack, myxomatous degeneration or syphilis.

Congenital heart defects are one of the significant risk factors for the development of IE. High risk is conferred by certain complex cyanotic congenital heart diseases, such as transposition of great arteries and Tetralogy of Fallot (TOF).

The predominant cause of acquired valvular abnormality is rheumatic heart disease (RHD). In developing countries, RHD, which occurs primarily among young people, remains the most frequent underlying cardiac condition predisposing the patients to IE (Mylonakis and Calderwood, 2001). In fact, RHD is the commonest heart disease among all cardiovascular diseases in Nepal (Limbu *et al.,* 2002). In developed countries, rheumatic valvulopathy was the most frequent predisposing factor for IE only until the end of 1970s (Hoen, 2006). Currently, it accounts for less than 20% of cases and only 6% of patients with RHD eventually develop IE (Brusch, 2007). Instead, degenerative heart disease secondary to atherosclerosis is now more important cause of acquired valvular abnormality particularly in elderly people (Eykyn, 1998).

Myxomatous degeneration such as Marfan's syndrome is an autosomal dominant disorder of connective tissues resulting in abnormalities of musculoskeletal system, cardiovascular system and eyes. It is an important risk factor for cardiovascular abnormalities which may be further complicated by IE (Jaiswal *et al.,* 2003).

Expanding use of invasive procedures together with implanted endovascular and intra-cardiac devices such as pacemaker, defibrillators, catheters and prosthetic valves are considerable risk factors (Hoen, 2006; Renzulli *et al.,* 2000). Infection with human immunodeficiency virus (HIV) may independently increase the risk of IE (Manoff *et al.,* 2004). In addition, diabetes mellitus, use of immunosuppressive drugs, invasive procedures of oral cavity, genitourinary and gastrointestinal system in susceptible individuals, pregnancy and excessive alcohol consumption are possible risk factors (Baum, 2001; Mylonakis and Calderwood, 2001).

3.9 PATHOLOGY

The pathology of infective endocarditis may be local (cardiac), including valvular and perivalvular destruction or systemic (non-cardiac) due to the detachment of septic vegetation with embolism, metastatic infection and septicemia (Thiene, 2006).

Local consequences: They occur in the valve itself or in perivalvular region. Vegetations are characteristic cardiac lesions. In the acute phase**,** it consists of septic thrombus

entrapping microorganisms and neutrophil infiltrates. While in subacute-chronic phase, microorganisms may disappear, granulomatous inflammation including giant cells occurs and vegetations may transform into coarse calcific deposits. Apart from vegetation, frequently there is cusp disruption with loss of substance that account for tearing, fraying, perforation and bulging, especially when the microorganism is *Staphylococcus aureus*. Valve incompetence with left ventricular decompensation and congestive heart failure are usual hemodynamic complications. Local spread of infection includes extension to aortic wall that may lead to the development of sinus of Valsalva aneurysms and ring abscess. In addition, infection of atrioventricular valves may also affect subvalvular apparatus like chordae tendinae and papillary muscle (Thiene, 2006).

Systemic consequences: They are primarily due to embolization of infected material from heart valve and, particularly in chronic infection, immune-mediated phenomena (Pelletier, 2006). Systemic consequences differ in whether endocarditis is right-sided or left sided, and whether emboli from vegetations are septic or non-infected. Right- sided endocarditis typically produces septic pulmonary emboli which may result in pulmonary infarction, empyema, pneumonia or lung abscess. Left-sided endocarditis may be complicated with systemic embolism that may involve any tissue, particularly spleen, kidney, meninges, bone, pericardium, synovium, or vitreous humor (Pelletier, 2006; Thiene, 2006).

Many of the extracardiac manifestations particularly in subacute endocarditis are due to various immunological mechanisms. Among these include glomerulonephritis, peripheral manifestations (e.g., Osler nodes, Roth spots, subungual hemorrhages), and possibly, various musculoskeletal abnormalities (Brusch, 2007).

3.10 CLINICAL MANIFESTATIONS

The clinical manifestations of IE are usually diverse and occur within 2 weeks of bacteraemia (Pelletier, 2006). They may develop slowly and insidiously (in subacute IE) or suddenly and aggressively (in acute IE). Fever is a hallmark of both acute and subacute IE (Prendergast, 2002). Among the presenting symptoms, it is a non-specific but the most frequent one. It may be absent or minimal in patients with congestive heart failure, severe debility, chronic renal failure, previous use of antimicrobial drugs, or IE caused by less

virulent organisms (Mylonakis and Calderwood, 2001). During physical examination, cardiac murmurs, due to a preexisting valvular abnormality or infection itself, are heard in more than 90% of patients (Pelletier, 2006).

Low grade fever, night sweat and weight loss are cardinal manifestations of subacute IE. When appropriate therapy is delayed for weeks or months, additional clinical features, embolic or immunologic in origin, develop (Brusch, 2007). Symptoms secondary to emboli include acute meningitis, hemiplegia, hematuria, infarction of kidney, spleen or retina. Manifestations representing immunologic phenomena are glomerulonephritis, Osler's nodes, Roth spots, lumbosacral back pain and positive rheumatoid factor. About 40% of patients present peripheral manifestations like petechiae, Janeway lesions, splinter hemorrhages, clubbing of the fingers and toes and generalized rashes (Brusch, 2007; Giessel *et al.,* 2000; Keys, 2008; Mornos and Ionar, 2006).

In acute IE, patients notice an acute onset of high-grade fever, chill and sometimes a septic shock. Complications develop within a week. These include dyspnea, shortness of breadth and fatigue of severe congestive heart failure and a wide spectrum of neuropsychiatric complications.

In right heart IE, general clinical presentations include chill, fever, night sweat, malaise and signs of septic pulmonary emboli like cough, pleuritic chest pain, hemoptysis and dyspnoea. Occasionally lung injury is so extensive that respiratory insufficiency supervenes. In contrast to left-sided IE, peripheral stigmata and immunologic vascular phenomena are usually absent (Bayer *et al.,* 1998; Horstkotte *et al.*, 2004).

3.11 DIAGNOSIS

3.11.1 DIAGNOSTIC CRITERIA FOR INFECTIVE ENDOCARDITIS

The variability in clinical presentation of IE requires a diagnostic strategy that will be both sensitive for disease detection and specific for its exclusion across all forms of the disease (Bayer *et al.,* 1998).

The need for such robust diagnostic criteria was shown by the original Von Reyn criteria, published in the early 1980s. However these criteria were designed to be very stringent (Bayer *et al.*, 1998). Because of these limitations, in 1994, Durack *et al.* of the Duke University proposed standardized criteria called 'The Duke criteria'(Appendix-VII) for assessing the patients with suspected IE by combining predisposing factors, the clinical, microbiological, pathological, and echocardiographic characteristics (Bayer *et al.*, 1998; Brusch, 2007; Mylonakis and Calderwood, 2001).

3.11.2 DIAGNOSIS OF INFECTIVE ENDOCARDITIS

The diagnosis of IE requires a multifaceted approach involving the clinical suspicion and examination, imaging with echocardiography and laboratory investigation by means of microbiological analysis and inflammatory markers (Beynon *et al.*, 2006).

3.11.2.1 CLINICAL DIAGNOSIS

Clinical manifestations of bacterial endocarditis are highly variable and frequently nonspecific. However, some degree of fever is common. Findings on physical examination including signs of congestive heart failure, heart murmur consistent with valvular regurgitation, petechiae, splenomegaly and retinal hemorrhages can supplement in the diagnosis of IE. Physicians must have a high index of suspicion for IE, particularly in patients with predisposing conditions or high-risk behaviors (Giessel *et al.*, 2000).

3.11.2.2 ECHOCARDIOGRAPHY

Echocardiography has become an indirect diagnostic method of choice, especially in patients who present with a clinical picture of IE but who have nondiagnostic blood culture results (Brusch, 2007). It can guide both medical management and discussion of an appropriate timing for intervention (Mornos and Ionar, 2006). Echocardiography should always be performed in all patients suspected of having IE (Bayer *et al.*, 1998).

Characteristic vegetation, abscess, new prosthetic valve dehiscence, or new regurgitation are four powerful echocardiographic identifiers of IE in combination with other clinical parameters (Bayer *et al.*, 1998). However it does not allow reliable differentiation between

vegetations of active and healed IE. Frequent causes of false-positive echocardiographic findings are non-infected valve-attached vegetations (e.g., in Libman-Sacks endocarditis, carcinoid heart disease, acute rheumatic fever) and filiform tumors.

3.11.2.3 LABORATORY DIAGNOSIS

3.11.2.3.a CULTURE

Given the need for prolonged antibiotic treatment in most patients with infective endocarditis, positive microbiological cultures from blood and/or resected valvular vegetations and their antibiotic sensitivities are vitally important for successful management (Beynon *et al.,* 2006).

Blood Culture

The standard test for diagnosing IE is the documentation of a continuous bacteraemia based on blood culture results (Brusch, 2007). Because bacteraemia is a common feature during IE, the causative organism can be isolated in blood cultures in more than 90% of endocarditis cases (Lepidi *et al.,* 2005). Current guidelines recommend that three sets of blood cultures are drawn one hour apart from different vein puncture sites over 24 hours and not through intravenous lines, before the introduction of antibiotic treatment (Beynon *et al.,* 2006). Each set should include for one aerobic and one anaerobic culture. Constant bacteraemia typical of IE allows drawing of blood for cultures at any time (Horstkotte *et al.,* 2004). Organisms that rarely cause endocarditis but commonly contaminate blood cultures (e.g., diptheroids and CONS) must be isolated repeatedly if their isolation is to serve as a major criterion (Kandel, 2004).

Culture of resected vegetations and infected valvular tissue

IE is definitively diagnosed when microorganisms are cultured from or seen histologically in heart valves or endocardial vegetations obtained during cardiac surgery, embolectomy, or autopsy (Pelletier, 2006). European Society of Cardiology (ESC) has recommended that especially in culture-negative endocarditis, all materials excised during cardiac surgery in patients with active IE should be cultured and examined. It is a common practice to culture vegetations and any valvular tissue that appears suspicious of being infected at intra-operative findings in order to isolate the microbes involved in the infection and to establish

the appropriate postoperative antibiotic treatment (Bayer *et al.,* 1998; Chuard *et al.,* 1998; Renzulli *et al.,* 2000; Wallet *et al.,* 2001).

However, bacterial isolation from valvular tissue is not always possible: healed endocarditis, broad-spectrum perioperative antibiotic therapy, systemic hypothermia, hemodilution, cold cardioplegia and the attitude to prime the oxygenator with crystalloid solution and antibiotics reduce the possibility of bacterial isolation from the infected tissue. The use of crystalloid cardioplegia may cause an osmotic and physical shock to the bacteria still living in the vegetations (Renzulli *et al.,* 2000). Another drawback of valve culture is preoperative antibiotic therapy giving a poor yield. Further, if the valve has undergone a chronic/sequellar process, the culture will not be informative (Wallet *et al.,* 2001). It has been estimated that culture of vegetations allows bacterial identification in less than 25% of cases of bacterial endocarditis; nevertheless it can isolate bacteria undetected by blood cultures (Renzulli *et al.,* 2000). The only proven case of *Mycoplasma hominis* endocarditis was detected by valve culture (Horstkotte *et al.,* 2004).

During open heart surgery, the operating cardiac surgeon selects and excises suspected valvular tissue and is aseptically handed to the scrub nurse. The nurse then aseptically transports the tissue in a sterile vial without any medium or that containing sterile normal saline. The valve sample is processed immediately under laminar flow in microbiology laboratory. Prior grinding helps in recovery of pathogens. The tissue may be ground in mortar and pestle with 1ml of BHI broth (Wallet *et al.,* 2001), in automated pummeling instruments like stomacher (York *et al.,* 2004), in commercially available sterile disposable plastic grinders (Campbell *et al.,* 2000) or the most easily by sterile surgical blade as in Sterile-Scalpel method (York *et al.,* 2004). The sample is then inoculated on chocolate agar, 5% sheep blood agar, MacConkey agar and in an enrichment broth (Brain Heart Infusion broth or Thioglycollate broth or Todd Hewitt broth). The inoculated plates are incubated at 37ºC in 5-10% CO_2 for 72 hours except the MacConkey agar which is incubated aerobically. A piece of valve should be deep frozen at -80°C. Standard microbiological techniques are performed to identify the bacteria.

3.11.2.3.b NON-SPECIFIC NON-DIAGNOSTIC TESTS

Various non specific abnormalities are quite common among IE patients which may supplement in the diagnosis of IE. Common nonspecific abnormalities include normocytic-normochromic anemia, leukocytosis, elevated ESR, elevated C-reactive protein level, proteinuria, microscopic hematuria and acute hemolysis in PVE case (Mornos and Ionar, 2006; Mylonakis and Calderwood, 2001; Pelletier, 2006). Occasionally, false-positive non-treponemal serologic tests for syphilis may occur.

3.11.2.3.c NON-CULTURAL DIAGNOSTIC TECHNIQUES

The current approach to diagnose IE involves echocardiography to detect vegetations and culture of blood and/or excised valvular tissue to isolate the pathogen. However vegetations may be difficult to be detected on echocardiography and despite improved culture methods, blood cultures remain negative in approximately 14% of IE cases (Beynon *et al.,* 2006; Lepidi *et al.,* 2008). Further the incidence of positive cultures from vegetations and tissue retrieved at operation is even lower (Renzulli *et al.,* 2000). Alternative noncultural diagnostic methods include histopathologic examination of excised tissue, molecular techniques, and serological tests (Lepidi *et al.,* 2005).

Histological examination of resected valvular tissue

Histologic examination of resected valve tissues remains a gold standard for the diagnosis of IE when these specimens are available. Histological findings are included in the Duke criteria and the von Reyn criteria and are considered to be criteria of definite IE.

The presence of vegetations, extensive neutrophil-rich inflammation and microorganisms in valve tissue are three well-known criteria for the histological diagnosis of IE. However, the last criterion does not apply in all cases because microorganisms may be destroyed by preoperative antibiotic treatment and consequently may not be detectable by histological methods. When vegetations are lacking and microorganisms fail to be detected in valve tissues, the pattern of inflammation may be considered to be a key in formulating the diagnosis. Acute inflammation has been defined as predominant presence of polymorphonuclear leukocytes (PMLs) in valvular inflammatory infiltrates, while chronic inflammation, as the presence of inflammatory infiltrates composed mainly of mononuclear

cells (macrophages and lymphocytes) in the absence of neutrophils. In contrast to T-lymphocytes and macrophages, PMLs are the only inflammatory cells whose presence allows the differentiation of infective endocarditis from other inflammatory but non-infective processes. Tissue of native cardiac valves is normally avascular. However when there is an infection in valve tissue, it always creates neovascularization (vascular proliferation) of variable abundance that allows the entry of leukocytes into valve tissue and the development of an inflammatory reaction. Thus, neovascularization may be a new histological criterion that could aid pathologists in the recognition of infective processes in valve tissue.

Histological examination is an effective back-up procedure as it may disclose a concealed infection in clinically undiagnosed case of endocarditis. It may also guide antimicrobial treatment if specific causative agent can be identified by means of special stains or immunohistological techniques (Chuard *et al.,* 1998; Prendergast, 2004; Zrubrugg *et al.,* 1996). It is more often positive than cultures in treated patients since nonviable microorganisms are visible for sometimes before being cleared by macrophages. Histological evidence is also essential to validate the results of valve culture in catagorising the isolate as a pathogen or a probable contaminant (Wallet *et al.,* 2001). More importantly, it allows distinction of IE from other conditions like rheumatoid nodules and fibroelastomas whose echocardiographic features mimic IE (Prendergast, 2004).

For histological examination, formalin fixed tissue is dehydrated, molded, embedded, sectioned and finally stained by various tissue stains (Appendix- VII) e.g., Hematoxylin-Eosin, Gram, Gomori or Periodic Acid Shiff stains. The Hematoxylin-Eosin stain allows classifying inflammatory response of the host as acute or chronic/sequellar process. The other stains give information about the presence or absence of microorganisms (Wallet *et al.,* 2001).

Molecular techniques

The advent of molecular techniques, notably polymerase chain reaction (PCR), has much improved the detection of fastidious and non-culturable agents. This approach has been used to diagnose IE due to *Tropheryma whipplei* and *Bartonella* spp. It is particularly useful

when negative cultures are caused by previous administration of antibiotics or when phenotypic characterization is essential after two or more organisms are isolated in separate cultures (Prendergast, 2002). It may be done on blood or tissue samples though PCR-based tests have low sensitivity unless they are performed directly on resected cardiac valvular tissue. Broad spectrum PCR uses nucleic acid target for the amplification of bacterial 16S rRNA genes (Beynon *et al.,* 2006).

Serological techniques

Another non-cultural diagnostic technique is the use of serologic tests for certain microorganisms. Such tests, associated with cell cultures, are now recommended for patients with blood culture-negative endocarditis for which *Coxiella burnetii, Brucella, Chlamydia* and *Bartonella* spp. are the suspected causative organisms.

3.12 MEDICAL TREATMENT

Antibiotics remain the mainstay of treatment for IE. However medical cure of IE may be difficult for several reasons. The vegetation that characterizes IE is composed of a mixture of fibrin and platelets, contains a large inoculum of metabolically less active bacteria i.e., 10-100 billion bacteria per gram of tissue, and is often enclosed in a layer of exopolysaccharide that hampers antibiotic penetration (Hoen, 2006).

To ensure complete cure, the concentration of antibiotics within the vegetation must be sufficiently high and sustained. For this, the antibiotic treatment should be bactericidal and the bactericidal activity should ideally be obtained rapidly and maintained until cure is obtained. The intravenous route of antibiotic administration is the best since it provides maximal bioavailability. Most antibiotics are administered as short infusions (30 minutes) and the treatment is usually continued for 4-6 weeks depending on the organism. The chosen antibiotic must be specific for the causative organism which is determined by culture and sensitivity tests. In the absence of clinical clues to a specific cause, therapy for culture-negative native-valve endocarditis should be individualized and empirical therapy is started which generally includes ampicillin, ceftriaxone or vancomycin often in combination with an aminoglycoside (Mylonakis and Calderwood, 2001). In recent years, hemodynamically stable patients with bacterial endocarditis have been treated as outpatients after an initial

course of inpatient therapy. Ceftriaxone therapy in a single daily dose is particularly amenable to this treatment strategy (Giessel *et al.,* 2000). Most IE patients respond to appropriate antibiotic treatment within 72 hours with a definitive loss of fever and improvement in general well being (Oakley and Hall, 2001). The most recent recommendations for the antibiotic treatment of IE have been issued by the European Society of Cardiology (ESC) and the American Heart Association (AHA).

3.13 SURGICAL TREATMENT

Antibiotic therapy remains the first line treatment of IE (Dodge *et al.,* 1995). Yet, despite the availability of new and potent antibiotics, the management of patients with acute IE remains a difficult therapeutic challenge. Eradication of septic focus, abolition of the accompanying systemic manifestations and prevention of further hemodynamic deterioration and valvular destruction by the infectious process frequently require early surgical intervention (Alexiou *et al.,* 2000; Delay *et al.,* 2000; Murashita *et al.,* 2004; Yoshida *et al.,* 1991). Surgery is mandatory in at least 30% of cases with active IE and in 20-40% of patients after healing (Horstkotte *et al.,* 2004). Since the first implantation of an artificial cardiac valve in 1960, valve replacement has become a routine and successful practice for various heart conditions like degenerative, rheumatic, or congenital valve diseases. However IE accounts for only a small fraction of total number of valves that are replaced but is an important indication for surgery, since the procedure usually is life-saving in patients with intractable infection or acute hemodynamic decompensation (Chuard *et al.,* 1998). Various indications for surgical intervention in patients with native valve endocarditis are as follows:

1. Congestive heart failure, which is the most common indication for surgery,
2. Persistent sepsis for more than 7-10 days despite adequate antibiotic treatment indicating failure of conservative management,
3. Endocarditis due to microorganisms that are not normally cured by antimicrobial therapy alone, e.g., fungi, *Pseudomonas aeruginosa*, *Brucella* spp. and *Coxiella* spp., probably Enterococci or those that have a potential for rapid destruction of cardiac structures, e.g., *Staphylococcus aureus, S. lugdunensis*,
4. Relapses due to multi-drug resistant (MDR) or difficult-to-treat microorganisms,

5. Locally uncontrolled infections as indicated by perivalvular involvement like in cellulitis, abscess, pseudoaneurysm, fistulas or rupture of one or more valves, conduction disturbances, myocarditis,
6. Recurrent septic emboli following adequate antibiotic treatment,
7. Vegetations that are mobile and larger than 10-15mm on the mitral valve,
8. Kissing infection of the anterior mitral leaflet (AML),
9. Severe native valve obstruction,
10. Often in case of metastatic infections (e.g., cerebral and other types of aneurysms and macroabscesses of the brain and spleen).

The indications for surgery in patients with prosthetic valve endocarditis are the same as those for patients with NVE, with addition of conditions like valvular dehiscence and early PVE.

Operating during an inactive or healed phase of disease process in a sterile field is highly desirable (Alexiou *et al.,* 2000). Hence in most stable patients, surgery is best delayed until standard antibiotics therapy (SAT) is completed to reduce the risk of perioperative complications and early prosthetic valve endocarditis (Beynon *et al.,* 2006; Prendergast, 2006). However it is not always possible to wait for surgical treatment until active phase transforms to healed endocarditis for the patients with significant valve lesions and who are unresponsive to medical treatment (Yoshida *et al.,* 1991). The optimal time to perform surgery is before severe hemodynamic disability or spread of infection to perivalvular tissue (Burma *et al.,* 2008). The two primary objectives of surgery are control of infection through debridement with removal of infected and necrotic tissue and reconstruction of cardiac morphology including repair or replacement of the affected valve/s (Horstkotte *et al.,* 2004).

CHAPTER-IV

4. MATERIALS AND METHODS

The present study was conducted at Shahid Gangalal National Heart Centre (SGNHC), Bansbari, Kathmandu from November 2007 to October 2008. SGNHC is a tertiary care referral centre for heart patients in the nation with 150 beds and well equipped cardiovascular surgical department.

4.1 MATERIALS

All materials required for the present study are listed in Appendix-I.

4.2 METHODS

4.2.1 Patients

Patients of all age groups and both sexes undergoing heart valve replacement surgery with pre-operative diagnosis or intra-operative suspicion for IE were included in the study. A total of 31 heart valves resected from 27 patients were studied.

4.2.2 Case definition

The patients studied were categorized into two study groups based on whether they had pre-operative diagnosis of IE and hence whether they had been on antibiotic therapy before surgery.

Those patients who had pre-operative diagnosis of IE and had completed their standard course of antibiotic therapy before undergoing heart valve replacement surgery were included in the study group of 'Treated IE'. All cases in this study group underwent surgery for the correction of valvular dysfunction and hemodynamic decompensation and not for an intractable infection. 12 patients from whom 15 resected heart valves were studied fell in this study group.

Those patients in whom IE was not suspected before surgery and hence had not received pre-operative antibiotic therapy for IE but were intraoperatively suspected for IE were included in the study group of 'Suspected IE'. Different signs for intra-operative suspicion of possibly infected valves were: presence of vegetation, perforation, tearing or rupture of valve cusps, annular or periannular abscess, and/or rupture of chordae tendiniae. These

cases underwent surgery for the reasons other than IE, the most common of them being for the correction of valvular insufficiency or stenosis due to degenerative, rheumatic or congenital diseases. 15 patients from whom 16 resected heart valves were studied fell in this study group.

4.2.3 Collection of resected heart valve samples

Infected or possibly infected heart valves were selected and resected by the operating cardiac surgeon from the patients undergoing valve replacement surgery. From each patient, two samples were collected: one in a sterile vial for bacteriological study and the next sample fixed in 10% formalin for histopathological study. The samples were immediately transported and processed in the laboratory.

4.2.4 Methods for bacteriological study

All 31 resected heart valves were processed for bacteriological study at microbiology department of SGNHC laboratory.

4.2.4.1 Processing of samples

The resected heart valve sample in the sterile vial was aseptically transferred onto a sterile petri dish by a sterile forcep. It was then cut aseptically into small pieces using a sterile surgical scalpel, the process being called Sterile Scalpel Method of tissue homogenization.

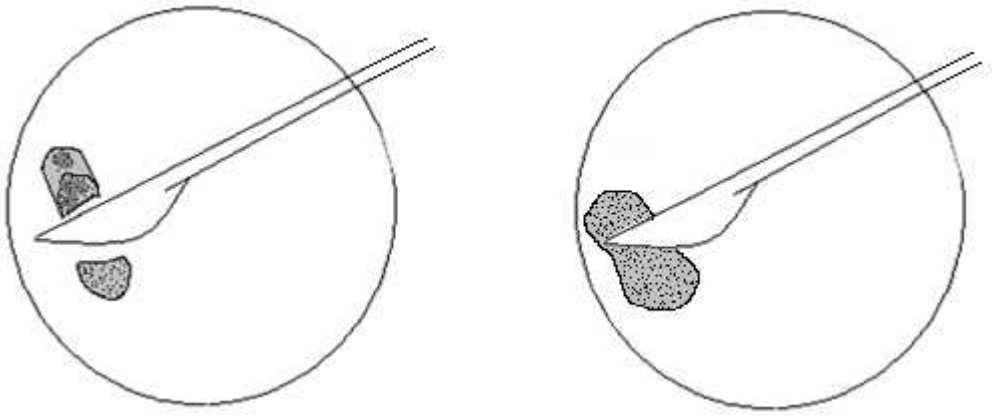


Figure 2: Illustration of Sterile-Scalpel Method of tissue homogenization (York *et al.*, 2004)

4.2.4.2 Culture

Using a sterile forcep, small pieces of cut tissue were aseptically transferred onto and then streaked over whole surface of chocolate agar, 5% sheep blood agar and MacConkey agar plates. The inoculated chocolate agar and blood agar plates were incubated at 35-37ºC in 5% CO_2 for 48 hours while MacConkey agar plate was incubated aerobically at 35-37ºC. Rest of the cut tissue pieces were transferred aseptically by a sterile forcep into an enrichment Brain Heart Infusion (BHI) broth. The inoculated broth was incubated aerobically at 35-37ºC for a week, with a loop full of broth being subcultured on chocolate agar, 5% sheep blood agar and MacConkey agar plates at an interval of every 24 hours of incubation.

4.2.4.3 Identification of the isolate

The inoculated culture plates were examined after 24 and 48 hours of incubation and the organism showing growth on inoculated area was identified using standard microbiological techniques which included observation of colony characteristics, staining reactions and biochemical properties (Cheesbrough, 1984; Collee and Marr, 2006).

Appropriate biochemical tests were performed for the confirmed identification of the bacterial isolates. Gram-positive organisms were identified primarily on the basis of their response to gram's staining, catalase test and coagulase test and their colony characteristics on various culture media.

The composition and the method for preparation of culture media, biochemical media and reagents used in biochemical tests are mentioned in Appendix-II. The procedures for biochemical tests and gram staining are mentioned in Appendix- III.

4.2.4.4 Antibiotic susceptibility test of bacterial isolates

The antibiotic susceptibility test of the bacterial isolate towards various antibiotics was done by Kirby-Bauer disk diffusion method as recommended by Clinical & Laboratory Standards Institute (CLSI-M100-2007) using Mueller Hinton Agar (MHA).

The inoculum was prepared by direct colony suspension method by transferring several isolated colonies from fresh non-selective agar plate to normal saline (Moody, 2004). The suspension was vortexed well and its turbidity was adjusted visually with sterile normal saline to match a 0.5 McFarland standard. The bacterial suspension was then swabbed

uniformly over entire surface of MHA plate. Within a sterile forcep, appropriate antibiotic discs were applied manually no closer than 24mm from center to center. The plate was incubated aerobically at 35-37ºC for 18 hours within 15 minutes of application of discs. Zone of inhibition around each disc was measured and was qualitatively reported as susceptible, intermediate or resistant on the basis of standard interpretative chart (Appendix-VI).

All tests were performed with regular quality control. During the study, sterility of each batch of the test medium was confirmed by incubating uninoculated plates overnight at 37ºC. Whole batch of media was discarded if the incubated media showed an evidence of significant bacterial growth. Along with test organisms, *Staphylococcus aureus* (ATCC 25923) was used to check the quality of the medium and performance of the test.

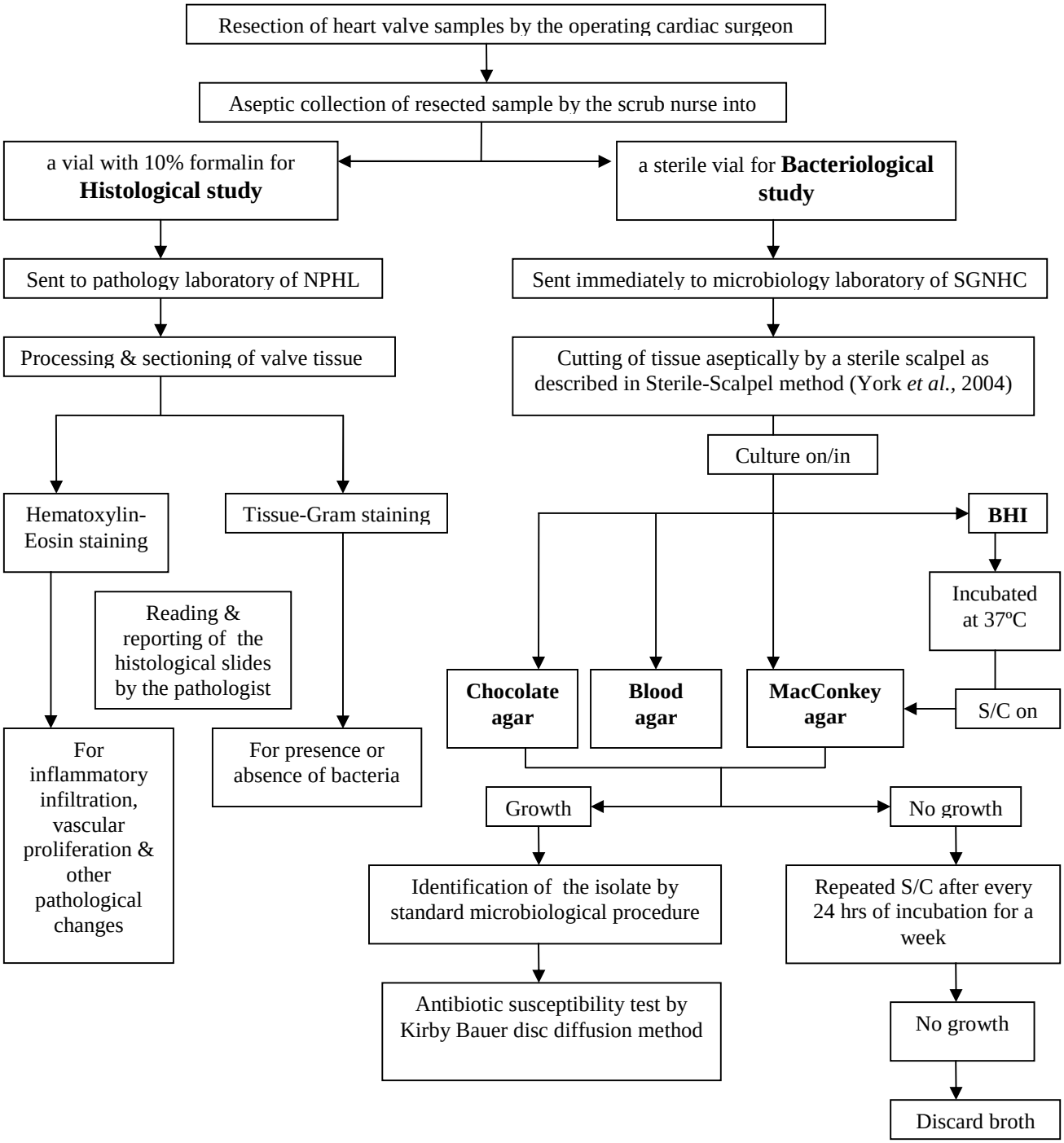
4.2.5 Methods for histological study

Of total 31 resected heart valve samples, only 21 (11 from 'Treated IE' and 10 from 'Suspected IE' group) samples were subjected to histological study because rest 10 samples were not available in formalin-fixed form.

The formalin-fixed heart valve samples were sent to the pathology department of National Public Health Laboratory (NPHL) for entire histological part of the present study.

According to the methodology practiced at NPHL, the tissue sample was firstly examined grossly. Then it was transferred to a cassette and dehydrated by immersing into multiple baths of progressively more concentrated ethanol followed by xylene and finally in hot molten paraffin, the process being called tissue processing. Then in the embedding process, the tissue was set in a mold to create a paraffin block which was then sectioned into thin (2-7µm) sections using microtome. Finally, the tissue sections were stained by Tissue-Gram stain and Hematoxylin-Eosin stain. The methodology for histological staining is mentioned in Appendix-IV. The stained histological slides were examined microscopically by the pathologist and were reported for the presence or absence of microorganisms in Tissue-Gram staining and for the evidence of acute inflammation, chronic inflammation and/or other findings like: loss of normal architecture, calcification, vascular proliferation, degeneration or fibrosis in Hematoxylin-Eosin staining.

FLOW CHART FOR RESECTED HEART VALVE EXAMINATION



BHI: Brain Heart Infusion; NPHL: National Public Health Laboratory; S/C: Subculture; SGNHC: Shahid Gangalal National Heart Centre

CHAPTER-V

5. RESULTS

5.1 CLINICAL PATTERN OF PATIENTS

During the study period, 27 patients were included. The mean age of the patients was 29.53 (± 14.26) years. The frequency of the patients was the highest in the age group 15-35 years representing young population and the lowest in the age group >50 years representing elderly people.

Table 2: Age and gender wise distribution of the patients in the study groups 'Treated IE' and 'Suspected IE'

Age group (years)	Gender		No. of cases in the study groups		Total No. (%)
	No. of males	No. of females	Treated IE	Suspected IE	
<15	4	4	3	5	8(29.62)
15-35	7	4	5	6	11(40.74)
35-50	5	1	3	3	6(22.22)
>50	1	1	1	1	2(7.40)
Total	**17**	**10**	**12**	**15**	**27 (100)**

As shown in the table 2, of 27 patients studied, 17 (62.96%) were males and 10 (37.30%) were females with the male: female ratio of 1.7: 1. Among total patients, 12 were from the study group 'Treated IE' and rest 15 patients were from the study group 'Suspected IE'.

Among the risk factors, rheumatic heart disease (RHD) was found to be the most frequent one being present in 20 (74.07%) cases. Congenital heart defects (CHD) were found in four (14.81%) cases. Remaining three (11.11%) cases were of without any identifiable risk factors.

Among intra-operative surgical findings suspicious of infective endocarditis being noted by the operating cardiac surgeon, the presence of vegetation was the most common being noted in 13 (41.93%) valve samples, followed by valve thickening in eight (25.80%) samples and

valve cusp perforation in six (19.35%) samples. The presence of intra-cardiac abscess was the least common finding being noted in four (12.90%) of samples, three of them being from treated IE patients and one being from suspected IE patient.

5.2 RESULTS OF BACTERIOLOGICAL STUDY

5.2.1 Bacteriological cultural results

All 31 resected heart valves were subjected to bacteriological study. Of these, only three (9.67%) samples were culture positive and remaining 28 (90.32%) samples were culture negative.

Table 3: Pattern of cultural results of heart valve samples resected from 'Treated IE' and 'Suspected IE' cases

Study groups	Total No. of resected heart valve samples	No. of culture positive samples (%)	Overall No. of culture positive samples (%)
Treated IE	15	2 (13.33)	3 (9.67)
Suspected IE	16	1 (6.25)	

Of the culture-positive samples, two (13.33%) samples were from 'Treated IE' study group and one (6.25%) sample was from 'Suspected IE' group.

5.2.2 Microbiological profile

Bacteria of two different genera were isolated from culture-positive samples, both of them being gram-positive cocci. They were *Staphylococcus aureus* from two samples, one being from 'Treated IE' case and the next from 'Suspected IE' case and *Enterococcus faecalis* from one sample of 'Treated IE' group.

5.2.3 Antimicrobial susceptibility pattern of the bacterial isolates

Enterococcus faecalis isolated from heart valve sample of 'Treated IE' group was in vitro sensitive to the antibiotics Ciprofloxacin, Erythromycin, Gentamicin and Vancomycin while resistant to the antibiotics Amoxycillin, Ceftriaxone, Cloxacillin, Cotrimoxazole and Penicillin. *Staphylococcus aureus* isolated from the heart valve sample of 'Treated IE' group was found to be sensitive to the antibiotics Ciprofloxacin, Cotrimoxazole and

Vancomycin; intermediate to Ceftriaxone; and resistant to Amoxycillin , Cloxacillin, Methicillin and Penicillin. Another isolate of *S. aureus* obtained from the sample of 'Suspected IE' group was sensitive to Ceftriaxone, Ciprofloxacin, Cloxacillin, Cotrimoxazole, Methicillin and Vancomycin and resistant to Amoxycillin and Penicillin.

5.3 RESULTS OF HISTOLOGICAL STUDY

Of total 31 samples, only 21 samples (11 samples from 'Treated IE' cases and 10 samples from "Suspected IE' case) were examined histologically in the pathology laboratory of NPHL. Overall histological results of Hematoxylin-Eosin and Tissue-Gram staining, as per the histology reports of NPHL, are presented in the table 4.

Table 4: Pattern of histological results of resected heart valve samples in Hematoxylin-Eosin staining and Tissue-Gram staining

Study Groups	No. (%) of samples revealing following histological results in					Total (%)
	Hematoxylin-Eosin staining			Tissue-Gram staining		
	Acute inflam	Chronic inflam	Calcified degeneration with no signs of inflam	Presence of bacteria	Absence of bacteria	
Treated IE	5 (45.45)	3 (27.27)	3 (27.27)	5 (45.45)	6 (54.54)	**11 (100)**
Suspected IE	1 (10)	1 (10)	8 (80)	0 (0)	10 (100)	**10 (100)**
Total	**6 (28.57)**	**4 (19.04)**	**11 (52.38)**	**5 (23.80)**	**16(76.19)**	**21**

inflam: inflammation

5.3.1 Histological results of Hematoxylin-Eosin stained tissue sections

As shown in the table 4, of total 21 samples examined histologically in Hematoxylin-Eosin staining, acute inflammation was revealed in overall six (28.57%) samples, chronic inflammation in four (19.04%) samples and calcified degeneration with no signs of inflammation in 11 (52.38%) samples.

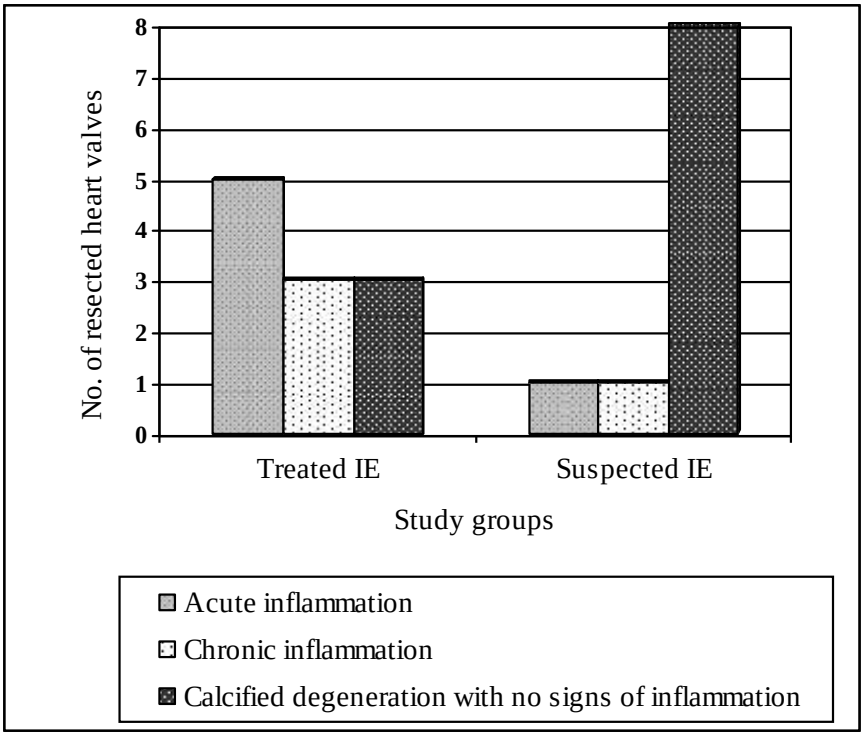


Figure 3: Histological results of Hematoxylin-Eosin stained heart valve sections from 'Treated IE' and 'Suspected IE' cases

As shown in the figure 3, of 11 samples from 'Treated IE' cases, five (45.45%) samples revealed acute inflammation, three (27.27%) samples revealed chronic inflammation and rest three (27.27%) samples showed calcified degeneration with no signs of inflammation. Regarding 10 samples from 'Suspected IE' cases, majority of samples i.e., eight (80%) samples showed just calcified degeneration with no signs of inflammation. Remaining two samples revealed acute and chronic inflammations each.

5.3.2 Histological results of Tissue-Gram stained tissue sections

According to the table 4, of total samples examined histologically in Tissue-Gram staining, bacteria were revealed in five (23.80%) samples. Of these, two samples showed gram-positive cocci while rest three samples revealed gram-negative bacilli.

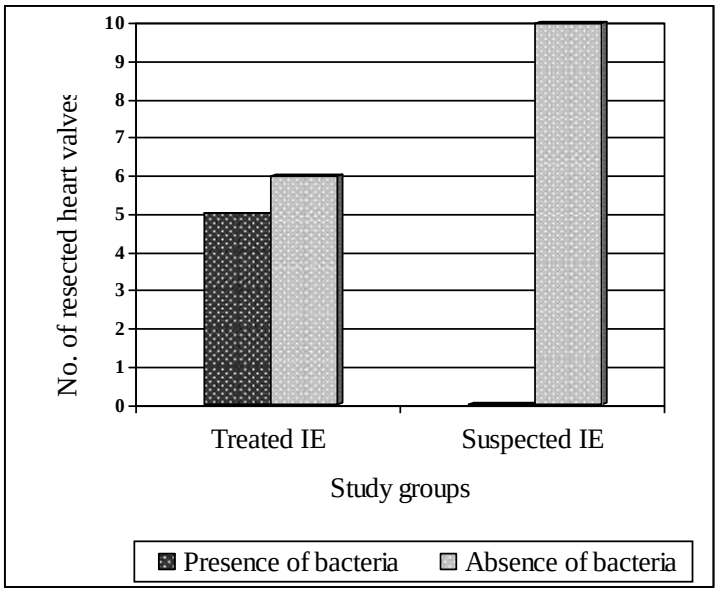


Figure 4: Histological results of Tissue-Gram stained heart valve sections from 'Treated IE' and 'Suspected IE' cases

As presented in the figure 4, all five samples revealing bacteria were from 'Treated IE' cases constituting 45.45% of samples of this study group. None of the samples from 'Suspected IE' group was positive for bacteria in tissue-gram staining.

5.4 INTRA-OPERATIVE AND HISTOLOGICAL RESULTS OF CULTURE-POSITIVE SAMPLES

Table 5: Intra-operative and histological results of culture-positive heart valve samples

Study groups	Intra-operative surgical findings		Histological results		Bacteriological cultural results
	vegetation	Abscess	Acute Inflam	Bacteria	
Treated IE	+	+	+	+ (gram positive cocci in short chains)	*Enterococcus faecalis*
	+	+	+	+ (gram positive cocci)	*Staphylococcus aureus*
Suspected IE	+	-	Histological examination not performed		*Staphylococcus aureus*

+ : presence of; - : absence of; Inflam: Inflammation

As shown in the table 5, only two samples from ‘Treated IE’ cases were culture positive. Intraoperatively, both of the samples revealed abscess along with vegetation. In histological examination, Hematoxylin-Eosin staining showed acute inflammation and Tissue-Gram staining showed gram positive cocci in both of the culture-positive samples. From the sample revealing gram positive cocci, *Staphylococcus aureus* was isolated in culture, while from the next sample showing gram positive cocci in short chains, *Enterococcus faecalis* was isolated.

Only one sample from ‘Suspected IE’ group was culture positive giving *Staphylococcus aureus*. Intraoperatively, vegetation was noted by the operating surgeon. Histological data of this sample was not available to verify the cultural result because of the unavailability of formalin fixed sample.

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

The present study was conducted among the patients having pre-operative diagnosis or intra-operative suspicion for IE who underwent heart valve replacement surgery at SGNHC, in order to obtain bacteriological and histopathological profile of the resected heart valves. A total of 31 resected heart valve samples from 27 patients were studied. A small sample size was one of the limitations of this study.

In the present study, mean age of the patients was 29.53 (±14.26) years ranging from eight to 68 years. The highest number (40.74%) of patients was from the age group 15-35 years representing the young population and the least (7.40%) was from the age group >50 years representing elderly people which was comparable with the study done by Tibrewal (1999) and Kandel (2004).

As found in this study, the higher incidence of IE among young people is also true in other developing countries like Nepal. This indicated RHD to be the most common factor among younger population predisposing them to the development of IE.

However the epidemiologic features of IE has changed in most of the developed countries. The mean age of the patients used to be between 15-35 years during pre-antibiotic era which has more recently been between 40-60 years. The mean age of people developing IE in western countries has notably increased over time as recorded 54.4 (±17.3) years in the study conducted in U.K during 1980 to 2006 by Heiro *et al.,* 43.3(±9.1) years as reported by Peri *et al.* in 2000, 44.95 (±0.3) years as reported by Renzulli *et al.* in 2000. This shift in mean age of IE patients is partly because of the dramatic reduction in the incidence of rheumatic disease in western world and partly as a result of increasing longevity, new predisposing factors and an increase in nosocomial cases.

In the present study, male patients were more frequent than female patients with a male: female ratio of 1.7: 1. Renzuli *et al.* (2000) found this ratio to be 1.9: 1 in their study conducted on IE during 1978 to 1998. Kandel in 2004 reported the ratio to be 2.4:1. Higher male: female ratio among the IE patients has been reported in many studies. Heiro *et al.* in 2006 reported the ratio to be 2.5: 1 and Peri *et al.* in 2000 reported it to be 4.1: 1.

In this study, rheumatic heart disease (RHD) was found to be the most common underlying risk factor for the development of IE being presented by 74.07% of the cases. Kandel in 2004 as well reported that RHD was the most common underlying cardiac disease being present in 91.20 % of patients. In fact RHD is the most common heart disease of developing countries like Nepal as reported by Limbu *et al.,* in 2002. Congenital heart defects were found in 14.81% of our patients, the most common of them being bicuspid aortic valve followed by septal defects, tetralogy of fallot (TOF) and patent ductus arteriosus (PDA). Rest 11.11% of cases had no identifiable risk factors.

In present study, as in the study conducted by Kandel in 2004, none were the cases of intravenous drug abuse. According to the data of SGNHC, intravenous drug abusers comprised negligible proportion of IE cases in its setting though were considered a significant risk group for IE in developed countries. This could have been either because the number of IVDA in our country was lower than that in the western world or IE among IVDA had remained underdiagnosed in our country or because the IVDA patients did not report to the hospital due to social and/or economic reasons.

The presence of valve prostheses and other intra-cardiac devices is another risk factor for IE development. In this study, none were the cases of prosthetic valve endocarditis. In the study conducted by Kandel (2004), PVE comprised only a single case. According to the data of SGNHC, early PVE was a rare incidence in its setting. This indicated the minimal intra-operative contamination and hence maintenance of satisfactory level of sterility in operation theater (which was also indicated by nil major surgical wound infection rate according to The Annual Report-2007 of SGNHC). Another reason for this could be because of the practice of attempting valve replacement surgery among IE patients only at the healed stage of infection as far as possible.

Among various predisposing conditions of IE mentioned by Heiro *et al.* in 2006, acquired valvular diseases comprised 23%; prosthetic valve 20.60%; bicuspid aortic valve 11.70%; mitral valve prolapse 10% and congenital defects 3.10%. 31.60% had no underlying cardiac conditions, 7.70% had history of IVDU and 31% were chronic dialysis patients. Peri *et al.* (2000) reported RHD to be present in majority (63%) of patients, while degenerative heart disease to be less frequent (17.40%). 6.50% of cases were with no single identifiable predisposing condition for IE.

Various intra-operative surgical findings noted by the operating cardiac surgeons like: vegetation, cusp perforation, valvular tear, thickened cusps, ruptured chordae tendinae, annular/periannular abscess etc. were important for this study because it was theses findings based on which the surgeons intraoperatively suspected infective endocarditis and sent samples from such cases for the study. In this study, valvular vegetation was found to be the most common intra-operative finding in both treated and suspected cases, being noted in 41.93% of samples, followed by valve thickening in 25.80%, cusp perforation in 19.35% of samples. Abscess in aortic root or on mitral valve leaflet was noted more among treated cases (20%) than among suspected cases (6.25%).

Though IE may be one of the reasons for various intraoperataively revealed pathologies like: thickened/torn/ruptured/perforated valve cusps, ruptured chordae tendinae or valvular vegetation, other non-infectious pathological conditions such as RHD, congenital defects or degenerative changes as well can be the contributing factors. However the presence of intracardiac abscess is always an indicative of IE irrespective of pre-operative diagnosis or cultural result. In this study, abscess was noted in three of 15 samples from treated cases. Of these, only two samples were culture positive both giving gram positive cocci. The remaining culture negative sample revealed acute inflammation at histologic level giving an evidence of infection. This indicated that the abscess in this sample was sterile which was because of the six weeks of pre-operative intravenous antibiotic therapy received by the patient.

Since none of the patients in the ‘Suspected IE’ study group had received pre-operative antibiotic therapy for IE, in any case of unexpected infection, they were supposed to give positive culture of their resected valve tissue. In this study, among intraoperatively

suspected cases, an abscess was detected intraoperatively in only one case which was found to be culture negative. Histopathology also failed to reveal any signs of inflammation. This false negative result could have been the result of errors in tissue sampling procedure. Since an infective process might be confined to a small area of valve tissue, the sample selected and provided for this study might be devoid of infective process and hence gave false negative cultural and histological results.

Culture of resected heart valves and other valvular tissue constitutes a standard post-surgery microbiological diagnostic approach. It has been mentioned in literature that culture of resected valvular tissue is infrequently positive ranging from 15-25%. In the present study, out of 31 valve samples cultured, only three (9.67%) samples were culture positive. Renzulli *et al.* (2000) reported 15.08% culture positivity of resected valve tissue from IE cases. In 2001, Wallet *et al.* reported that 13 out of 53 heart valve samples were culture positive which comprised a culture positivity of 24.52%.

Such a lower rate of culture positivity from valvular tissue from IE cases could be because of perioperative antibiotic prophylaxis, healed endocarditis, the procedure of priming the oxygenator with crystalloid solution and antibiotics, the use of crystalloid cardioplegia that might cause an osmotic and physical shock to bacteria still surviving in vegetation (though blood cardioplegia was used at SGNHC) and most importantly because of broad range of preoperative antibiotic therapy. In this study, the use of routine microbiological technique that was insufficient for the isolation of fastidious bacteria and non bacterial causative agents could also be an important reason of such low culture positivity and was an important limitation of this study.

In the present study, of three culture positive samples, two were from treated cases and one was from intraoperatively suspected case. All treated IE cases had completed their four to six weeks of preoperative antibiotic therapy and all of them had undergone surgery to correct hemodynamic decompensation resulting from IE-related valvular damage or dysfunction and not because of known cause of uncontrolled infection. Hence, theoretically they were supposed to have healed endocarditis. Out of 15 samples from 12 treated cases, only two (13.3%) samples were culture positive giving *Staphylococcus aureus* and *Enterococcus faecalis*. Morris *et al.* (2003) reported 9.0% recovery of organisms from valve

culture among the patients who had completed their standard course of preoperative antibiotic therapy. The isolates reported were *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Streptococcus sanguis*, *Brucella abortus*, *Candida albicans* and *Aspergillus fumigatus*.

Both of these culture positive valve samples from treated cases were evaluable with histological report corresponding to the presence of gram positive cocci and acute inflammation. Intraoperatively, valvular vegetation and abscess were noted in both of these samples. In addition, cusp perforation too was noted in one of the samples.

In addition, one more sample from 'Treated IE' group, showed growth (turbidity) in BHI broth, but it failed to give growth in subculture on any of the agar culture media (chocolate agar, blood agar or MacConkey agar). Gram stain of an aliquot of BHI broth showed gram negative rods. Intraoperatively, there were valve destruction, vegetation and rupture of chordae tendinae of anterior mitral leaflet which could be due to bacterial invasion. Histologically, the valve sections showed acute inflammation and vascular proliferation, which were the signs of bacterial infection, in Hematoxylin-Eosin staining and gram negative bacilli in Tissue-Gram staining. On this background, it could be regarded as the case of culture negative endocarditis which could have been because the organism was fastidious for the routine cultural conditions to support its recovery. It could be fastidious gram negative bacilli such as HACEK group of bacteria, *Bartonella* spp., *Brucella* spp., or others.

Among 16 resected heart valve samples cultured from intraoperatively suspected IE cases, only one (6.25%) sample was culture positive from which *Staphylococcus aureus* was isolated. The patient had cusp perforation and vegetation in intra-operative surgical findings which might be due to infection. However histopathological data for this sample was lacking because of unavailability of formalin fixed tissue, to verify the evidence of microbial infection of the valve.

Viridans streptococci are considered the most common etiological agents of infective endocarditis accounting for approximately 30-40% cases of sub-acute bacterial endocarditis. However in the present study, *Staphylococcus aureus* and *Enterococcus faecalis* were the

only isolates. Viridans streptococci were isolated from none of the samples. It could be because of the sterilization of heart valves by the standard course of preoperative intravenous antibiotic therapy received by all the cases of 'Treated IE' group. Upton *et al.* (2004) in the study "Culture results of heart valves resected because of streptococcal endocarditis: Insights into duration of treatment to achieve valve sterilization" reported that two weeks of continuous combination therapy or four weeks of penicillin therapy was sufficient for valve sterilization in more than 95% of streptococcal endocarditis cases.

In antibiotic susceptibility test, all of the isolated gram positive cocci were sensitive to Ciprofloxacin and Vancomycin and resistant to Amoxycillin and Penicillin. In addition to this, *Enterococcus faecalis* was sensitive also to Erythromycin and Gentamicin; and exhibited resistance towards Ceftriaxone, Cloxacillin and Cotrimoxazole. The strain of *Staphylococcus aureus* isolated from the treated case was sensitive also to Cotrimoxazole; intermediately sensitive to Ceftriaxone; and resistant to Cloxacillin and Methicillin. While the strain isolated from the suspected IE case was sensitive also to Ceftriaxone, Cloxacillin, Cotrimoxazole and Methicillin.

In histopathological examination of stained tissue sections, the presence of bacteria in Tissue-Gram staining and inflammatory infiltration and neovascularization in Hematoxylin-Eosin staining were considered histological evidence of infection of heart valves. An acute inflammation as described as infiltration of tissue predominantly by neutrophils, could be taken as an evidence of active infectious process in the absence of microorganisms. A chronic inflammation as described as the infiltration of tissue predominantly by mononuclear inflammatory cells (lymphocytes, macrophages, plasma cells) could be the sign of infectious process especially after the termination of active infection. In addition, the chronic inflammation could also be present in the tissue undergoing chronic damage by non-infectious processes like rheumatic, congenital or degenerative diseases. For example histological examination of heart valves in RHD had been shown to have Aschoff bodies which were perivascular foci of eosinophilic collagen surrounded by lymphocytes, plasma cells and macrophages.

In the present study, histopathological examination was carried out in only 21 heart valve samples, 11 samples from 10 treated cases and 10 samples from 10 suspected cases. The

inability of performing histological examination of all 31 samples was an important limitation of this study.

Of 11 heart valve samples from treated IE cases, only five (45.45%) samples showed bacteria with or without acute inflammation, three (27.27%) samples showed chronic inflammation without bacteria and rest three (27.27%) samples revealed no signs of inflammation. Of bacteria revealed in five samples in Tissue-Gram staining, two samples showed gram positive cocci and rest three showed gram negative bacilli. Lepidi *et al.* (2005) reported the presence of bacteria and/or acute inflammation in 70.58% of heart valves resected from definite IE cases, followed by chronic inflammation without bacteria in 17.64% and no signs of inflammation in 11.76% of resected valve samples from such patients.

The lower occurrence of bacteria and/or acute inflammation in our study might be because all of the cases in the 'Treated IE' group had completed their full course of preoperative antibiotic therapy which could have destroyed the infecting bacteria. Further some of the cases in this study group had undergone valve replacement surgery after as long as six months of active infection. Bacteria might have been removed by the immune system and acute inflammation might have been replaced by granulomatous inflammation during this extended period before surgery. Moreover, Tissue-Gram staining alone was used in this study which might be insufficient to reveal the infecting bacterial and non-bacterial agents. The use of supplemental special tissue stains like: Periodic acid Schiff (PAS), Giemsa, Grocott-Gomori methenamine silver and Warthin-Starry stains and advanced techniques like immunohistochemistry and auto-immunohistochemistry as practiced in the study of Lepidi *et al.* in 2005, would have increased the sensitivity of detecting microorganisms in infected valve tissue.

Of the five samples revealing bacteria with or without acute inflammation at histological level, only two samples were culture positive in bacteriological study. From the sample showing gram positive cocci in short chains, *Enterococcus faecalis* was isolated and from the next sample showing gram positive cocci, *Staphylococcus aureus* was isolated. One more sample that grew possibly fastidious bacteria in BHI enrichment broth showed gram negative bacilli, acute inflammation and vascular proliferation at histological level. Rest of

the two samples showing gram negative bacilli histologically remained culture negative. This indicated that the bacteria seen histologically were non-viable because of the preoperative antibiotic therapy and they were yet to be removed from the sterilized valve by phagocytosis and/or bacterial cell lysis which could take months. Being nonviable, the bacteria posed no infection risk to the newly implanted valve prosthesis.

Regarding 10 valve samples from 'Suspected IE' cases subjected to histological examination, majority i.e., 80% of samples revealed no signs of inflammation but only extensive fibrosis. One i.e., 10% sample showed chronic inflammation and rest one i.e., 10% sample showed acute inflammation. Lepidi *et al.* (2005) in their study reported the presence of bacteria and/or acute inflammation in rare i.e., 1.63% of valve samples resected from cases without pre-operative diagnosis or suspicion of IE, followed by chronic inflammation without bacteria in 37% and just fibrosis with no signs of inflammation in 60.65% of samples from such cases.

In the present study, one (10%) culture negative sample from suspected IE case showed acute inflammation histologically giving an evidence of infection. Since this patient had no pre-operative indications of infective endocarditis, this could be considered as Asymptomatic, Concealed, Incubating or Latent IE case. The study "Asymptomatic Endocarditis: Are consequent histological studies useful in valve surgery" conducted by Zurbrugg *et al.* (1996) reported a similar finding of histological evidence of IE in 11.4% of heart valves resected from the patients with no pre-operative diagnosis of IE. All valve samples were culture negative. Saitoh *et al.* (1997) in their study titled "Concealed infective endocarditis" reported that 11.3% of excised heart valve samples from cases with no pre-operative diagnosis of IE revealed acute inflammation, all samples being culture negative.

6.2 CONCLUSION

The bacteriological and histopathological study of the resected heart valve samples showed that valve culture was more often positive among preoperatively treated IE cases than intraoperatively suspected cases isolating *Staphylococcus aureus* and *Enterococcus faecalis*. The only culture positive sample from suspected IE case gave *Staphylococcus aureus* in culture. Almost half of the samples from treated infective endocarditis cases revealed

bacteria with or without acute inflammation at histological level. Most of the samples from suspected infective endocarditis cases showed only fibrosis and calcified degeneration with no signs of inflammation. Both culture positive samples from treated cases revealed bacteria and acute inflammation histologically.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. Altogether, 31 resected heart valve samples from 27 patients (15 valve samples from 12 ‘Treated IE’ patients and 16 valve samples from 15 ‘Suspected IE’ patients) were included in the study. All 31 valve samples were subjected to bacteriological study while only 21 samples (11 valve samples from 10 ‘Treated IE’ cases and 10 valve samples from 10 ‘Suspected IE’ cases) were examined histopathologically because of unavailability of formalin-fixed tissue in rest 10 samples.

2. The mean age of the patients was 29.53(± 14.26) years. Most of the patients fell in the age group of 15-35 years representing young people.

3. The frequency of male patients was higher than that of female patients with a male to female ratio of 1.7: 1.

4. Most (74.07%) of the patients had rheumatic heart disease as underlying cardiac risk factor. Minority (14.81%) of the patients had congenital heart defects.

5. Pre-operative echocardiography revealed valvular vegetation in 15 (55.55%) cases. All 12 (100%) cases of ‘Treated IE’ group showed vegetation in echocardiography, of which two cases also revealed abscess.

6. Aortic valve was the most commonly affected valve accounting for 54.83% of total resected valves studied. This was followed by mitral valve (35.48%), pulmonary valve (6.45%) and tricuspid valve (3.22%). Of 27 patients, four (14.8%) patients had undergone double valve replacement surgery.

7. Among typical signs of infected or possibly infected valves found intraoperatively, the presence of vegetation was the most common being noted in 41.93% of samples, followed by valve thickening in 25.80% and valve cusp perforation in 19.35% of

samples. The least common finding was the presence of intra-cardiac abscess which was noted in 12.90% of samples.

8. Of 31 heart valve samples cultured, only three (9.67%) samples were culture positive. All three isolates were gram positive cocci.

9. Of 15 samples from treated IE cases, only two (13.33%) samples were culture positive giving *Enterococcus faecalis* and *Staphylococcus aureus*. Of 16 samples from 'Suspected IE' cases, only one (6.25%) sample was culture positive giviing *Staphylococcus aureus.*

10. In antibiotic susceptibility test, all three gram positive isolates were sensitive to Ciprofloxacin and Vancomycin and resistant to Penicillin and Amoxycillin. *Enterococcus faecalis* exhibited resistance towards most of the antibiotics.

11. Of 21 samples, histology revealed bacteria in only five (23.80%) samples in Tissue-Gram staining and acute inflammation in six (28.57%) samples, chronic inflammation in four (19.04%) samples and calcified degeneration in 11 (52.38%) samples in Hematoxylin-Eosin staining.

12. Majority (45.45%) of samples from treated cases histologically showed bacteria with or without acute inflammation. Of these, two samples were culture positive.

13. Majority (80%) of samples from suspected IE cases showed only fibrosis and calcified degeneration with no signs of inflammation. None of the samples histologically showed bacteria.

14. Concealed endocarditis was histologically revealed in 10% of suspected IE cases.

7.2 RECOMMENDATIONS

1. It is recommended that all valvular tissue resected during valve replacement surgery from the patients with pre-operative diagnosis of IE should be routinely cultured irrespective of pre-operative blood culture result or antibiotic therapy.

2. All infected or possibly infected cardiac tissue suspected intraoperatively should be routinely cultured irrespective of pre-operative diagnosis and indications for surgery. If culture becomes positive, the patient should be treated for IE according to its antibiotic susceptibility profile.

3. All resected valves requested for culture should be examined histologically.

4. The valve culture result should be reported only along with its histological report in order to exclude the false-positive culture results.

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