IMMUNOLOGICAL RESPONSES IN SEXUALLY TRANSMITTED DISEASES

BY

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DECLARATION

The author hereby declares that the work presented here for the Master of Science Degree in IMMUNOLOGY (MEDICINE) was conducted solely by himself under the supervision of Prof. G. C. Onyemelukwe - Head of Dept, Dept of Medicine, Faculty of Medicine, and Dr (Mrs) Y. O. Elegba - Consultant Medical Microbiologist, Dept of Medical Microbiology, Faculty of Medicine, Ahmadu Bello University, Zaria.

No part of it has been presented for any award of degree and reference to be published literature is adequately acknowledged.

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CERTIFICATION

This thesis meets the regulation governing the degree of Master of Science of Ahmadu Bello University and is approved for its Contribution to Scientific Medical Knowledge and literary presentation.

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DEDICATION

This work is dedicated to all patients with sexually transmitted diseases and all who have contributed to this work in the hope that lasting cure and possible vaccine would be found for their diseases in the foreseeable future.

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ABBREVIATIONS

VD.	Venereal diseases.
STD.	Sexually transmitted diseases.
ON.	Ophthalmia neonatorum.
GUM.	Genito-urinary medicine.
CDC.	Centre for Disease Control.
MIF.	Macrophage Migration Inhibition Factor.
APC.	Antigen Presenting Cells.
PF.	Polymorph function.
NF.	Neutrophil function.
PMN.	Polymorphonuclear leukocytes.
DGI.	Disseminated gonococcal infection.
TRIC.	Trachoma-Inclusion Conjunctivitis.
LGV.	Lymphogranuloma Venereum.
EB.	Elementary body.
RB.	Reticulate body.
MEMS.	Minimum Essential Medium Solution.
CD.	Cluster of differentiation.
	Cluster of determinant.
BKI.	Bacterial Killing Index.
CMI.	Cell Mediated Immunity.
HIV.	Human Immunodeficiency Virus.
PID.	Pelvic Inflammatory Disease.

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ABSTRACT

The lymphocyte sub-populations cd3, CD4, CDS, CDI9 were determined in the blood of fifty patients each with acute uncomplicated Neisseria gonorrhoea: Chlamydia trachomatis and Haemophilus ducreyi genital infection. Macrophage migration inhibition factor (MIF) test to purified protein derivative (PPD) to assess functional cell mediated immunity and the polymorph function test for the evaluation of phagocytosis and bactericidal activity were also assessed in the same patients. Comparison was made of the above results with thirty (30) normal healthy age. and sex matched controls.

Neisseria gonorrhoea studies

The mean lymphocyte subsets CD3, CD8, CD19 absolute numbers were raised compared to control subjects, although not significantly ($p > 0.05$) in all the patients. CD4 cells percentages in thirteen patients (26%) were below the value in the normal healthy control, although not significantly ($p=0.01$). There is tendency to reversal of the CD4/CD8 cell ratio in this disease. The study suggests that T-cell were activated during this disease, and that they may have a role in the containment of this infection. Macrophage inhibition factor test, indicated that there is a partial acquired cell mediated immunity in gonococcal urethritis and cervicitis.

The polymorph function using Staphylococcus aureus as assay test indicated that there is no impairment in the polymorphonuclear leucocyte cell function in comparison to controls.

Chlamydia trachomatis studies.

The study in Chlamydia trachomatis genital infection showed that there is no significant increase in lymphocyte subsets of CD3, *CD8,* CD19, $p > 0.05$. Fourteen (14) or 28% of the patients

had CD4 cells below the mean of the control subjects, though none significantly, $p > 0.05$. The CD4/CD8 cell ratio was not inverted in this disease.

Acquired protective cell mediated immunity in Chlamydia trachomatis genital infection was assessed by the Macrophage Migration Inhibition Factor (MIF), a cytokine. The study showed that there is complete acquired protective cell mediated immunity in chlamydia genital infection, though $p < 0.05$.

The Bacterial Killing Index (BKI) was assayed by the ability of the neutrophil to ingest Staphylococcus aureus. There was no impairment in the polymorphonuclear cells to eliminate Staphylococcus aureus of capacity. Therefore, there is no impairment of polymorph function in Chlamydia genital infection.

Haemophilus ducreyi studies.

The study in Haemophilus ducreyi genital infection indicated that nine (9) or. 18% of the patients' CD3 cells were below the control value: Fourteen (14) or 28% of the patients' CD8 cells were below the control value Eleven (11I) or 22% of the patients' CD8 cells below the control value, all these differences were not significant $p > 0.05$. The CD1.9 cells of all the patients were above, the normal control subjects, though not significant, $p > 0.05$.

The cell mediated immunity was assessed by measurement of the Macrophage Migration Inhibition Factor (MIF), a lymphocytokine. The study indicated that there is a incomplete cell mediated acquired immunity in H. ducreyi genital ulcer. The polymorph function was assessed and found to be intact.

CHAPTER ONE

1.00 GENERAL INTRODUCTION

Sexually transmitted diseases are a group of communicable diseases that are transferred predominantly by sexual contact (1).

A sexually transmitted disease (STD) is defined as one in which sexual contact is epidemiologically important but is not the only mode of acquisition (2). Venereal diseases and sexually transmitted diseases are the same disease, transmitted through sexual contact or by venereal route. For several years the term venereal disease was used for STD. Because of social distaste, embarrassment, disgust, shame, horror, immoral connotation and social stigmata, the term sexually transmitted disease has replaced venereal disease, which is less acceptable to the public. However the change in nomenclature has not changed the definitions and the route of transmission of the disease.

It is evident that the world is currently witnessing a profound change and increase in sexually transmitted diseases. The advent of Acquired Immune Deficiency Syndrome (AIDS) has complicated the issue and made matters worse. In previous years, sexually transmitted diseases included mainly the classical types - gonorrhoea, syphilis, chancroid, lymphogranuloma inguinale and donovanosis - and they have been called the first generation of STD (3). The second generation STD's are Herpes virus Hepatitis B virus, Chlamydia trachomatis infection. Third generation of STD is Acquired Immune Deficiency Syndrome as a result of infection with HIV. See Tables 1 & 2.

TABLE I

"TRADITIONAL" SEXUALLY TRANSMITTED DISEASES

FIRST GENERATION SEXUALLY TRANSMITTED DISEASES

Micro-organism	Diseases
Bacteria:-	
Treponema pallidum	Syphilis
Neisseria gonorrhoea	Gonorrhoea
Haemophilus ducreyi	Chancroid
Chlamydia Trachomatis	Lymphogranuloma Venereum (LGV)
Donovania granulomatis	Granuloma inguinale
Protozoa:-	
Trichomonas vaginalis	Trichomoniasis
Ectoparasites:	
Phthirius pubis	Pediculosis
Sarcoptes scabies	Scabies

TABLE 2

SECOND GENERATION SEXUALLY TRANSMITTED DISEASES

Microorganism	Diseases
Bacteria	
Chlamydia trachomatis (genital strains)	Non-gonococcal urethritis, epididymitis, cervicitis, salpingitis, neonatal conjunctivitis
Ureaplasma urealyticum	Non-gonococcal urethritis
Shigella	Shigellosis (Homosexual men)
Viruses	
Herpes simplex virus	Genital herpes
Human Papilloma virus	Genital warts
Cytomegalovirus	Neonatal disease
Hepatitis B and C viruses	Hepatitis
Protozoa	
Entamoeba histolytica	Amoebiasis (Homosexual men)
Giardia lamblia	Giardiasis
Trichomonas vaginalis	Vaginitis

Because of the improve road net works it is now possible to contact STD in the rural area and transport it to the cities, and vice visa. Air travel, because of airports, built in states many of the Federation has aided migration from one part of the country to another, and casual sexual

intercourse is recognized risk factor associated with travelling (4). In many communities in Nigeria, virginity is no longer a strong virtuous factor in marriage.(5) The increase in the number of higher Institutions of learning - Polytechnic, Colleges of Education and Universities has led to increase in social contact of all age groups. Furthermore, the National Youth Service Corps (NYSC), caused the sudden increase in mobility of the young age groups susceptible to STD. The Nigeria civil war of 1968 - 1970, led to the stranded women taking to prostitution. In Nigeria there are few specialists in venereology or genitourinary medicine, leading to increase in and complications of STD (4).

Bello 1983 observed that the sex incidence in STD follows the global pattern, with male to female ratio of 15:1 to 10:1. Because in Nigeria males are mobile and adventurous and enjoy early social contact than female (6). Beside in Northern Nigeria for example men are more readily symptomatic than women, and the males are more likely to seek medical attention because of cultural barriers.

At the West African College of Physician, conference in Kaduna (1993) whose theme was Life style diseases Onyemelukwe emphasized that life styles were risk factors for STD, as well as for non-communicable diseases like obesity, hypertension, diabetes mellitus etc (7). Although Nigerians are highly religious and ethnically oriented these dispositions seem not to have affected the transmission of STD, as pre-marital and extra -marital sexual contacts are very rampant leading to high incidence of STD, whose mode of transmission is largely heterosexual in Nigeria (8).

The use of contraceptive particularly condoms is limited to a small (5%) percentage of the Nigerian population (5). Most treatment of STD in the rural areas is done by alternative medical practitioner, and been poorly managed, their spread is consequently increased (4).

1.01 AIMS AND OBJECTIVES OF THE STUDY

There is the need to investigate sexually transmitted diseases which have plagued man for centuries in order to,

a. advance medical knowledge of the diseases and understand the state of immunity in patients who get these diseases. Such knowledge could assist in the control and treatment of STD and lead to development of vaccines to prevent and eliminate these diseases.

The aims of the study are therefore

1. To determine the cellular immunological responses of patients with specified STD by determining lymphocyte sub-populations, CD3, CD4, CD8, CD19;

2. Determine the macrophage migration inhibition factor (MIF) test as a reflection of the functional integrity of the cellular immunity of the these patients;

3. To assay the neutrophil bactericidal index of these patients in order to determine whether there are defects in function of the neutrophils of infected patients.

The Sexually transmitted diseases studied were:

Neisseria gonorrhoea genital Infection.

Chlamydia trachomatis genital Infection.

Haemophilus ducreyi genital Ulcer.

CHAPTER TWO

2.00 LITERATURE REVIEW.

2.01 Epidemiology of STD.

The marked increase in sexually transmitted infections during the last decade has led to renewed interest in the immunology of these diseases (9,10). The spectrum of sexually transmitted diseases has widened, as more diseases are being included into the list of STD. Oriel in 1982 divided the STD into the "traditional" STD and the second Generation STD (3). The Centre for Diseases Control in Atlanta in 1993 listed 20 diseases (11). At that time, this excluded Acquired Immuno-Deficiency Syndrome (AIDS). Just as the list is increasing or being enlarged, the incidence of STD is increasing world wide. (12,13).

The true incidence of STD is difficult to determine. In some countries, they are not reported at all because of social economic implications and impact, especially, on religion, moral behaviour tourism and hotel industry (14, 15). In other countries, like Norway and Sweden only the traditional STD are reported, while others go unreported or ignored. Under reporting is a world wide problem, particularly in countries where substantial numbers of patients are treated privately or by quacks (16). However in Scandinavia and United Kingdom, 90% of the patients with STD are treated in hospital clinics and the statistics are most reliable (17).

National Statistics give a reasonable idea of the trend of infections. In United States of America, it is believed that 80% of patients with STD are treated privately and most cases go unreported (16). In countries e.g. Britain and France there is accurate reporting (18). Also the cause of under reporting may also be due to cultural reasons, males cannot examine female genitals especially where female patients are only examined by female doctors (18).

The year 1989 marked the centenary of Ducrey's discovery of the bacillus, Haemophilus ducreyi

(20). During the first two decades following his discovery, at least a score of microbiologists and clinicians studied both the organism and the disease chancroid(21).

However, during the next seven decades, there was a dearth of interest, with fewer than thirty significant publications. During the tenth decade since the H. ducreyi discovery, interest has re-awakened because of chancroid outbreaks in several parts of the globe, like in Winnipeg, Canada, Greenland, Southern California and more recently in Dallas, Texas, Boston, Massachussets, New York and Orlando (20). Chancroid is regarded as a tropical sexually transmitted disease, and therefore efforts tended to be concentrated more on its treatment and management and not on the mechanism of the disease (22, 23, 24, 25).

Thus investigation on the humoral or cell mediated immune responses is scarce or non-existent.

2.02 Agents of Sexually transmitted Disease:

The Centre for Disease Control, Atlanta has identified twenty pathogens known to be implicated in STD(10). These pathogens and the diseases they cause are shown in tables 1 and 2.

2.03 Causes of global increase in STD.

Sexually transmitted diseases have always existed side by side with man, as sex is one of the social and biological obligations of man and woman. Lack of sexual desire is abnormal or may be symptomatic of a psychosomatic disorder (26). However, the present high level of which can be termed hyperendemic may be due to the following important contributing factors:

a. Demographic factor.

The baby boom of the seventies, and increase of the population after the world war II in most countries, has led to increase of the young population of adults (26). The age of menarche has

decreased in western world and some third world countries including Nigeria(27). Therefore, there are more young people at risk of acquired sexually transmitted pathogens.

In Europe, the estimated number of immigrants was about 4 million in 1982 consisting mainly unmarried men and women and these people have settled into new cultural environment, and acquired STD through casual sex encounters.

The world has become a global village, which has been made possible by increasing air travel, and availability of fast airplanes. Business and holiday trips which were not possible in previous years are now possible. During such trips, casual sex encounters are possible.

2.04 **Behavioural Factor:**

Extra-marital sexual encounters, which were frowned at in olden days, are rampant now. In fact in western countries, a satisfactory sex life is seen as basic human right. Premarital sexual intercourse is more accepted now. It is therefore possible for man or woman to have sexual intercourse with several people before marriage and the consequence could be STD (28).

Pornographic films, sexual content of newspapers and magazines, sex boutiques, films, television programmes, and cable satellite television introduce young people to direct sexual experience. Commercial sex workers previously called prostitutes also contribute to the spread and increase of STD. For example, in United Kingdom, 10 - 15% of gonorrhoea are spread by commercial sex workers (29). In developing countries, the influence of indigenous culture and religious impact may still have inhibiting influence on the rural population (29).

The increasing wave of drug addiction and consumption of alcohol have led to the increase in casual sexual experience after the consumption of such drugs and alcohol particularly western societies and westernising societies.

The introduction of contraceptive has been claimed to encourage sexual experiences and increase in STD. The role of contraceptive remains controversial as there are proponents and opponents(5). In western societies, homosexual practices have contributed to increase in STD, and indeed the first cases of AIDS were diagnosed in homosexual men (30).

Homosexual sex practices are not yet significant contributing factors in African and some Third World countries, where it remains a 'taboo' to be a homosexual. Pathogens such as Entamoeba histolytica, Giardia lamblia, Shigella species, have been spread sexually by homosexuals (31).

2.05 **Medical factors**:

The availability of over-the-counter drugs particularly antibiotics without prescription has encouraged self-medication of STD, and therefore introducing resistant strains. This practice is common in Third World countries particularly in Nigeria(32). Self-medication increases the spread of resistant strains (33). Before the advent of AIDS, scanty hours were devoted to the teaching of STD in most medical schools in United States of America while in some other medical schools it was non-existent (34). In developing countries where there is shortage of medical specialists in the area of genitourinary medicine (venereology) the problem is even more enormous as most medical professional have scanty knowledge of the management of STD (34).

Another important factor in promotion of STD is co-infection with multiple organisms. Patients with STD are more likely than others to have multiple sexual partners, therefore simultaneous infections are common. For example gonorrhoea can co-exist in 23% of women with trichomoniasis while Trichomonas vaginalis can be recovered from 4-32% of men and 27% - 63% of women with acute gonococcal infection (35).

The abuse of antimicrobial drugs has earlier been mentioned as one of the contributing factors to the spread of STD. Osoba and co-workers in 1977, in Ibadan obtained a reduced sensitivity rate of 82.5% for 80 strains of Neisseria gonorrhoea to penicillin (36). Antimicrobial are freely available without medical prescription. The sad consequences of this bacterial resistance phenomenon is that the treatment of venereal infections is becoming more difficult and expensive, and in a country where healthcare is low down in priority in the budget, the implication are palpable (37). In Nigeria it was postulated that the yaws caused by Treponema pertenue, which was prevalent in the south in early 1940's protected the southern Nigeria population from syphilis unlike in Northern Nigeria(37). As yaws has been eradicated by WHO in Nigeria, the cross-immunity it confers in the southern Nigeria has been lost in the present generation.

2.06 **Complications of sexually transmitted diseases.**

Neonatal Complications:

Ophthalmia Neonatorum. This is defined as conjunctivitis that occurs in the first 4 weeks of life, and though it is not life threatening it poses considerable risk of ocular damage of blindness if not diagnosed and treated appropriately (38).

In developed countries, the incidence of ophthalmia neonatorum varies from 0.2 - 05% of live births (38). There is little information from the third world but in African countries, it is as high as 10 to 34% (39).

Sexually transmitted diseases have been implicated as cause of ophthalmia neonatorum among which Neisseria gonorrhoea and Chlamydia trachomatis have attracted most attention(39). Chlamydia may be acquired from the vaginal route during delivery from mothers who are

symptomatic or asymptomatic. For example 18% to 74% of babies born to women infected with Chlamydia trachomatis will develop ophthalmia neonatorum(40). Other pathogens not causing sexually transmitted diseases have been implicated such as, Group B streptococcus, Staphylococcus aureus, Haemophilus influenza and Streptococcus pneumonia(40).

In developed countries, Chlamydia trachomatis is responsible for up to 50% of cases of ophthalmia neonatorum(40). In contrast, Neisseria gonorrhoea is a rare cause of ophthalmia in many western European countries (41). In third world and African countries, gonorrhoea is responsible for between 20% and 73% cases of ophthalmia (42). African studies have shown C. trachomatis to be responsible for 7.3% ophthalmia neonatorum in Kenya; 5% in Gambia 27% in Gabon 19% in Central Africa (43).

Before the advent of antimicrobial, physicians and surgeons spent major part of their medical practice in management of complications of STD such as tertiary syphilis, urethral strictures and infertility. The incidence of these complications has reduced to some extent in developed countries, but in third world and African countries, these complications still constitute major medical problem (44).

2.07 **Local Complications:**

(a) Epididymitis: This is a common complication of gonorrhoea and many patients become infertile as a result. Today gonococcal epididymitis is uncommon in the developed world. Currently Chlamydia trachomatis infection has overtaken gonorrhoea, as the major cause of acute epididymitis in young men in United State of America (45).

(b) Acute salpingitis: This is caused by ascending infection from cervix and is a disease of sexually active young women. The commonest cause of this is gonorrhoea. Just as in epididymitis, chlamydia infection has overtaken gonorrhoea in western countries as the major

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cause of acute salpingitis (46). Other STD's causing acute salpingitis include candidiasis, Mycoplasma hominis or M urealyticum. Primary infection may be Neisseria gonorrhoea or Chlamydia trachomatis, complicated by secondary infection with anaerobes and other vaginal commensals.

Westrom (1975) has estimated that even with antibiotic treatment, tubal occlusion occurs in 13% of women after one attack of salpingitis, in 36% after two attacks, and in 75% after three (47). A woman who has had salpingitis six times is more likely to have an ectopic pregnancy.

(c) **Infertility**:

Infertility rate had risen sharply in recent times in parallel with increasing incidence of STD (48). In African countries, infertility consumes a major proportion of reproductive health care resources. In sub-Saharan African countries, the prevalence of female infertility may be as high as 30% while the rate in males is up to 20% (23). In Nigeria, STD accounts for 50% of infertility in males and females (49). In Britain and United States of America, the prevalence of infertility is between 30% and 80%. Salpingitis accounts for 150,000-200,000 per year infertile women in United States of America (50).

(d) **Systemic Diseases**:

Sexually transmitted diseases may spread and cause diseases which are remote from the initially infected genital sites. These complications affect large numbers of patients globally, for example, gonococcal infection if untreated leads to septicaemia (51). The complications of STD include many medical syndromes like: 1) Reiter's syndrome which has been associated with Chlamydia trachomatis; 2) Urethral syndrome; 3) Fitz-Hugh-Cutis syndrome.

(e) **Cervical neoplasm:**

The main epidemiological determinants of carcinoma of the cervix are age of first intercourse and multiple sex partners (52). It is being thought that a sexually transmitted oncogenic pathogen may be implicated in the pathogenesis of the neoplasm (53). In recent years there has been association between Herpes simplex virus type 2 and papilloma virus in the aetiology of carcinoma of the cervix (54). It has been recommended that women attending STD clinics should have cervical cytological examination regularly (55).

2.08 **Syndrome Associated with Sexually Transmitted Diseases.**

(a). **Reiter's syndrome.**

Definition: Reiter's syndrome, also called Reiter's disease, infectious uroarthritis, venereal arthritis, idiopathic blennorrheal arthritis, arthritis urethritica, is a triad or tetrad consisting of (a) arthritis, (b) non gonococcal urethritis (c) conjunctivitis, (d) mucocutaneous manifestations. It is a disease of the young age group affecting men and women of 15 - 20 years. Reiter's disease is believed to be precipitated by urogenital or gastro-intestinal infections in susceptible individuals of HLA-B27 (56).

The symptoms and signs: The triad of Reiter's syndrome usually develop over 10 days to 21 days. Urethritis usually develop first, followed by conjunctivitis, then arthritis. An attack of dysentery or sexually transmitted diseases may precipitate the syndrome. Causative organisms implicated are Chlamydia trachomatis, Neisseria gonorrhoea, T-ureaplasma urealyticum. Other lesser causes are Haemophilus vaginalis Trichomonas vaginalis, Candida species, Schistosoma haematobium(56).

The condition is not usually recognized so that the incidence is virtually unknown. However, in Ibadan, Nigeria, 33% of patients with STD and in Sudan 35% of men with STD in Khartoum had non-specific urethritis (57).

(b) **Urethral Syndrome**

This is also called abacterial cystitis, a bacteriauric frequency. The syndrome consist of dysuria, frequency, urgency, feeling of incomplete bladder emptying in the apparent absence of significant bacteriuria. It may be present after treatment of STD. The causes are usually an aftermath of sexual intercourse other causes are, allergy to nylon underwear, biological washing powder, cold bubble baths, non significant bacteriuria (10^3 colony forming units/ml)(58). Fastidious or difficult to culture organism or anaerobes have being implicated(58). Chlamydia trachomatis or a virus have also been implicated (58). Patients of 40 years age and above are usually affected and females are predominantly involved. Cystoscopy shows typical inflammatory changes with ulceration of the bladder base. Initial management of urethral syndrome is by prophylactic antimicrobial therapy for 6 months to prevent bacteriuria recurrences (59).

(c) **Behcet's Disease.**

The clinical triad of recurrent oral and genital ulceration, uveitis and ocular inflammation and involvement of the central nervous system are typical. Central nervous involvement includes cranial nerve palsies, seizure, mental disturbance, aphasia, hemiparesis, cerebral ataxia (60). Behcet's disease is typically characterized by oral and genital ulceration, iritis, polyarthritis of variable distribution that may be either chronic or episodic. Less common features are erythema nodosum, pustular skin lesions, neurological and gastro intestinal manifestations and HLA - B 27 may be predisposing feature. Behcet's disease is more common in the Eastern Mediterranean Countries, Japan and associated organisms are Herpes simplex, Epstein-Barr virus, enterovirus (60).

(d) **Fitz-Hugh-Cutis Syndrome**

STD may spread from the vaginal and cervical area to the Fallopian tubes and into the peritoneal cavity and up the right colonic gutter to produce a perihepatitis. This is an unusual complication of genital infection in the female. It occurs when gonococci spread from the Fallopian tube and produce a localized peritonitis. Any area of the peritoneal cavity may be involved but the right upper quadrant is the commonest site. The organisms implicated are mainly gonococcus and chlamydia trachomatis (61).

2.09 SIGNS AND SYMPTOMS OF GONOCOCCAL URETHRITIS AND CHLAMYDIA GENITAL INFECTION.

Gonococcal and chlamydia genital infections are strikingly similar in signs and symptoms. The incubation period of chlamydia infection is however longer than that of gonorrhoea infection (which is 7 days to several weeks). Chlamydia infection causes watery, mucoid discharge with presence of many polymorphonuclear leukocytes but without gram-negative diplococci in the smear. Clinical symptoms in women include vaginal discharge, dysuria, increased urinary frequency and soreness in the vagina. Further clinical signs clinical signs depends on part of the lower genital tract infected and include evidence of cervical soreness and visible redness; cervical erosion with red colour and presence of thick mucoid discharge on the ecto-cervix and sometimes extending to the endo-cervix, acute cervicitis with cervical congestion erythema, abnormal exudate, oedema, hypertrophic erosion or cervical lymphoid follicular formation.

In men, however with uncomplicated infection, profuse or mild purulent urethral discharge may in both chlamydia and gonorrhoea urethritis persistent or recurrent urethritis may occur after treatment of gonorrhoea. Particularly disturbing is pain on micturition while the associated signs and symptoms may be due to complications.

2.10 SIGNS AND SYMPTOMS OF GENITAL ULCER DUE TO H. DUCREYI

Following a previous history of sexual exposure which may be for 2 days and 17 days, a genital ulcer may appear. This time interval may be reduced if there was previous genital lesion. A small papilla, which may be painful at first appears on the genital area and breaks down to form an ulcer. The ulcer is similar to syphilitic chancre and occurs mostly on the coronal sulcus and other parts of the glans penis. (55,62,63).

On examination by retraction of the foreskin, a lesion of multiple shallow ulcers may be observed about 0.3 - 2.5 cm diameter. It may have a ragged border or a purulent base.

Unilateral or bilateral tender inguinal adenopathy may be seen, in addition to the genital ulcer(s). If the inguinal lymph nodes are aspirated, they may yield H. ducreyi and this forms part of the diagnostic test for this sexually transmitted disease (64).

2.11 PATHOLOGY

Superficial necrosis pervaded with polymorphonuclear leukocytes and a peripheral zone rich in plasma cells, lymphocytes and epithelial and endothelial leukocytes were described by Dienst (65). They placed responsibility for the superficial necrosis on the existence of acute perivasculitis and endovasculitis with swelling of the endothelium. The granulomatous reaction seen in chancroidal ulcers is highly suggestive of the diagnosis and in many cases may be diagnostic.

2.12 Geographical Distribution of Sexually Transmitted Diseases

STD is found in all parts of the world and recent data indicate a steady rise in the statistics (12). Figure 1 shows steady global increase; Figure 2 depicts incidence of diseases in

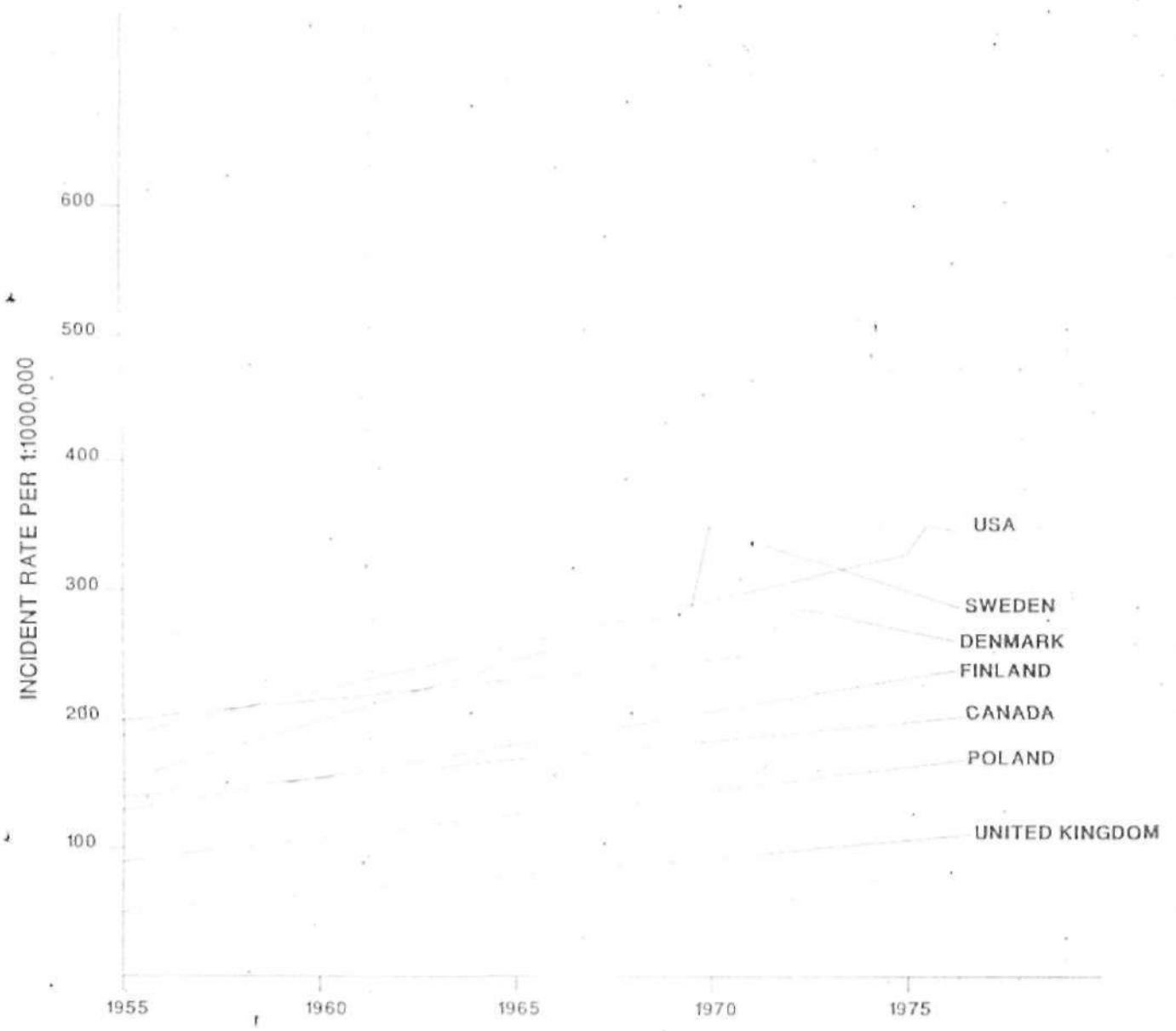


FIGURE 1

Reported Gonorrhea Incidence per 1:1000,000 population 1955-75
WHO GENEVA 1993. WHO BULL.

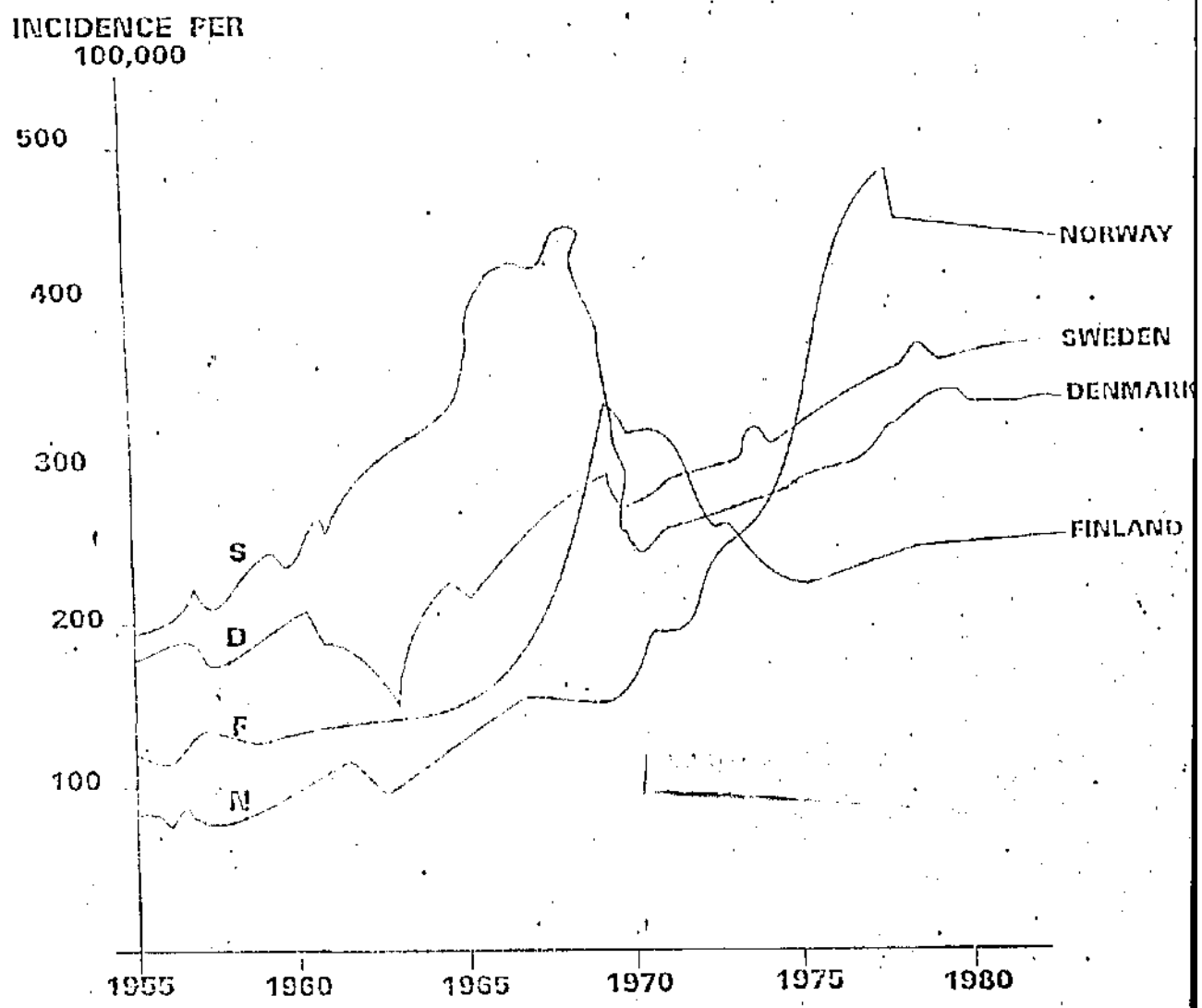


Figure 2

Report Incidence of Gonorrhea per 100,000 population in Four Scandinavian Countries 1955 - 80. From Statens Serumingtunt Copenhagen

LIBRARY

developed countries like USA, Britain, Sweden, and the Western European countries. In developed countries, with more medical facilities, higher income, more educated populations, readily available medical services, incorporation of adequate drugs, trained staff and laboratory facilities, STD has a favourable outcome. The study of the epidemiology of STD in developing countries of the tropical countries is a more difficult task, (66) as it highlights the differences in developed and poor nations in the areas of differential wealth, poverty, knowledge or lack of knowledge, good or poor communication, stable or migrant population, and predominance of urban or predominant rural populations.

Additionally, the geographical distribution of STD varies within tropical, subtropical developing countries as illustrated in Table 3. In Nigeria, the distribution of STD also differs between Northern States and Southern States (67). Studies by Bello and Elegba in Zaria (68), Osoba in Ibadan (69), Acholonu in Owerri (70) illustrate this trend. The differences are due to low literacy level, poor personal hygiene, poverty, inequality of distribution of medical facilities.

It is hoped that the high incidence of STD may be slowed down or decreased with the advent of AIDS epidemic which places demands upon man and woman to modify their sexual behaviour in various ways. The modification may be brought about by intense sex education directed to sexually active age groups in the 18-50 years of age bracket.

Fig 1 Reported gonorrhoea incidence per 100,000 population in some industrialized countries

Fig 2 Reported incidence of gonorrhoea per 100,000 population in four Scandinavian countries 1955 - 80

2.13 Epidemiology of Neisseria gonorrhoea

Gonorrhoea which is one of the commonest traditional sexually transmitted diseases is present in all countries of the world. Attempts at control of this STD has not proved successful. Man himself is to be blamed being the only natural host. In 1972, Roberts, estimated the incidence to be about 70 million and Morton in 1972, estimated the gonorrhoea cases to be 200 million (72,73).

(a) Developed Countries.

The statistics of gonorrhoea and other STD's in developed and Western European countries are less than accurate (74). Notification of STD is more complete in some countries than in others. For example in Denmark, most cultures are performed in one laboratory (75). In United Kingdom, 80%-90% of cases are treated at STD clinics and are therefore reported, whilst 95% of general practitioners refer men with urethritis to STD clinics (76). In United States of America, 1 million out of 2.7 million cases were reported to the health authorities in 1976, that is 62% under-reporting (77). Figure 2 shows the numbers of reported cases of gonorrhoea for some countries. The fall in incidence rate in Sweden between 1970-1977, was due to increased used of condoms, more open attitudes to STD and the increased efficacy in the detection of female cases.

Morton in 1976 and Wiesner and co-workers in 1976 drew attention to seasonal variation with third quarter (July - September) peaks in United Kingdom and United States of America (77,78). This was due to climatic conditions, sociosexual behaviour, virulence of gonococci and control programmes.

(b) Tropical, Subtropical, Developing Nations.

Gonococcal cases reported by some countries are grossly inaccurate due to political and social implications (74). Various study groups in Ibadan (79); Zaria (80) and Lagos (81) have given incidences of STD. Selected population studies in Africa and Asia reveal very high prevalence rates within range of 3% to 18% (82). In Uganda, Arya, and co-workers in 1973 and Arya & Taber in 1975 found 8.9% of men and 18.3% of women respectively in Tego district had Neisseria gonorrhoea (83,84). WHO considered N. gonorrhoea to be a major underlying cause of high prevalence of infertility in parts of Africa (85).

In Nigeria, there were 600 per 1000 infection rate of gonorrhoea in Nigeria troops (86). Gonorrhoea accounted for 4907.6 per 10,000 cases in Lagos (87). Other studies are 97 per 1000 cases in Sokoto (88); 17% of hospital patients in Ibadan (79); and 5.5% in Zaria (80). In South Africa, Ballard et al 1977, found out that the incidence of Neisseria gonorrhoea was 54% of 212 male white population attending STD clinic in Johannesburg (89).

2.14 Epidemiology of Chlamydia trachomatis genital infection

Information about the incidence of chlamydia genital infection has come mostly from developed countries as the rather complex diagnostic techniques are not yet widely available. There are few epidemiological reports from South Africa and Nigeria (90,91). At present it

appears the chlamydia genital infection is the most common cause of sexually transmitted diseases in Britain, North America, Scandinavian countries (92,93). Probably because genital chlamydia infection are often neither recognised, correctly diagnosed or properly treated, there seemed to be virtual epidemics in many countries of the world (94). See Table 3

The study of the epidemiology of chlamydia genital infection assumed definitive trend, from 1965, when Gordon & Quan introduced accurate diagnostic and isolation method (95). Previously it was classified under, various names such as non-gonococcal urethritis, post-gonococcal urethritis (96).

2.15 Epidemiology of H. ducreyi

There is a paucity of information concerning the incidence and epidemiology of chancroid, primarily due to difficulties inherent in making an accurate diagnosis of chancroid.

In the five years before 1981, the number of cases of soft sore reported annually in California was 21 per 100,000. In the United States during the same time period, this figure was 646 per 100,000. In recent years there have been outbreaks of chancroid in Winnipeg Canada, Greenland, and in United States of America. The following states have reported outbreaks, California, Texas (Dallas) Massachussets (Boston), New York, Florida (Orlando) (20). In United Kingdom (Sheffield), chancroid has been reported from

TABLE 3

Prevalence of Chlamydia trachomatis genital infections in certain Tropical and Subtropical Countries (Male & Female) WHO Geneva 1990 (12)

Men with Urethritis	Chlamydia trachomatis	
Countries	Isolated Antibodies %	
South Africa	19.2	
Swaziland	2.7	
Singapore	30.2	
Gambia	15.4	
Iran	8.8	15.7
Gabon	16.3	
Men Attending STD Clinic		
Ethiopia		45.3
Nigeria		18.7
Sudan		4.4
Women Attending STD Clinic		
South Africa		13.3
Ethiopia		32.4
Nigeria		26.7
Sudan		10.4

merchant sea men and businessmen returning from endemic areas of Asia and Africa (97).

In the developing countries, chancroid remains a common sexually transmitted disease. It has been reported in Southeast Asia, Singapore, Vietnam, Korea and Philippines (98). Field studies in Gambia, Kenya, Swaziland and Uganda have identified chancroid as one of the common sexually transmitted diseases (23). In Nigeria, few studies have been conducted (99).

In California, 95% of confirmed cases were men while 5% were women (20). In Kenya, prostitutes are the most frequently named source of contacts. The most commonly affected age groups is 18-30 years in men (23).

2.16 SUSCEPTIBILITY TO INFECTION COLONIZATION OF MUCOSAL SURFACES EXAMPLE OF GONOCOCCUS:

Once deposited on a mucosal surface in a new host gonococci must establish themselves rapidly to avoid being washed away by mucosal secretions. It is now accepted that micro - organism must be able to adhere to epithelial cells if they are to colonize mucosal surface successfully (100). Electron microscopy of urethral scrapings from men with gonorrhoea show gonococci attached to the surface of urethral epithelial cells, and entering them apparently by phagocytosis (100).

The mechanism by which gonococci attach to and invade epithelial cells is known, as it is likely to involve interaction between components on the surface of both gonococci and epithelial cells. When cultured in the laboratory, virulent gonococcal cells produce filamentous surface projections termed pili, which promote their attachment to cells and to Fallopian tube mucosa. However the importance of pili in naturally occurring gonococcal infections has been questioned since pili are rarely seen on electron microscopy of gonococcus in the specimen patient (100).

Furthermore, studies with genital tissue maintained in organ culture have shown that gonococci that lack pili may nevertheless attach to and invade epithelial cells, demonstrating that pili are not essential for attachment and invasion (100).

Some preliminary evidence suggest that sugar residues on the gonococcal surfaces are involved in the attachment process, but whether these sugars are components of gonococcal endotoxin or other molecule such as surface glycoprotein, remain to be determined (101). With regard to epithelial cells, gonococci attach to organ cultures of human genital mucosa of non-human origin, which suggest that there may be receptors for gonococci that are found on only human cells (102). At present there is no direct evidence for such receptors sites. This is an attractive hypothesis as it is in keeping with the finding that man is the only known natural host for gonococcus.

2.17 HUMORAL RESPONSES.

Several studies have been directed towards humoral mechanisms in sexually transmitted diseases. Some have attempted to refine serological assays (103). Serological assays have generally been of limited value because of lack of purified antigens and or because the sensitivity of the assay was so slight. Buchanan and co-workers in 1973, using gonococcal pili provided a specific and sensitive assay for detecting recent gonococcal infection (104). While piliated gonococci are associated with virulence in humans, the presence of circulating anti-pilar antibodies does not protect against subsequent infection (104, 105, 106).

2.17.1 HUMORAL RESPONSES IN GONORRHOEA INFECTION:

Specific humoral immune responses develop following gonococcal infection. The presence in serum of antibody to N. gonorrhoea has been demonstrated using a variety of techniques including immunofluorescence (107); radioimmunoassay (108); and enzyme - linked immunosorbent assay (109). Furthermore, some of these techniques have also been used to demonstrate the presence of anti-gonococcal antibody in urethral exudate from males (110), and genital secretions from females (111). Development of humoral immune responses does not prevent patients from repeated infections of gonorrhoea (112). The reasons for the susceptibility of patients to re-infections may be connected with the antigenic heterogeneity of N. gonorrhoea (106, 111). It is not known at present which gonococcal antigens are important in these repeated infections, but those investigated have been shown to be serologically heterogenous.

These antigens include pili, the other membrane protein, and acidic polysaccharide antigens (112). Other systems using whole organism rather than specific system have also demonstrated that gonococci are serologically diverse (112). Thus, the possibility exists that following gonococcal infection, a patient may be immune to re-infection with organisms of the homologous serotype, but susceptible to infection with organisms showing different antigenic characteristics. Evidence to support this concept has come from studies of experimental infection of chimpanzees and from chambers implanted subcutaneously in guinea pigs where immunity produced by vaccination was strain specific (105,113).

Another factor that may contribute to the susceptibility of patients to re-infection with N. gonorrhoea is the ability of gonococci to elaborate a proteolytic enzyme which cleave human immunoglobulin of IgA_1 (but not IgA_2) subclass at the hinge region (114). However, while this enzyme may inhibit the activity of IgA, it is inactive against immunoglobulin G and M classes

that are also found in genital tract secretions, thus the significance of this enzyme now called IgA protease in the pathogenesis of gonococcal infection is at present becoming clear, when is now called IgA protease (115,116).

Secretion of proteins into the extracellular medium by Gram-negative bacteria requires that they pass across a cell envelop consisting of two membranes,- the cytoplasmic membrane and the outer membrane. To achieve this, bacterial exoproteins utilise a variety of strategies, most of which rely on separately encoded accessory transport proteins for translocation through the outer membrane (117). The IgA protease family of proteins also enzymes are classified as auto-transporters and thus an exception to this rule. Neisseria IgA protease are synthesized as large pre-proteins (Iga) consisting of a N-terminal leader sequence followed by the IgA protease domain (IgAß), the α-protein (Igaα), which is secreted in conjunction with the protease and the C-terminal IgAß (116). The export of the secretory domains is accomplished by their stepwise transport across the cytoplasmic and the outer membranes. Transport of IgA across the cytoplasmic membrane is accomplished by the processing of the leader sequence. Extracellular transport requires only IgAß, whose proposed role is to extrude the Igap/α polypeptide chain across the outer membrane. That Igaß surface is demonstrated by the fact that the cloned structural Iga gene alone is sufficient for IgA protease export from Escherichia coli and Salmonella species. Auto-proteolytic cleavage at specific processing sites within Iga leads to the uncoupling of Igap/α from the outer membrane-assembled Igaß and mediates further maturation to the extracellular products Igap and Igaα. This model of IgA protease secretion which is mainly based on studies with the Iga gene product of N. gonorrhoea strain Ms11 also applies to Iga proteases from other N. gonorrhoea and N. meningitides strains, and it appears that the related enzymes from Haemophilus influenza, and a serine protease from Serratia marcescens,

utilize similar export principles (117).

There are three phases in the outer membrane transport of IgA protease (1) outer membrane sorting and assembly, (2) the transportation phase and (3) the extracellular release phase.

2.17.2 Humoral responses in chlamydia infections

Less is known about the roles of immune responses in genital chlamydia infection than in ocular infection (118). While it may seem reasonable to expect the humoral and cell mediated immune responses evoked to have roles similar to those in ocular infection, this may not necessarily be so (119,120). Infections with chlamydiae in humans result in the formation of antibodies directed against certain surface proteins -- MOMP, and polysaccharides LPS. The elementary bodies (EB) of chlamydia contain these surface components (MOMP and LPS) which are important in the pathogenesis and evocation of humoral immune responses (121). In men with chlamydia urethritis IgG antibodies are present in blood (122). While chlamydia infections usually evoke antibody responses, there are some patients who do not appear to respond. Some women with microbiologically proved chlamydia urethritis have serum antibodies while some do not (123).

The presence of anti-chlamydia antibodies in blood and local discharges is used as a diagnostic test for chlamydia genital infections. In women, with chlamydia cervicitis IgM and IgG are present in the blood, and IgG and IgA antibodies are detected in the secretions (124). In men with chlamydia urethritis IgG antibodies may be present in blood (125). This may be possible if the antibody - negative patients have been tested too early, before antibodies develop, but it appears that chlamydia infection may not always evoke an antibody response.

Chlamydia may conceivably have periods of latency and is non-multiplication m vivo. This may account for above phenomenon; but if this occurs in the mucosae, there must either be period of growth to maintain the infected state, since epithelial cells are constantly shed or organism must be capable ot persisting into deeper cell layers or remaining in some non-mucosal site (125).

2.18 ROLE OF SERUM IRON IN INFECTION.

A further factor that may influence the susceptibility to infection is the ability of the micro-organism to assimilate serum iron, as addition of exogenous iron has been shown to protect gonococci against killing by serum (126). Iron in serum and body fluid does not exist in the free form but is closely associated with iron-binding proteins such as transferrin (TF)or lactoferrin(LF). To acquire iron, gonococci must be able to obtain iron from the iron-binding proteins of the host and gonococci had been shown to be capable of producing an iron-binding siderophere tentatively named "gonobactum" (126). Further understanding of the mechanism by which gonococci acquire iron could be of value as the prevention of iron assimilation might be a means of modifying or even preventing gonococcal infection.

The low concentration of Irce iron in body fluids creates bacteriostatic condition lor many micro-organisms and is an important defence factor against invading bacteria. Consequently, pathogenic organisms have developed diverse and elaborate system to obtain the iron required for growth. Many mjero-organisin synthesize high-affinity iron chelators (siderophores) as well as specific transport systems, for the transport of siderophore-Fe^3 complexes into the cell. Some

organisms can acquire iron directly from host iron-binding proteins by highly specific recognition and transport system. With the human host potential iron sources available to pathogenic bacteria include transferrin (TF) found in serum and lactoferrin (LF) present on mucosal surfaces (127). In addition, haem-containing compounds are a particularly abundant source of in vivo iron. Pathogens that occupy intracellular niches in vivo can utilize the iron in haem compounds only after the haem is released. This typically occurs by some form of tissue damage resulting in the release of intracellular material. The ability to utilize haemin and haemin-containing compounds for nutritional iron uptake has been documented for several pathogenic bacteria, including the following Vibrio cholerae, Klebsiella pneumonia, Yersinia pestis, Shigella flexeri, Bordetella pertussis, Streptococcus pneumonia, Haemophilus ducreyi (128-132).

The gram-negative pathogen Neisseria gonorrhoea is capable of utilizing a wide variety of iron sources, including TF, LF and haem-containing compounds (126). Although haemoglobin can be used as an iron source by most gonococcal strains, haem cannot be used when it is complexed with hemopoxin or albumin Neisseria gonorrhoea does not produce siderophores, and acquisition of iron from TF and LF involves a family of distinct iron-regulated outer membrane receptors. These include the well-characterized TF-binding protein Tbpl and Tbp2 and the LF-binding protein, Lbp. Iron is removed from TF and LF in an energy-dependent manner; however neither TF nor LF is internalized. Once the iron from TF has been internalized, it appears to be transported onto the ferric binding protein (Fbp). Fbp appears to function within the periplasm, shuttling iron from TF through the periplasm and to the cytoplasmic membrane. The gene for the gonococcal and meningococcal Fbp has been cloned and sequenced, and the promoter region has been shown to contain two regions that exhibit homology with the

Escherichia coli ferric uptake regulator protein (Fur) binding sequence. Fbp appears to be regulated by a Fur-like mechanism and gonococcal and meningococcal Fur homologs have been identified (133).

Although the ability of N. gonorrhoea to utilize haemin and haemin-containing compounds have been documented, the mechanism whereby this organism acquires haemin are poorly understood. Two haemin binding proteins, 97 and 44 kDa, have been purified from N. gonorrhoea by haemin-affinity chromatography. However, the role of these proteins in haemin haemoglobin binding receptor (HmbR) have been described for Neisseria meningitides (134). It is not known if similar homologs exit in N. gonorrhoea. The ability to acquire haem iron may be particularly important for mucosal pathogen like N. gonorrhoea since a large amount of haemin is present at mucosal sites because of the desquamation of epithelial cell.

2.19 ROLE OF COMPLEMENT IN INFECTION

Phagocytosis is the primary defence mechanism against most bacteria as well as many viruses and protozoa. To facilitate ingestion, the microbial membrane is coated with complement and antibody to make it make "edible", (opsonization). Subsequently, host cell receptors for these proteins bind and immobilize the microbe (immune adherence). Complement and immunoglobulins are the two most critical opsonic system of human blood and are products of independent but cooperative parts of the immune system (135). The major goal of the complement system is to deposit clusters of C3b on the target. This targeting is mediated by IgM or IgG, in the case classical of pathway and by the absence of convertase regulatory proteins, in the case of the alternative pathway. Regulation of C3 activation and destruction of C3b coated particles are therefore salient issues in understanding the complement system. The current

concept is that there are complement receptors (CR) that bind to the complement components. These receptors are group into types. For example complement receptor type one (CR1) are present for the C3b/C4b to be effective, and this has been identified in primates. The Membrane Cofactor Protein (MCP) also the receptor for measles virus and group A beta haemolytic streptococci has also been identified (136).

The classical activity ascribed to complement depends upon the operation of nine protein components (C1-C9) acting in sequence of which the first consist of three major subfractions termed C1q, C1r, C1s. The terminal components of the complement cascade have the ability to punch a "Functional hole" through the cell membrane on which they are fixed, perhaps through transformation into an active phospholipase, that leads to cell death. The full complement system leading to membrane damage can cause bacteriolysis in Gram-negative organisms by allowing lysozyme to reach the plasma membrane where it destroys the mucopeptide layer of the cell wall.

About 3% of patients with gonorrhoea develop disseminated gonococci infection (DGI) with haematogenous spread of gonococci from the primary site of infection. Such patients develop skin lesions. Septic arthritis, endocarditis and meningitis have also been reported (137). Patients deficient in components of complement C7C8C9 systems are particularly at risk of developing DGI, which indicate that the bactericidal activity of complement is important in preventing haematogenous spread of gonococci (137). This idea is supported by finding most strains of gonococci isolated from patients with DGI are resistant to the complement dependent bactericidal activity of normal human serum while strains of gonococci from patients with uncomplicated infection are often susceptible (148).

It has recently been shown that the susceptibility of gonococci to killing by serum is associated with the phenotypic characteristic of the colonies produced when the organism are grown on defined agar medium. Organism producing transparent colonies are relatively resistant to serum whereas organism producing opaque colonies are more readily inactivated. Gonococci isolated from the cervix vary in their tendency to produce opaque or transparent colonies, depending on which day of the patient's menstrual cycle they are isolated (139). Transparent variants tend to be isolated from cervix about the time of menstruation, which is the time when disseminated infection in female is most common.

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2.20 ROLE OF POLYMORPHONUCLEAR LEUKOCYTES

Once established on a mucosal surface, gonococci penetrate into the submucosal tissue where they stimulate the host defence mechanism. In symptomatically infected patients, the initial response consist of an inflammatory reaction with a large numbers of polymorphonuclear (PMN) leukocytes infiltrating the site of infection. The factors that initiate the inflammatory response are not known, but laboratory studies indicate that gonococcus may attract PMN leukocytes either by generating a cytotoxin or by activating complement (106).

Although many species of micro-organisms have surface structures such as capsules, which inhibit phagocytosis, gonococci appear to be unique in that many strains have a surface protein termed "Leukocyte Association Factor" (LAF) which promotes their attachment to and ingestion by PMN leukocytes. While the presence or absence of LAF is probably the major factor influencing the association of gonococci with PMN leukocytes, its influence on the overall severity of the disease has not yet been investigated. Other surface components which exert minor anti-phagocytic activity include pili and an EDTA soluble material (110). Although the

production of capsules by gonococci has been reported, recent studies have shown that the capsule-like material seen surrounding gonococcal cells is probably an artefact caused by part or all the outer membrane becoming detached from the plasma membrane.

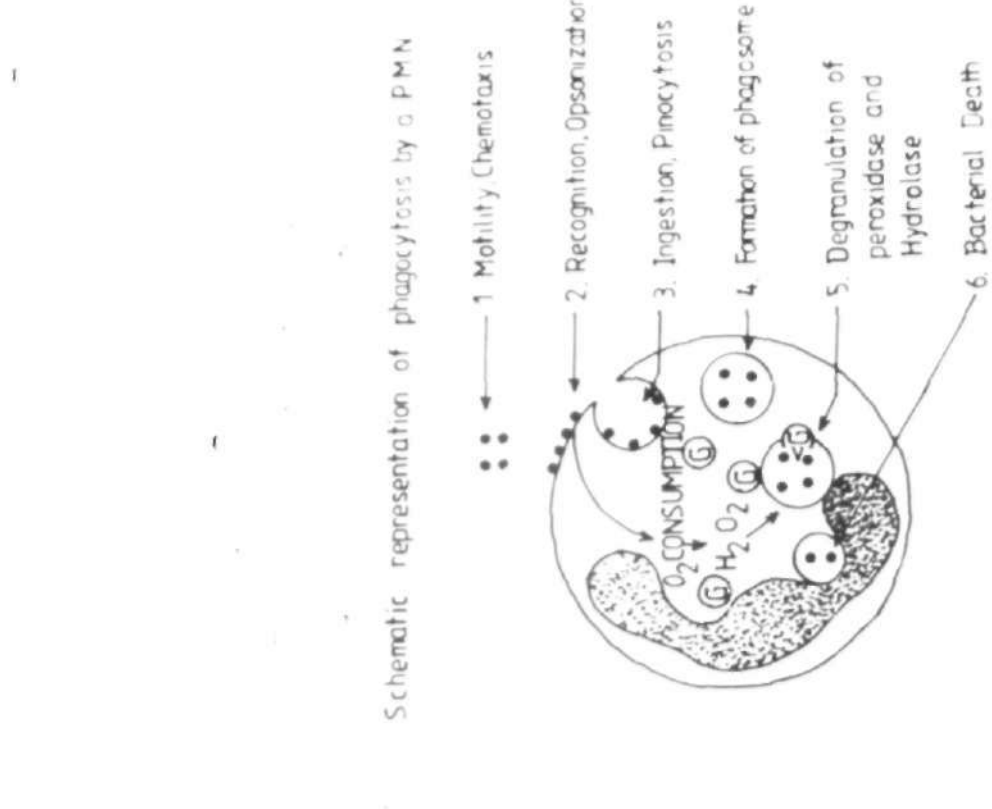
Electron microscopic examination of gonorrhoea exudate shows that PMN leukocytes phagocytose gonococci and that many ingested bacteria are killed (106). It also appears that some gonococci can survive and grow within phagocytic leukocytes which are then destroyed. The resulting clusters of gonococci surrounded by the remains of dead leukocytes have been called "Infection Units" and it has been suggested that gonococcal infection units may be protected from both the cellular and humoral defence of the host (105,106).

The inflammatory responses as a result of Chlamydia trachomatis infection in the genital, is similar for both male and female (140, 141). The inflammatory polymorphonuclear leukocyte response are similar to other acute inflammation in other sites of the body. Inflammation of the site is characterized by infiltration with PMN leukocytes, hyperaemia, oedema and haemorrhagic changes. Purulent and sero-purulent exudate issue from the orifices for example in salpingitis; muco-purulent in urethral and cervical orifices. In chlamydia salpingitis, the tubal mucosa is infiltrated and destroyed by inflammatory cells, mainly PMN leukocytes and also seen are some lymphocytes.

Chlamydia-macrophage interactions are dependent on multiplicity of interleukins (142), and high dose ingestion leads to cytotoxic damage. At low doses, macrophages actually support chlamydia multiplication but on continued stimulation by lymphokine, chlamydia growth is inhibited (143). PMN leukocyte accumulation is an early indication of chlamydia infection of mucosae. Human blood PMN cells are highly effective in killing chlamydia by oxygen dependent and independent mechanisms (144).

See Plate I below.

Plate I Schematic representation of phagocytosis by a PMN



From The Phagocytic Cell in Host Resistance
Bellanti J A, Dayton DH (editors). Raven Press. 1975

2.21 Role of ABO Blood group in Sexually Transmitted Diseases.

In 1976, Foster and Lobrum reported increase susceptibility of individuals of Blood group B to infection with Neisseria gonorrhoea (145). The presence or absence of anti-B isohaemagglutin may be the causative factor for the reported increase in the susceptibility to gonorrhoea of individual with blood group B. In 1984, Kinane and co-workers reported that individuals of blood group B are more susceptible to gonococcal infection and further suggest that the absence of anti-B isohaemagglutin is important (146). In 1997, report from Ibadan, Nigeria has further confirmed above findings and other studies on other STD's are awaited (147).

2.22 Role of Polymorphomuclear leukocytes in phagocytosis

One of the most important function of the blood cells and tissue macrophages is the ingestion or phagocytosis of IgG-coated microorganisms. Receptors for the constant region of IgG, the Fcγ receptors, enable these cells to detect and destroy IgG coated microorganisms during infection and IgG coated blood cells in autoimmune disorders (148). There are three major classes of Fcγ receptors, designated FcγR1, FcγR11, and FcγR111. They are encoded by at least 8 genes localised on the chromosome 1 at q21-23 and additional isoforms within the three classes are generated through alternative splicing (149). The three classes of Fcγ receptors contain highly conserved extracellular Ig domains, but their cytoplasmic regions are distinct from one another, suggesting that they may not all be involved in transmitting a phagocytic signal. Because multiple Fcγ receptors isoforms are expressed in tissue macrophages and other phagocytic cells, it has been difficult to ascertain which Fcγ receptors induce phagocytosis in the absence of other Fcγ receptors and what molecular structures are required.

The requirement for phagocytosis are as follows; a single class of human Fcγ receptor in the absence of other Fc receptors can induce phagocytosis of IgG-sensitized microorganisms or red blood cells. Isoforms of all three Fcγ receptor classes are able to transmit a phagocytic signal, although each has particular requirement. In electron micrographs, erythrocytes ingested by Fcγ receptor cells appear within well defined membrane vacuoles similar to phagocytosis by traditional phagocytes such as macrophages which express endogenous Fcγ receptors (150). Degradation of ingested particles is observed within discrete vacuoles and is consistent with the effect of intracellular lysosomal enzymes (see Plate I). The ingestion of microorganisms does not proceed at 0°C and is inhibited by cytochalasin D, which interrupts the assembly of actin filaments essential for phagocytosis.

Phagocytosis may be induced in certain non-phagocytic cells by the addition of appropriate Fcγ receptor (151). Phagocytosis mediated through FcγR11A as well as through FcγR1/γ and FcγR111A/k receptors requires PI-3 kinase activity, in other respects, the mechanisms for Fcγ receptor mediated phagocytosis differ. FcγR11A induces phagocytosis associated with the phosphorylation of tyrosine within the conserved motif of its own cytoplasmic domain (152), whereas both FcγR1 and FcγR111A require the tyrosinases within the conserved motif of its cytoplasmic region of their subunit for phagocytosis (153). The protein tyrosine kinase Syk markedly enhances the phagocytic signal in the two Fcγ receptor associated with the γ chain (152). Enhancement of FcγR11A mediated phagocytosis by Syk kinase is modest compared with that of FcγR1/γ mediated or FcγR111A/γ mediated phagocytosis. Efficient phagocytosis by FcγR11A is mediated by the endogenous kinase and such efficiency is necessary to prevent or contain infection by sexually transmitted infective agents.

2.23 STUDIES OF CELL MEDIATED IMMUNITY IN STD

The broadening knowledge of cell mediate immunity has led to recognition of its role in chronic intracellular infections (154,155). This situation coupled with the lack of success in defining protective immunity in antibody studies has led to the study of cell mediated immunity. Also considerable interest has been shown in the cellular changes which occur in immune status of individuals who are infected with various diseases.

Many microorganisms live inside host cells where it is impossible for humoral antibody to reach and destroy them. Obligate intracellular parasites like viruses, have to replicate inside cells; facultative intracellular parasites such as Mycobacterium tuberculosis, Mycobacterium leprae, Listeria monocytogenes, Brucella abortus and Leishmania species can replicate within cells, particularly in macrophages. Their intracellular mode of abode offers them protection against humoral immune response (155).

A specific immune response, the cell mediated immunity CMI has been evolved to deal with these microorganisms. These cells are distinct lymphocyte sub-population made up of T-cells, because they differentiate within the milieu of the thymus gland. They operate against cells bearing intracellular organisms> T-cells only recognize antigen when it is on the surface of a body cell. The surface receptors, recognize antigens plus a surface marker, which informs the T-lymphocyte that it is making contact with another cell. These cell markers belong to molecules known as Major Histocompatibility Complex (MHC) Class I & II. Several sub-populations of the T-lymphocytes, called T-helper/inducer, T-cytotoxic/suppressor, pan-B T-cells, also called CD4, CD8, CD19, Total-T-cell, CD3 respectively exist.

For example, helper T-cells make lymphokines called Interleukins, or cytokines, that stimulate the other major population of the lymphocytes, B cells, to make more antibodies.

Antibodies bind selectively to antigens. In fact they are soluble forms of the antigen receptor molecules on the B cells. Yet the binding of an antigen to B cell's receptor is not sufficient for activation; the B cell needs help from a T cell. Toward that end the B cell internalizes the antigen, digest it as a presenting cell would and displays the pieces on its surface bound to MHC class I or II molecules. If a T cell recognizes the displayed antigen, it becomes active and produces lymphokines and other molecules. These chemicals in turn complete the stimulation of the B cell. As a result antibodies are released into the circulation to find and neutralize infectious agents throughout the body. Lymphokines from helper T cells kill cells in tissues infected with viruses and bacteria. These antibody and T-cell responses form two prongs of the immunologic defence against invading pathogens.

The T-helper cells, CD4, primed to the antigen will recognize and bind to the combination of antigen with class II MHC molecules on the Antigen Presenting Cells (APC) to produce lymphokines. Similarly, T-cytotoxic cell, CD8 will function in contest with Class I MHC. The antigen presenting cells are macrophages, neutrophils, monocytes, polymorphonuclear cells, the Natural Killer (NK) cells and the dendritic cells. There are various methods available to determine cell mediated immunity in bacterial infection, the earliest method was lymphocyte transformation (156). Previous reports on lymphocyte transformation in gonorrhoea leave no doubt as to the existence of CMI response in this infection (157,158). However, several aspects of gonococcal CMI response need clarification. The changes in lymphocyte sub-populations CD3, CD4, CD8, CD19, the leukocyte macrophage migration index (MIF), have not been investigated.

2.24 IMMUNITY (CHANCROID):

There is no true immunity to chancroid. Numerous separate infections are commonly seen in the same patients (22,25. The Ito-Reenstierm intracutaneous reaction with a killed suspension of the Ducreyi bacillus shows that some degree of cellular hypersensitivity develops. A positive complement fixation reaction also develops (159). It is peculiar that the allergic behaviour of the skin as evidenced by the intracutaneous test makes no change in the susceptibility of the individual towards inoculation and re-infection.

This allergic phenomenon can not be interpreted as indicating immunity but merely as a phenomenon of hypersensitivity.

2.25 Interleukins in infections

The methods of intercellular communication fall into two broad groups (a) those in which cells make direct physical contact by means of adhesion (or contact) molecules (b) those involving indirect communication via a variety of soluble compounds. In the case of indirect communication, the remote messengers (extracellular signalling proteins or ESP) cannot deliver their message directly to the genes in the nucleus of the cell they are targeting. Instead, they interact with receptors located on the outer surface of the target cell (transmembrane receptors). Some other types of soluble messengers such as corticosteroids, pass straight through the membrane and team up with their receptors in the cellular cytoplasm. Once an ESP has bound on to its specific receptor, one or more intracellular signal transduction pathways are activated or blocked. At the end of these pathways lies the on-and-off switch, for one (or various)target genes; activation or inhibition will result in a change of cell state or the expression of a particular function of that cell (160 Changes of state of the cell include cell growth,

differentiation, division and suicide or the altered set-up and expression of cell-surface molecules and receptors. The number of receptors present at any one time for a particular ESP is therefore not static. Expression of functions includes the production and release of soluble factors (160).

A range of ESP's, receptors, signalling molecules and target genes have been identified. The ESP's include proteins often referred to as cytokines, for example, interleukins, colony stimulating factors, or growth factors, e.g. epidermal growth factor, platelet-derived growth factors, and Macrophage Inhibition Factor, MIF (161). The best understood receptor types are the tyrosine kinase receptors, the G-protein linked receptors (161). The various intracellular signal transducing messengers like G-proteins, phosphatases and transcription factors lead to the activation or inactivation of particular genes. Cytokines have illustrated interesting aspects of cell-to-cell communication such as specificity, diversity, and flexibility. A common feature of all cytokines is their activity at low concentrations and their diverse range of activities that each of them can display (160,161).

Interleukins are a group of polypeptides belonging to the family of cytokines, that is, hormone-like molecules which can affect various cell function thereby enabling communication between different cell types. The interleukins can be released by lymphoid cells in response to an antigen, for example, an infectious agent. But in contrast to the chemical composition of the antibodies their chemical composition is not determined by that of the stimulating antigen. Non-antigenic stimuli also induce synthesis of interleukins and many non-lymphoid nucleated cells can be producers of interleukins and or express functional interleukin receptors (162). Many interleukins can interact specifically with more than one cell surface receptor molecule (Plate II). The function of interleukines coupled with their availability as cloned purified entities suggests that they have an important therapeutic role in human disease (163). The IUIS/WHO Standing

Committee on Interleukins Designation in 1996 has designated IL-16 (164). See Table 4

Plate II Schematic presentation of mode of action of cytokine.

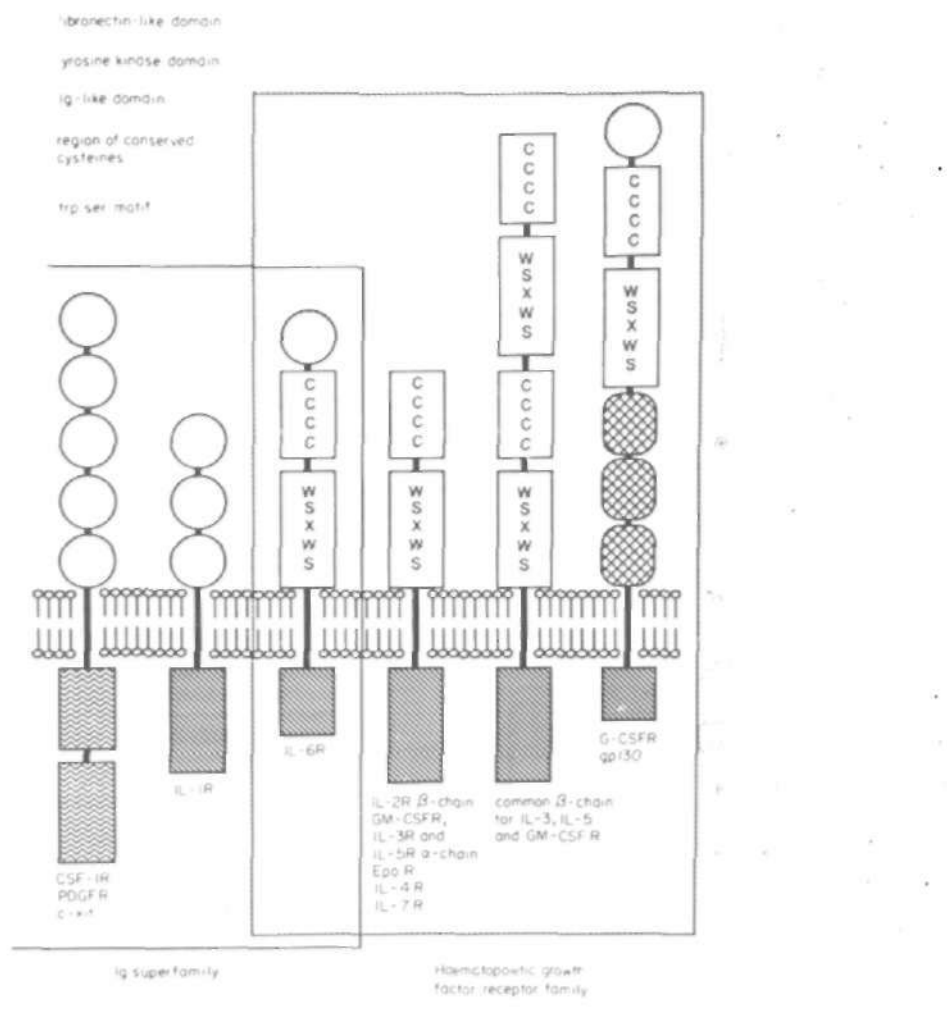


TABLE 4

Interleukines Group and Function.

Group 1:- Share a predominant function as growth and differentiation factors for T- cells and NK-cells. They include IL-2,II-4,IL-7.

Group 2:- Exert major effects on either of the immune system and various components of erthyropoesis. They are IL-2, IL-3, IL-6, IL-7.

Group 3:- Consist of heterogenous and are pleiotropic in their effects. They are IL-6, IL-10 and facilitate the growth of tumours.

IL-8 :- Key factor in the initial stages of inflammation. It is a major chemotactic factor for the influx of polymorphnuclear leucocytes into the sites of injury or inflammation; it was also called macrophage/leucocyte inhibition factor (MIF): IL-8 plays role in tumour invasion and metatasis; possibly growth.

IL-6:- Activation of T-cell; induction of cytotoxic T-lymphocyte, B-cell differentiation; increase in the number of platelets, neutrophils; stimulation of thymocytes; exhibit growth factor for tumour cells.

IL-10:- They main cytokine involved with anti-inflammatory and immunosppressive function.

2.26 MICROBIOLOGY

Neisseria gonorrhoea are spherical or oval cocci 0.8 x 0.6μm that tend to occur in pairs, only occasionally singly, in tetrads, octets, or clusters. When paired, the adjacent sides are flattened giving a coffee-bean shape to the cocci. They are readily stained by Gram's stain and are classically Gram-negative. They are non-capsulated, non-sporing and non-motile (165).

In direct smears from acute infections, gonococci are usually located within polymorphonuclear leukocytes with only a few scattered extracellular forms. In chronic infections, the cocci are predominantly extracellular. Gonococci are aerobic and facultatively anaerobic. A relatively high humidity and an ambient carbon dioxide concentration of 2-10% are required for growth. Narrow ranges of temperature between 35-36°C and pH between 7.2-7.6 are optimal. Although gonococci are the most fastidious of the Neisseria group of organisms, their nutritional requirements appear to be relatively simple and include glutamine and cocarboxylase.

Freshly isolated N. gonorrhoea are fairly uniform in colonial morphology. After 24 hours of incubation the colonies are round, 1 to 5 mm in diameter, convex, greyish-white, with a smooth glittering surface and a smooth edge.

(a) Types:- Colonial variants often appear on subculture and these have called type 1, 2, 3, and 4. Only types 1 and 2 are virulent in volunteers and these types have pili by electron microscopic viewing. Purified pili appear to be immunologically specific for gonococci. The gonococcal pili are important as antigenic site in antibody humoral response and potential site for synthesis of vaccine (104,166).

(b) Biochemistry of *Neisseria gonorrhoea*. Neisseria gonorrhoea is non-haemolytic, indole and oxidase positive. This is the basis of diagnostic test. It ferments glucose but not other sugars. This is another biochemical test of identification. Gonococci are fragile organisms, drying is dangerous to them, within 1 to 2 hours. This is important to note to ensure the success of isolation from genital swabs or specimens. Moist heat at 55°C sterilizes cultures in less than 1 hour. Ultraviolet light and a variety of antiseptics kill gonococci. Unsaturated fatty acids such

as those in the cotton used to prepare swab and in ordinary bacteriologic grade agar inhibit Neisseria gonorrhoea.

2.27 MICROBIOLOGY OF C. TRACHOMATIS

The genus chlamydia consists of prokaryotic organisms capable of intracellular multiplication in vertebrate host cells. It has two separate specialized cell forms, one that can survive extracellularly and one that lives within the host cell. The chlamydiae possess a metabolism deficient in energy production (167). They are particularly widespread among vertebrates and produce latent and long lasting infections. Chlamydia psittaci is a disease agent of non-primate vertebrates - birds, cats, dogs, cattle, sheep, goats, mice, rats, and other rodents and arthropods. Occasionally, humans are infected by C. psittaci.

(a) Taxonomy, species, serotypes

The genus chlamydia is presently subdivided into two species:- (1) C. trachomatis (2) C. psittaci and in this study only C. trachomatis will be considered.

C. trachomatis consists of three strains viz:

a. Trachoma-Inclusion Conjunctivitis (TRIC) agents.

b. Lympho-Granuloma-Venereum (LGV) agents.

c. the mouse pneumonitis agent.

C. trachomatis is subdivided into serogroups on the basis of difference in thermostable (100°C for 30 minutes) polysaccharide antigens. The TRIC agents belong to the serogroup A-K (plus BQ) and LGV organism to the serogroups I, II, III, also labelled L1, L2, L3. The basis

for the distinction of 15 serotypes is the microimmunofluorescence (MIF) test. Complement fixation and immunodiffusion also have been employed, but are not as sensitive as MIF technique. The immunodominant antigen appears to be 2-keto-3-deoxyoctamic acid or a similar sugar, which bears out the relationship of Gram-negative bacteria (167).

(b) **Cell morphology**

The chlamydiae are spherical and small with a diameter of 0.2 to 0.4 μm. Electron microscopy has demonstrated a trilamenar cytoplasmic membrane indistinguishable from that of bacteria. Outside the cytoplasmic membrane is another, slightly thicker trilamenar structure that represents a rudimentary cell wall, of a more loosely organized peptidoglycan structure than that found in typical eubacteria. Chlamydiae are considered Gram-negative but are stained weakly by soafranin (168).

(c) **Genome**

The genome is small, 6.6-9.5 x 10^5 dalton, approximately 6-8.5 x 10^3 base pairs. The genome compares in size to rickettsial DNA. It is half the size of the Neisseria DNA, and one-fifth the size of DNA in Escherichia coli (169).

The cytoplasm of chlamydia possesses ribosomes composed of 30S to 50S subunits, as in eubacteria but differing from 60S and 40S of vertebrates.

(d) **Surface receptors:**

The TRIC agents carry a surface neuraminidase which removes sialic acid from the host cell membranes and consequently, destroys host cell receptors. Neuraminidase has not been

demonstrated in the LGV strains (169). The TRIC organisms haemagglutinate when in elementary body (EB) stage, (see below) much as do myxoviruses, which likewise possess *neuraminidase*.

(e) **Growth cycle:**

The most outstanding aspect of chlamydia is its unique growth cycle, involving a characteristic two-stage infection and replication process. Chlamydia is only infective while in the metabolically inactive form designated the EB, which can survive outside the host cell but cannot replicate (169). The EB is small in size and possesses a relatively dense cell envelope constituting 15% of the cell weight. The EB begins the infection process by inducing a phagocytosis even in types of host cells that do not normally engulf whole particles in this way.

The passage of the infecting particle into the host cell, requires intimate contact. Significant for this event are receptors and binding site of the EB and complementary structure on the host cell cytoplasmic membrane. The receptor sites include heat labile structures on the EB and trypsin-sensitive sites on the cytoplasmic membrane of the host cell.

After incorporation of the EB form of chlamydiae into a cytoplasmic host cell vacuole, during the next 6-8 hours the chlamydia body increases in size to 3 to 4 times its original volume. During the next 8 to 12 hours the microbial cell envelope becomes increasingly permeable to allow more ready passage of such material as the needed ATP and essential precursors from the host cytoplasm. Ultimately, the cell envelope is reduced to only 5% of the total cell weight. It is during this stage that the form of chlamydia changes into that of the initial body, also called reticulate body (RB) (121). The RB microbial form is strictly intracellular and

is metabolically relatively active. The RB is more basophilic than the EB. It is fragile and cannot initiate any infection. The RB replicates by developing buds that eventually separate by repeated binary fission, resulting in a number of RB's within the host cell cytoplasm. In tissue cultures, this process continues for 24-72 hours after infection when nearly all RB's undergo reorganization into EB which are released eventually to infect new host.

One of the most striking changes during the growth cycle occurs in the cell envelope. During the transformation from EB to RB, the hexagonally arranged structures of the laminar layer of the cell envelope disappear. Although fragmented and diluted out during the transition from EB to RB, the chemical structures continue to be present. In the TRIC-agent serotype, the EB also losses the ability to haemagglutinate. The increased cell wall permeability of the RB readily allows passage of ATP, nutrients, and metabolic end-products into and away from the cells. Reconstruction of the cell wall appears to be a requirement for reversion from RB to EB (121).

(f) **Effect of drug on growth cycle**

Penicillin independently inhibit the reorganization of the inner cell envelope (170). They thereby force the microbe to remain in the intracellular latent state of the RB unable to develop into EB. Thus, under the influence of these drugs, the chlamydia growth cycle is temporarily terminated. Megagiant EB are formed. The penicillins do not affect the attachment of chlamydia to host cells, nor the penetration by EB into susceptible cells and they do not kill the RB (170). The penicillins do however inhibit binary fission of the RB buds and they inhibit the formation of the subunit envelope layer and consequently stop the reversion from RB to EB.

(g) **Origin of the term Inclusion Conjunctivitis**

It is the progression of cytoplasmic inclusions in the host cells that has inspired the term inclusion conjunctivitis. Such inclusion may be observed after Giemsa or iodine staining. The latter reflects the presence of glycogen in the vacuoles of *C. trachomatis*. The detection in culture studies of chlamydia inclusions after staining provides the basis for the diagnosis of chlamydia infection (171).

(h) **Metabolism of chlamydia**

The energy metabolism of chlamydiae is deficient principally in the capacity to generate ATP. Consequently, the chlamydiae depend on outside sources, that is, the host cells to supply ATP for the synthesis of their own structural components. Thus, they are energy parasites (121). The specialized chlamydia metabolism can produce only its own required cell constituent. As a consequence of their inadequate energy generation, chlamydiae are obligate intracellular parasites of vertebrate cells. In addition to ATP, needed as an energy source, a number of essential precursors or building blocks are similarly derived from the host system. For example the initial stage of glycogen requires the host-cell enzyme hexokinase (121). The DNA is produced from precursors derived from the host cell cytoplasm.

(i) **Chlamydia surface antigen**

The surface of the elementary body is exposed as a target to the host immune system and must also play a vital role in mediating attachment of chlamydia to the host cell and subsequent ingestion (172). The two most important antigens so far identified are the Major Outer

Membrane Protein (MOMP) and the lipopolysaccharide (LPS).

(j) **Major Outer Membrane Protein (MOMP)**

This accounts for 60% of the protein at the surface of the chlamydia EB and varies slightly in relative molecular mass in different serovars (173). Monoclonal antibodies to MOMP recognize species, subspecies, and type specific epitopes which shows that the protein has multiple immune determinant sites. Type-specific antibodies may have been associated with immunity (173). Structural rigidity of the organism is based on disulphide cross linking between adjacent polypeptide chains of the MOMP as well as of other proteins. Reduction of the disulphide bridges of the MOMP probably represent an early structural and metabolic adaptation needed for differentiation.

(k) **Lipopolysaccharide (LPS)**

This is found at the surface of the chlamydia EB, and RB, is endotoxic by the Limulus Lysate test and is structurally related to the lipid A and ketodeoxyoetonate core lipopolysaccharides of rough (RE) mutants of Salmonella typhirium and other bacteria. Antibody to chlamydia -Specific epitope present on LPS forms the basis of the chlamydia complement fixation test for psittacosis and lymphogranuloma venereum (LGV).

(l) **Chlamydia genetics**

Chlamydia lack the defined mutants, vectors and transformation systems necessary for classical genetic analysis (174). Progress has been made in cloning the genes for coding for chlamydia LPS biosynthetic enzymes and for fragments of MOMP. Bacteriophages have been described infecting C. psittaci RB, whilst C. trachomatis carries a common cryptic plasmid found in all serovars and apparently conserved through evolution (175). This chlamydia plasmid may be particularly useful for the gene probe diagnosis of chlamydia infection because multiple copies may be present in individual chlamydia, thus increasing the assay in sensitivity (175).

2.28 MICROBIOLOGY OF HAEMOPHILUS DUCREYI

Typical H. ducreyi, on microscopy is a Gram-negative bacillus, frequently occurring in clumps reminiscent of the cording of tubercle bacilli. They are non-motile, non-sporing and not acid fast. H. ducreyi colonies grow under required conditions, are 1-3 mm in diameter and darkish brown in colour. Contrasting clearly with other organisms grown from the same lesions, the most striking difference is the extreme coherence of colonies, which could be pushed intact across the plate. Cultures in liquid medium grow in clumps, which are attached to the sides of tubes. In 1982, Ursi, Dyck and other, isolated an unusual Gram-negative bacillus from clinically resembled chancroid on culture media used for H. ducreyi. These they called ducreyi-like bacteria (DLB) (176).

(a) **Biochemical test:** H. ducreyi is oxidase positive and catalase, urease, and ornithine decarboxylase negative. It does not produce indole or hydrogen sulphide and does not ferment glucose, sucrose, lactose, mannitol oxylase. It requires x factor haematin for growth.

(b) **Virulence:** The virulence of this organism for growth has been studied by some investigators, Odumeru, Wiseman, 1984; Hammond et al 1978. The only accepted test for

virulence has been the rabbit intradermal test, which results in necrosis and eschar formation. Isolated strains lose their virulence after repeated passages on artificial media (177). Virulence has been related to antibiotic resistance.

(c) **Electron microscopy of tissue injury by *H. ducreyi***

H. ducreyi is transmitted from one person to another during sexual contact. Presumably, the organism enters the skin through a break in the continuity of the epithelium. A papular lesion develops with epithelial cell swelling and polymorphonuclear cell infiltration. Within several days a pustule forms and ruptures, producing a non-indurated painful circumscribed ulcer with ragged edges.

On histological examination the ulcer can be divided into three zones: a narrow superficial zone of necrotic tissue, red blood cells, fibrin and degenerating polymorphonuclear cells; a middle zone that is broader and has oedematous tissue with extensive microvascularization and strands of endothelial cells; and a deep zone that merges with the middle zone and is infiltrated with plasma cells and lymphocytes. Bacteria are occasionally seen in the tissue that includes the superficial zone. H. ducreyi characteristically appear in clumps or as "shoals of fish" (178).

Electron microscopy (Marcsh, Stuttgen 1978), shows H. ducreyi in interstitial spaces at the base of chancroid ulcer (179). Published data on H. ducreyi lesions in humans are very limited and provide only indirect evidence that there is adherence of H. ducreyi to host cells at the base of the lesion. Entry into the cells and subsequent exit from the cells are important in the pathogenesis of chancroid (180). Adherence of H. ducreyi to host cells is likely to be an

important first step in the pathogenesis of chancroid and the form of interaction with host cells may be an important virulence factor for H. ducreyi (177).

2.29 PATHOLOGY

Superficial necrosis pervaded with polymorphonuclear leukocytes and a peripheral zone rich in plasma cells, lymphocytes and epithelial and endothelial leukocytes were described by Dienst (187). They placed responsibility for the superficial necrosis on the existence of acute perivasculitis and endovasculitis with swelling of the endothelium. The granulomatous reaction seen in chancroidal ulcers is highly suggestive of the diagnosis and in many cases may be diagnostic. Heymann, Beeson and Sheldon consider biopsy the most accurate diagnostic procedure (181).

CHAPTER THREE

3.00 MATERIALS AND METHODS

3.01 (a) Patient Selection and Control

The study was carried out from the Special Treatment Clinic (STC) and the General Out Patient Department of Ahmadu Bello University Teaching Hospital Zaria. From April 1993 to December 1995 Patients were included if they had signs and symptoms suggestive of the sexually transmitted disease under study. History taking and physical examination were also carried out. The age of patient ranged from 15 to 50 years. All males and females within this age bracket were included both single and married. The total number of patients studied was one hundred and fifty (150), composed of 25 men and 25 women for each disease under investigation, namely N. gonorrhoea; C. trachomatis; H. ducreyi. The controls numbering (30) thirty, composed of (15) fifteen males and females each were volunteers, age, and sex matched from the General Out Patient Clinic.

(b) *Patient Exclusion*

The following criteria were used to exclude patients; Patients with complications of STD, such as fever, joint manifestations, skin lesions, (b) those who were currently on antibiotics (c) females on contraceptive or family planning devices (d) patients receiving steroids or non steroidal anti-inflammatory drugs (e) patients suspected of or who had clinical symptoms of Acquired Immunodeficiency syndrome (AIDS). (f) commercial sex workers (g) patients with sexual trauma (h) those with multiple infections for example Neisseria gonorrhoea and Chlamydia trachomatis genital infections. (i) pregnant females (j) those who refused

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verbal/written consent to the study. The study was approved by the Ethical Committee of the Ahmadu Bello University Teaching Hospital Zaria. See Appendix.

3.02 SPECIMEN COLLECTION:

(a) MALES:

The tip of the penis is cleaned with sterile gauze. The meatus is than squeezed for the pus to be collected with a sterile cotton swab. For genital ulcers saline water is used to clean the surface gently before swab is collected.

(b) FEMALE/WOMEN:

Female patients are put in lithotomy position, Cusco's speculum without disinfectant is used to expose the cervix, which is illuminated with an angle poise lamp. The cervix is first gently cleaned, a gentle pressure is applied at the suprapubic area, and a sterile cotton swab is taken. Specimen were collected from the urethral, posterior vaginal fornix and endocervix. In females with genital ulcers, vulva, labial, and cervical, were swabbed as in males above.

3.03. LABORATORY DIAGNOSIS OF NEISSERIA GONORRHOEA

(a) GRAM STAIN

A Smear was made from the urethral of vaginal discharge collected from the patient. It was fixed on microscope slide by heat. After cooling for 5 mins the smear was staeved crystal violet for 60 seconds. It was then washed with water. Immediately after, the slide was covered with Gram's iodine for another 60 seconds. The n if was decolorized for 30 seconds with acetone of 30 ml mixed with 20 ml ethanol. The slide was washed again and covered with

safranin 2.5% solution, 95% ethanol for 30 seconds. It was washed with and air dried. Gram positive bacterial retain the crystal violet iodine colour; Gram negative bacterial are decolorized by the ethanol (181).

(b) NEISSERIA GONORRHOEA IDENTIFICATION

Inoculation of primary isolation plates with chocolate agar Thayer-Martin (Oxmoid) media was done. After 48 hours cultures were re-examined, before any specimen can be reported negative. A definitive diagnosis of gonorrhoea is made on the basis of (a) Gram negative, diplococci from urethra swab, for male: cervical or urethral swab, female. (b) glucose oxidase positive (c) sugar utilization reaction test of Neisseria gonorrhoea.

Observation of characteristic kidney shaped gram negative diplococci lying within polymorphonuclear leukocytes, with only a few scattered extracellularly is typical of gonorrhoea infection. This was taken as positive culture. Equivocal result: if the Gram-negative diplococci are seen only extracellularly; the result of the smear examination is equivocal. Negative result: if no Gram-negative diplococci are seen, it is negative. By this method 95% of all infected men, 55-60% of all infected women yielded positive smears.

Oxidase test: Plates were examined after 2 hours inoculation and suspected colonies were picked by touching with a cotton bud in oxidase reagent. Oxidase positive: the bacteria turned the contact area of the bud purple within 5 to 15 seconds.

SUGAR UTILIZATION TEST FOR NEISSERIA GONORRHOEA

Species	Glucose	Maltose	Fructose	Sucrose	Lactose
N. gonorrhoea	+	-	-	-	-
N. meningitides	+	+	-	-	-

3.04 ImmunoComb Chlamydia trachomatis Assay Procedure

Phosphate Buffered Saline for ImmunoComb Procedure

Preparation; 8:5% (w/v) NaCl was dissolved in 85 g NaCl in litre distilled water. (ii) 0.67 mol/l KH_2PO_4 was dissolved in 90.7 g KH_2PO_4 in 1 Litre distilled water. (iii) 0.67 mol/l KH_2PO_4, was dissolved in 116.2 g of K_2HPO_4 in 1 Litre distilled water. 1 litre working solution was prepared by mixing 100 ml stock solution; 2.33 ml stock solution, (ii) 47.67 ml stock solution and 85 ml distilled water was added to make up 1 litre. The solution was checked for pH 7.4.

10 μl of serum diluted in phosphate buffered saline pH 7.4, was placed over each group of antigens. (ORGENIC, YAUNE, 7650 ISRAEL) The slides were placed in a humidified box for 30 minutes at 37°C, washed in phosphate buffered solution for 0-10 minutes and it was air dried. Then an optimal dilution of florescent isothiocyanate FITC, conjugated anti-species immunoglobulin was placed over each spot for 30 minutes at 37°C. The slides were then washed in the buffered solution for 10 minutes. Evans blue 0.02% was added to the last wash as a counter-stain. Slides were air dried for 30 minutes. Chromogenic colour were compared with the control provided in the box. the slides were read as positive or negative.

3.05 Clinical and Laboratory Diagnosis of genital ulcer due to H. ducreyi

(a) **GRAM STAIN AS IN NEISSERIA GONORRHOEA**

(b) A simplified laboratory technique for the diagnosis of chancroid plus clinical appearance and history was used in this study.

Media agar: Protose peptone (Difco), 30 gm corn starch: 1.0 gm potassium phosphate dibasic, 4.0 gm; potassium phosphate monobasic, 1.0 gm; sodium chloride, 5.0 gm; Bacto agar, 10 gm; distilled water, 1 litre. The ingredients were dissolved in water and sterilized by autoclaving at 15 lb pressure at 121 for 15 minutes. A Stock solution of type III equine haemin (crystalline Sigma) was prepared by dissolving 250 mg of heamin in 2 ml of 0.2 mol/L KOH in 47.0% ethanol and bringing to a final volume of 10, litre with sterile distilled water.

After sterilization of the modified base 2% of defined supplement and 10 ml of haemin solution per litre was added aseptically, giving a final heamin concentration of 25ug/ml.Plates were poured and stored in the refrigerator at 4^{oC}. The plates were inoculated and incubated at 33^{oC} in atmosphere of 5% CO_2 for 4 days.

Colonial appearance and identification.

Heamophilus ducreyi colonies were 1-3mm in diameter, darkish, brown in colour. There was *extreme coherence of the colonies which was pushed intact across the plate.*

Biochemical test. Alkaline phosphate test positive. Does not ferment any carbohydrate. (b)

Biochemistry: required haematin (X-factor), they are oxidase positive and does not ferment any carbohydrate.

3.06 IMMUNOLOGICAL/HAEMATOLOGICAL ANALYSIS

All patients who have the three named STD's namely gonococcal urethritis, Chlamydia trachomatis genital infection and chancroid, had their blood taken for immunological and haematological analysis.

3.07 HAEMATOLOGICAL TEST

(a) Collection of Blood Specimens:

Venous blood of 15 ml was collected from gonococcal infection Chlamydia trachomatis genital infection, chancroid genital ulcer(s) patients and control. 9 ml was used for lymphocyte sub-populations enumeration, 2.5 ml of this blood was placed into sequestrene EDTA bottles for full blood count and differential count. The remaining 3.5 ml was placed into heparinized tube in the ratio of 1000 I.U of heparin to 1 ml of blood and this was allowed to stand for 2 hours, the supernatant used for MIF assays.

(b) Total White Blood Cell Count (total WBC):

A 1;20 dilution of the heparinized blood 20 ul in 400ul was made in white cell count dilution fluid (3% glacial acetic acid with a tinge of gentian violet). It was counted. Under a microscope at a magnification of 160. The white blood cells were expressed as WBC x 106/litre. (182)

(c) Differential lymphocyte count.

A small drop of blood in EDTA was placed on a microscope slide and spread out to give a thin film. This was air dried, stained with Leishman stain and the percentage of lymphocytes counted under a microscope at a magnification of 100. (182)

3.08 METHOD OF ISOLATION AND ENUMERATION OF LYMPHOCYTES.

(a) Isolation of T-lymphocytes: Method:

Method of Boyum (1966) was used in the separation of human lymphocyte as it produces a high yield with fairly high purity (183).

10 ml of fresh blood is obtained by plastic syringe from peripheral venous blood by venepuncture into a heparinized test tube (1ul heparin/ml). 9 ml of the whole blood is layered onto 3 ml of Ficoll-Hypaque medium. 3 ml of 0.9% NaCl is added in a narrow sterile plastic tube 16 mm. The plastic tube is then centrifuged at room temperature for 40 minutes at 400 g. After centrifugation, a white layer of lymphocytes is visible at the interface between the plasma and Ficoll-Hypaque. The lymphocyte is then removed with Pasteur pipette.

The cells are washed in minimum essential medium (MEM) solution then re-suspended in MEM and centrifuged finally. The cells were washed three times with MEN by re-suspending them in the medium and then centrifuging at 400g for 10 mins at room temperature. After the final wash the cells were resuspended in 1ml of MEN containg 5% inactivated absorbed foetal calf serum (FCS) and cell concentration adjusted to 4 x 10^6 ml. More than 95% of the cells were viable as tested by Tryphan blue dye exclusion test.

3.09 Enumeration of Lymphocyte Sub-populations CD3, CD4, CD8, CD19 using immunofluorescence Microscopy Technique.

The method described in Becton Dickinson brochure (1981) was adopted (184). Fifty microlitre (50 μl) aliquot of the previously prepared lymphocyte suspension was mixed with 20

μl of each monoclonal antibody CD3 - Pan -T- Cells: GD4- Cytotoxic cell, CD8 - Suppressor cells: CD19 - pan - B - cells.

This was incubated for 30 minutes at 4°C in crushed ice, after which it was centrifuged and washed at 1000 rpm. for 5 minutes at 4°C.

After the final wash, the lymphocytes were re-suspended in a drop of MEM, placed onto a glass slide, covered with cover slip and sealed in a colourless nail varnish. The slide was then left to equilibrate in a moisture chamber for 15 minutes at 4°C, before screening randomly of field for fluorescence using immunofluorescence microscope. A field was selected and viewed under normal light, and total number of cells in a visible area recorded. The UV light was then switched on and the number of cells in the same area that started bright peripherally with fluorescent dye was recorded separately as positive.

Several fields are scanned until a total of 100 lymphocytes both positive and negative were counted. The number of positive cells is expressed as percentage of total count. The absolute number was calculated using the below formula for positive cells. The method was used for all the samples under study.

Calculation of total lymphocytes:- This is calculated by the formula:

$$\frac{a}{100} \times \frac{b}{100} \times c \quad D \times 10^9 / L$$

a = % of T-cells that fluorescence

b = % peripheral blood lymphocytes

c = Total WBC x 10^9/L

D = The aggregate number of T-cells specific in the peripheral blood lymphocytes.

3.10 MACROPHAGE MIGRATION INHIBITION FACTOR (MIF) TEST.

The method of Rosenberg and David (1970) was used (185). Leukocytes were separated from 2.5 ml heparinized blood obtained by venepuncture and allowed to stand for 1 hour in 1 cm glass tube. After washing in Eagles Minimum Essential Medium they were suspended at a concentration of 1 x 10^6/ml in this medium containing 10% fetal calf serum (FCS) and used to fill 12.5 μl capillaries (half of 25 μl micro capillary). The tube were plugged with paraffin wax (BDH chemical, pole, England), spun at 800 g for 5 minutes, cut with a diamond pen, and the cell buttons were placed in plastic migration chambers (sterilin) containing medium (MEM) and 10% FCS with or without PPD antigen; sealed with coverslip and incubated at 37°C for 18 hours.

All procedures were carried out at room temperature before the final incubation. The area of cell migration was projected by a prism attached to a standing microscope, supported on graph paper. A ruler was used to measure the area, and the squares counted

$$MI \quad \frac{MX}{MO}$$

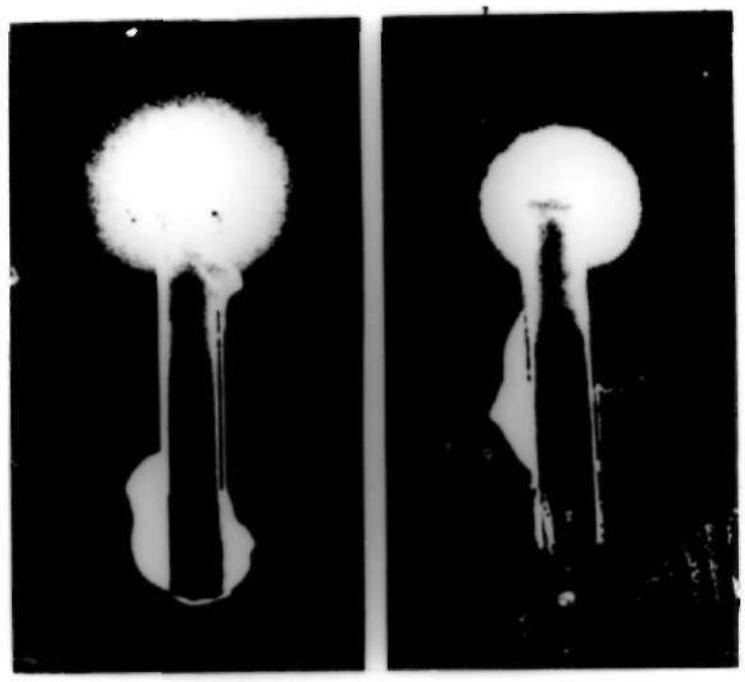
MI = Migration index

MX = Area or squares in presence of antigen PPD

MO = Area or squares in absence of antigen.

See Plate III.

Plate III Leukocyte migration inhibition test



3.11 NEUTROPHIL BACTERICIDAL ASSAY

The method of Philips et al. 1979 was adopted (186). 8ml of Ficoll-isopaque was layered and 10ml of heparinised (100U, Evans preservative free) venous blood. This was then centrifuged (1500 x g, 20 minutes) after which the layers containing the mononuclear cells, platelets, plasma and Ficoll-isopaque were discarded, retaining the bottom layer containing erythrocytes and neutrophils. This was then mixed with 5ml of 6% Dextran T 500 (Pharmacia) and 15ml of 0.05 M sodium phosphate butter (pH 7.4) containing 0.1M sodium chloride (PBS) and allowed to settle at its room temperature for 45 minutes. The neutrophils still in suspension were collected and washed 3 times with PBS by centrifugation (700 x g for 5 mins) and resuspended. The nucleated cells were counted in a cell counter (Coulter counter model FN) and adjusted to $2x10^7$ nucleated cells/ml. This technique yielded a cell suspension in which neutrophils accounted for greater than 90% of the nucleated cells.

(a) PREPARATION OF BACTERIAL SUSPENSION.

A stock of culture of Staphylococcus aureus NCTC 1530 was maintained on Columbia agar slopes. An isolated colony agar plate was inoculated into nutrient broth and incubated for 16 hours at 37°C. The bacteria were harvested and washed 3 times by centrifuging at 2500 rpm for 15 min in sterile Dulbecco's DPB. (Commonwealth serum laboratories). After the final wash the bacterial suspension was adjusted to an optical density of 1.8 at 540 mm using a spectophotometer (Varian Techtron, Model 635).

(b) ASSAY:

Ingestion of the bacteria by neutrophil was allowed to occur by incubating together a mixture of 600 μl of M 199 see text. Pooled human serum; 50 μl of bacterial suspension and 200 μl of the neutrophil (above) suspension for 10 minutes at 37°C. At the conclusion of this pre-incubation, the neutrophils were carefully washed three times with Dulbecco's PBS by centrifuging at 1500 rpm for 3 minutes. After the last wash the pellets were re-suspended in 1 ml of medium 199 containing penicillin (100 μl/ml) and streptomycin (100 μg/ml) to get rid of any remaining extracellular bacteria in the suspension.

At this time, zero time, 400 μl aliquot (t_0) was removed and the remaining suspension was further incubated at 37°C for 3 hours. Immediately upon removal, the aliquots were carefully washed once in Dulbecco's PBS at 1500 rpm for 3 minutes. The pellet was re-suspended in 4 ml of sterile distilled water and neutrophils were disrupted by sonication MSE 1-73 (15W, 3 seconds). The number of viable bacteria so released were counted using the following microtitre technique.

The wells of a sterile micro-titre tray were filled with 225 ul of sterile nutrient broth under aseptic conditions. 25 micro-litres of the disrupted neutrophil suspension was added to the first well of the first five columns and a 10 fold serial dilution of the inoculum was made down the column to a final titre of 10^7 nucleated cells/ml. A new sterile tip was used for each dilution to maintain aseptic condition. The next two columns served as blanks and contained only nutrient broth and were used as indication of contamination.

After completion of the 3 hour incubation at 37°C, the above procedure was repeated (t3).

See Plate IV.

The tray was incubated for 10 hours at 37° C and then examined under incident light for growth. Each wall was scored as either positive (growth) or negative (no growth) and an estimate of the number of viable bacteria made using the most probable number. See Appendix behind the text.

The bacterial killing index was calculated as follows:

$$KI = \frac{t3}{t0}$$

KI = Killing index

t3 = Number of bacteria after incubation

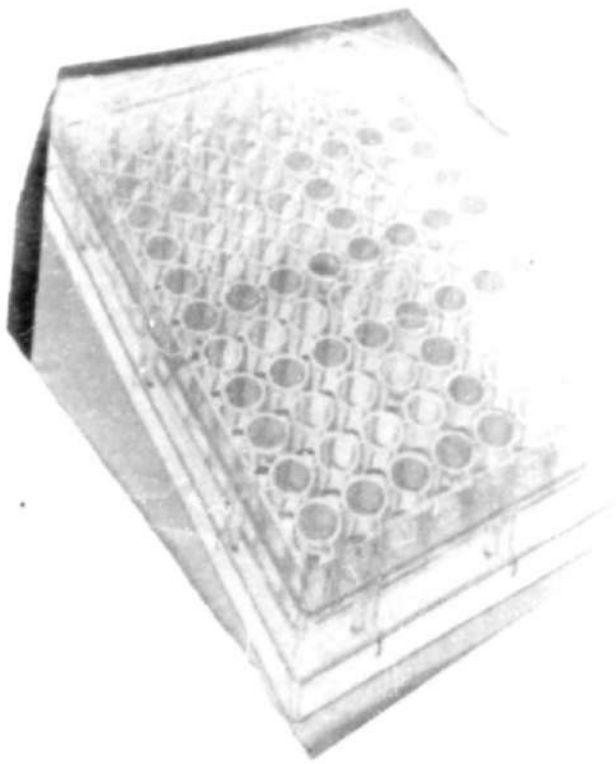
t0 = Number of bacteria ingested

The lower the index value obtained, the better was the killing ability.

3.12 STATISTICAL METHODS

Analysis of the results was done by using the X^2, chi square test; Results were significant if the p value was less than 0.005, 95% confident limit; mean expressed as ± 2SD.

Plate IV Microtitre plate for determination of bacterial killing index



CHAPTER FOUR

4.00 RESULTS

4.01 CD3 Lymphocyte sub-populations in Neisseria gonorrhoea, Chlamydia trachomatis, Haemophilus genital infection.

The lymphocyte sub-population CD3 in Neisseria gonorrhoea patients and controls are indicated in Table 5. The control subjects had mean of 63.93% SD ± 2.31%, absolute number 2.85 x 10^9/L. The patient infected with gonococcus had a mean ± SD of 69.88 ± 4.81% absolute number 3.17 x 10^9/L. Analysis of the result showed that all the patients' CD3 sub-population of lymphocytes were all above that of the controls Fig. 3,4, 5. The increase was however not statistically significant ($p > 0.05$). The distribution of CD3 subsets of lymphocytes in patients with chlamydia genital infection are shown in Table 5. The patients with chlamydia urethritis/cervicitis had mean ± SD of 69.70 ± 2.29%, mean absolute number 3.02 x 10^9/L. It was observed that the CD3 subsets were all above that of control subjects Fig. 3,4,5. Comparing the values of the patients with the controls, it was not statistically significant ($p > 0.05$). Circulating lymphocyte sub-population CD3 was investigated in genital ulcer due to Haemophilus ducreyi, the result is displayed in Table 5. The result indicated that nine (9) or 18.0% of the patients were below the control mean value - see figure 3,4,5. The mean ± SD for the patients was 66.68 ± 3.54%, absolute number 3.18 x 10^9/L, the range was 60-72% absolute number 2.69-3.83 x 10^9/L. The difference in the two means was not statistically significant ($p > .0.05$).

TABLE 5 LYMPHOCYTE SUBSETS IN PATIENTS WITH STD's AND CONTROL SUBJECTS IN ZARIA

STD	CD3 Mean ± 2SD Range% Absolute Nox10^9/L	CD4 Mean±2SD Range% Absolute Nox10^9/L	CD8 Mean±2SD Range% Absolute Nox10^9/L	CD19 Mean±2SD Range% Absolute Nox10^9/L	*p value
Neisseria gonorrhoea n=50	M=69.88±4.81% R=66-73% A=3.17x$10^{9/L}$ ± 4.81	M=37.84±2.51% R=35%-41% A= 1.17x10^9/L±2.51	M=29.04±1.56% R=26-33% A= 1.17x10^9/L ±1.56	M=26.52±2.81 R=22-31% A=0.74x10^9/L ±2.85	>0.05
Chlamydia trachomatis n=50	M=69.20±2.29% R=65-73% A= 3.02x10^9/L ±2.29	M=38.14±2.30 R=31-43% A= 1.43 x10^9/L±2.23	M=29.90±2.16% R=26-34% A= 1.19 x10^9/L±2.16	M=29.64±2.94 R=25-36% A= 1.10x 10^9/L ±1.92	>0.05
Haemophilus ducreyi n=50	M=66.68±3.54 R=60-72% A= 3.18x10^9/L ±3.5	M=38.52±2.36% R=35-43% A=1.61x10^9/L±2.36	M=24.38±3.80 R=19-32% A= 1.03x10^9/L ±2.16	M=25.58±2.90% R=21-30% A= 0.53 x10^9/L±2.9	>0.05
Control n=30	M=63.93±2.31 R=59-68% A=2.85 ± 2.31x10^9/L	M=37.43±2.08 R=35-41% A=1.54 ± 2.08x10^9/L	M=22.43±2.76% R=10-27% A= 0.93 ± 2.76x10^9/L	M=20.30±1.93% R=17-23% A= 0.81 ± 1.93x10^9/L	

* When Patients are compared with controls
M=Mean ±2SD
R=Range%
A=Absolute Value

4.02 CD4 Lymphocyte sub-populations in Neisseria gonorrhoea, Chlamydia trachomatis, Haemophilus ducreyi genital infection

CD4 Lymphocyte sub-populations in control subjects and Neisseria gonorrhoea patients were studied and are shown in Table 5. The mean and SD of CD4 cell were 37.43% ± 2.08 absolute number 1.54 x 10^9/L in controls. The CD4 subsets in gonococcal urethritis/cervicitis was 37.84% ± 2.51 mean, absolute number 1.17 x 10^9/L. Analysis of the result showed that thirteen (13) or 26% of the patients were below the mean control values. See Figures 3,4,5. The difference between the two means was not statistically significant, $p > 0.05$. The mean ± SD, the range of CD4 subsets in chlamydia genital infection are shown in Table 5. The result showed that the mean ± SD was 38.14% ± 2.30 mean absolute number 1.43 x 10^9/L. There were twelve (12) or 24% of the patients whose CD4 cell fell below the control values see Figures 3,4,5. Comparison of the two means showed that it was statistically insignificant, $p > 0.05$.

CD4 cells in the patients with genital ulcer due to Haemophilus ducreyi are shown in Table 5. The mean ± SD, is 38.52 ± 2.36% or 1.61 x 10^9/L absolute number and or Fourteen (14) or 28% of the patients CD4 cells were below the control mean values see Figures 3,4,5. The difference between the two means was statistically not-significant, $p > 0.05$.

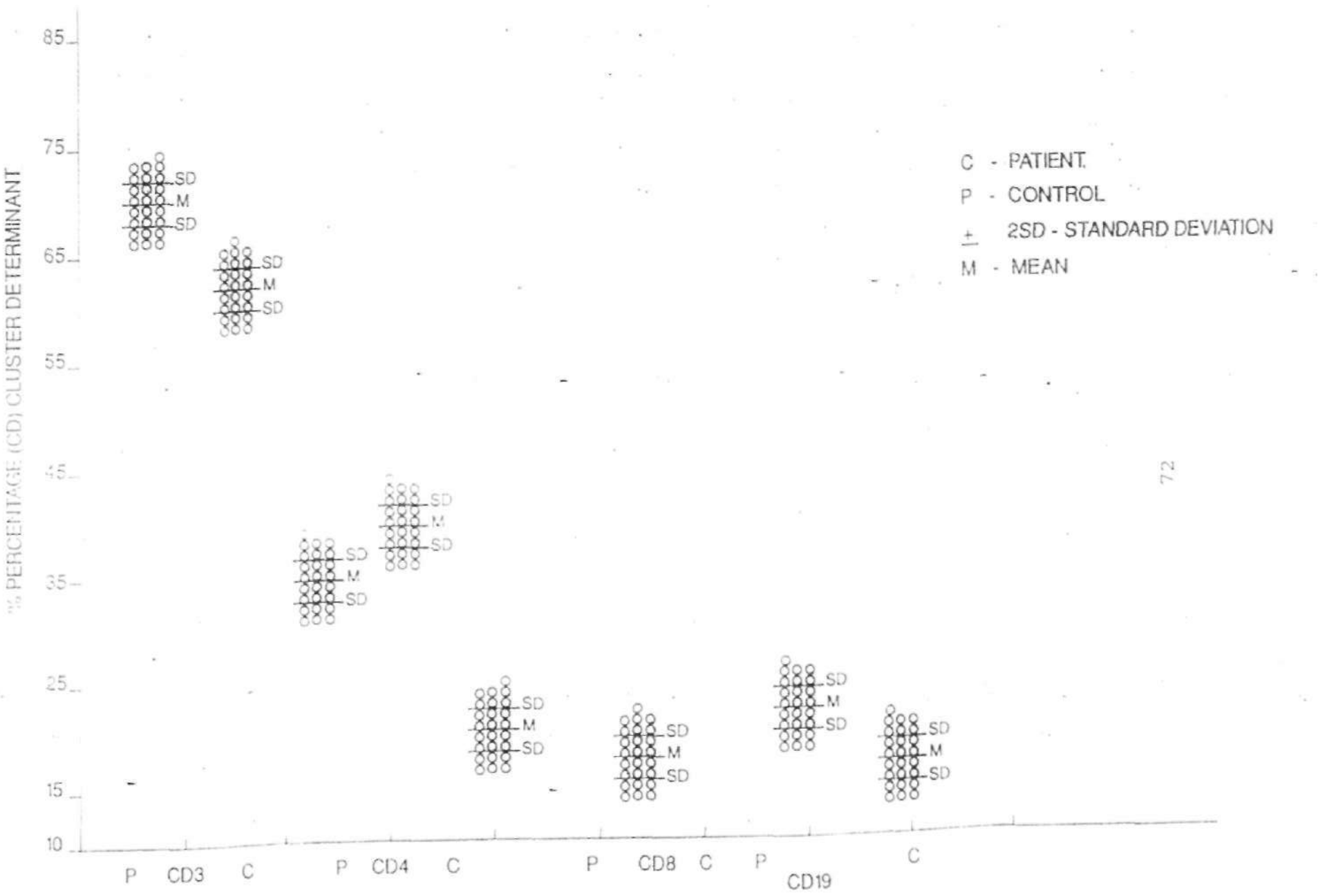


FIGURE 3

(CD) CLUSTER DETERMINANT PERCENTAGE IN CONTROL SUBJECTS AND GONOCOCCAL PATIENTS

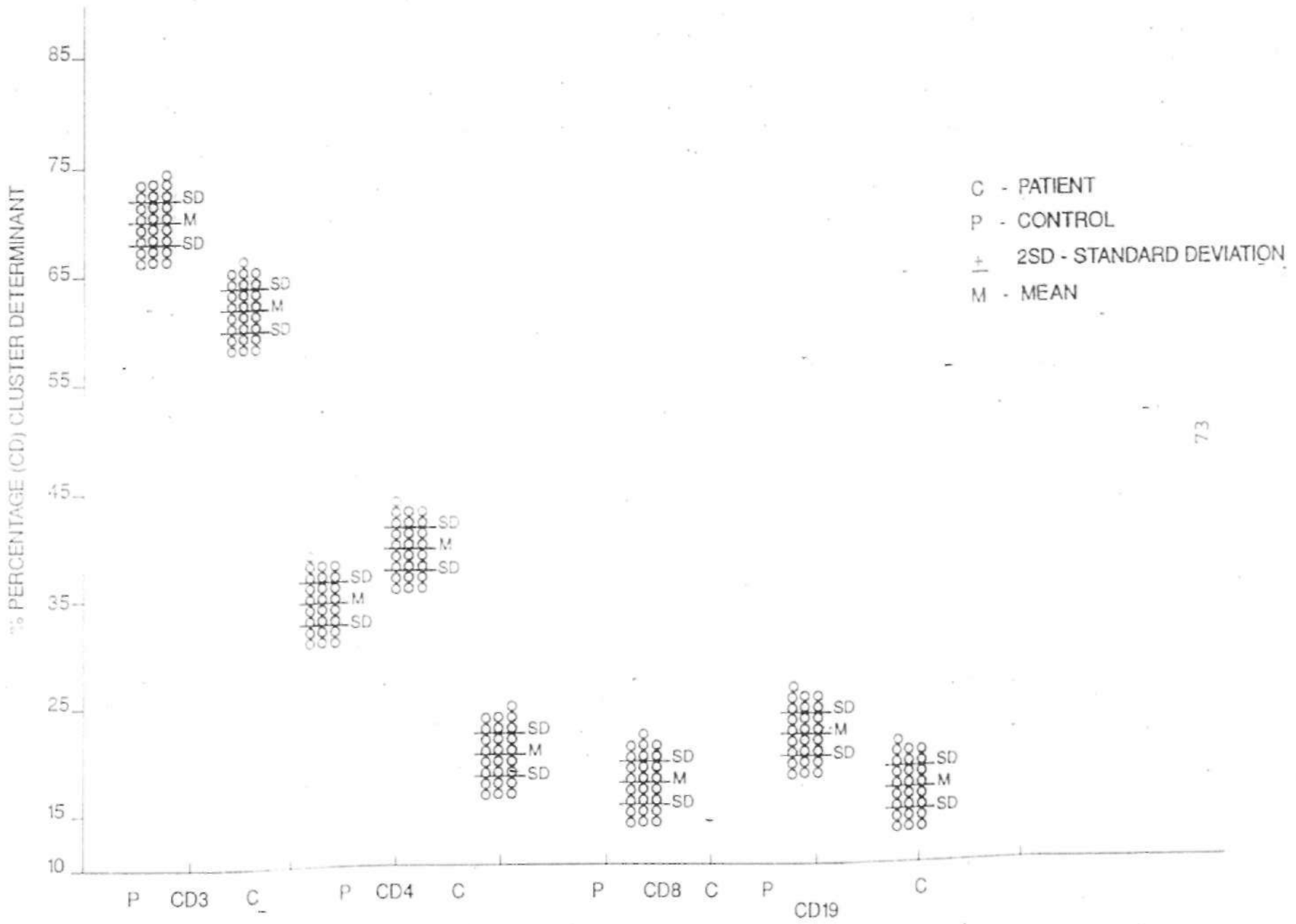


FIGURE 4

(CD) CLUSTER DETERMINANT PERCENTAGE IN CONTROL SUBJECTS AND CHALAMDIA TRACHOMATIGS PATIENTS

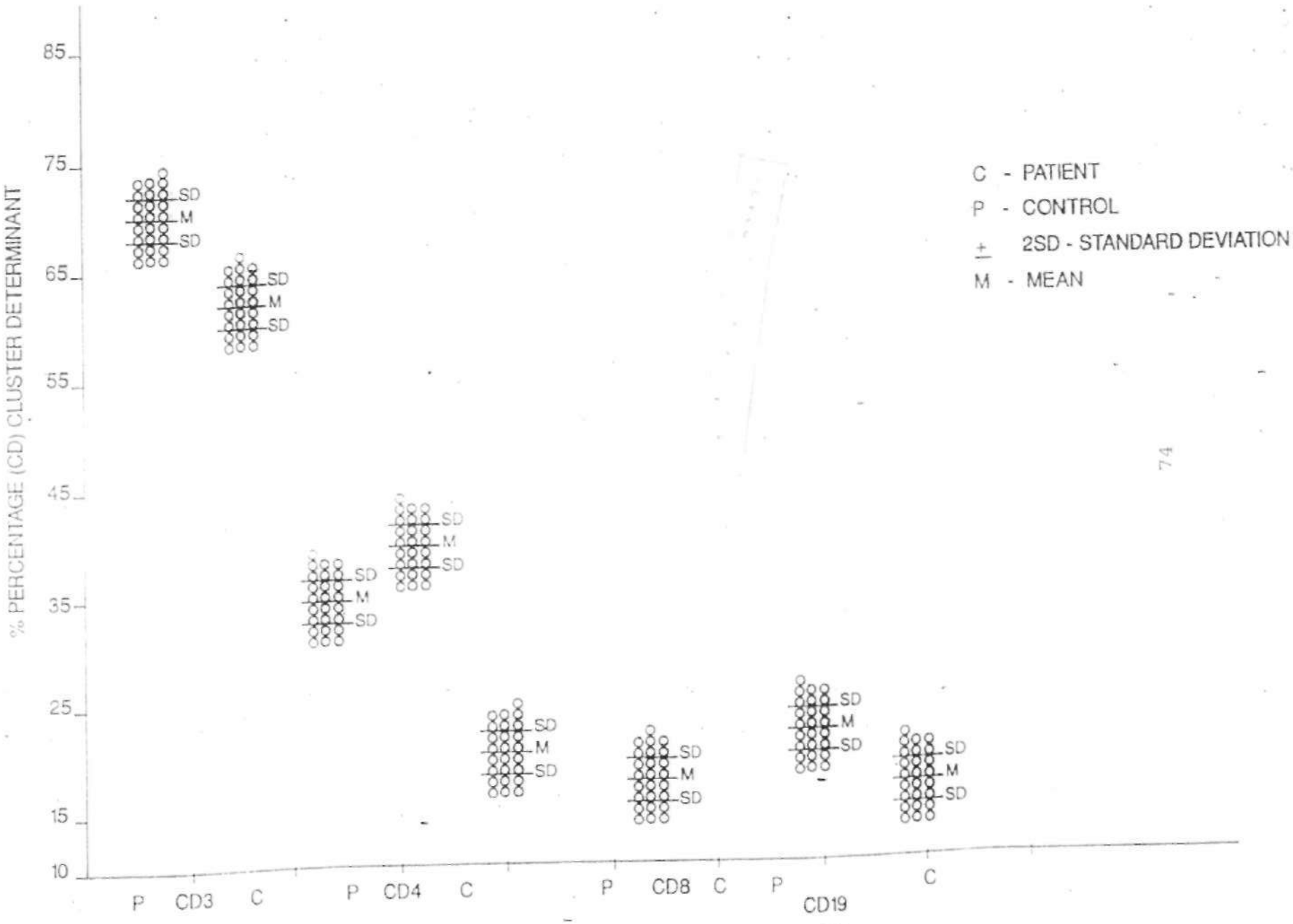


FIGURE 5

CLUSTER DETERMINANT (CD) PERCENTAGE IN CONTROL SUBJECTS AND GENITAL ULCER TO HDCREYI PATIENTS

4.03 CD8 Lymphocyte sub-populations in Neisseria gonorrhoea, Chlamydia trachomatis, Haemophilus ducreyi genital infections

Table 5 shows the lymphocyte sub-populations CD8 in patient and controls. The mean ± SD is 22.43% ± 2.76 mean absolute number 0.93 x 10^9/L. Table 5 shows the mean ± SD, and the range for the patients infected with gonococcal urethritis/cervicitis. The results are 29.04% ± 1.56% absolute number 1.17 x 10^9/L. Analysis of the result showed all the patients CD8 cells were above that of the control mean value, though it was statistically none significant, $p > 0.05$. The mean ± SD in chlamydia genital infection are 29.90% ± 2.16 mean absolute number 1.19 x 10^9/L. All the patients CD8 cells were above the mean control value. Comparing the two means showed that the difference between them was statistically not significant, $p > 0.05$.

The CD8 cell sub-population in Haemophilus ducreyi genital ulcer patients are shown in Table 5. The mean ± SD is 24.38% ± 3.80% mean absolute number 1.03 x 10^9/L. Eleven (11) or 22% of the patients' CD8 cells were below the control value. The difference between the two means indicated that it was statistically none significant, $p > 0.05$.

CD4/CD8 ratio in STD's

The mean CD4/CD8 lymphocyte subset ratio for normal subjects in this study was 1.6:1, that for the studied STD's were 1.3:1 for Neisseria gonorrhoea, 1.2:1 for Chlamydia trachomatis.

It was observed that there was tendency to reversal or inversion of the CD4/CD8 subsets of the lymphocytes of the studied STD's. See Table 6.

Table 6: **CD4/CD8 Ratio in STD's, Neisseria gonorrhoea, Chlamydia trachomatis, Haemophilus ducreyi in Zaria.**

CD4/CD 8 Ratio	Neisseria gonorrhoea 1.3:1	Chlamydia trachomatil.2:1	Haemophilus ducreyi 1.5:1	Control 1.6:1

4.04 CD19 Lymphocyte sub-population in Neisseria gonorrhoea, Chlamydia trachomatis, Haemophilus ducreyi genital infections

Table 5 showed control mean ± SD is 20.30 ± 1.93% mean absolute number 0.81 x 10^9/L. Investigation of CD19 sub-population of lymphocyte in gonococcal urethritis/cervicitis reveal that the CD19 sub-population had a mean ± SD of 26.52 ± 2.81% mean absolute number 0.74 x 10^9/L. See Table 5. Analysis of the result showed that all the patients' CD19 subsets were increased and rose above the mean control subjects value see Figures 3,4,5. The increase was none significant statistically, $p > 0.05$.

The lymphocyte subset CD19 in chlamydia genital infection showed that the mean ± SD 29.64 ± 2.94% mean absolute number 1.10 x 10^9/L - see Table 5. All the patients' CD19 were above the control value - see Figures 3,4,5.

The lymphocyte sub-population CD19 was investigated in patients with chancroid. The mean ± SD and the range are as follows: 25.58 ± 2.90% absolute number 0.53 x 10^9/L and absolute number 0.86-1.19 x 10^9/L. Analysis of the result indicated all the patients' CD19 cells were above the control values see Figures 3,4,5. The difference in the two means was none significant statistically, $p > 0.05$.

4.05 Macrophage Migration Inhibition Factor (MIF) test in Neisseria gonorrhoea, Chlamydia trachomatis, Haemophilus ducreyi genital infections

Neisseria gonorrhoea

Table 7 showed the mean ± SD and range in normal subjects which were 0.64 ± 0.18 and range 0.43-0.93 respectively. The macrophage migration inhibition factor in gonococcal urethritis/cervicitis were mean ± SD and range are 0.71 ± 0.12 and 0.52-0.92 respectively. Comparison of the two means showed that sixteen (16) or 32% of the patients' MIF were below the mean control value. From this study it appears that there is acquired cell mediated immune response in gonococcal urethritis/ cervicitis. The difference in the two means was statistically not significant, $p > 0.05$.

Chlamydia infection

The result is displayed in Table 7. The mean ± SD and the range are as follows: 0.44 ± 0.09 and 0.34-0.57 respectively. Analysis of the result indicated that all patients' MIF values were below the mean control subjects. See Figure 7

The mean ± SD and the range are shown in Table 7. The mean ± SD and the range are as follows: 0.62 ± 0.54 and 0.52-0.74.

TABLE 7 MACROPHAGE MIGRATION INHIBITION FACTOR (MIF) TEST IN STD's versus CONTROL SUBJECTS IN ZARIA

STD	MIF mean±SD (range)	p value
Neisseria gonorrhoea n=50	0.71±0.12 (0.52-0.92)	> 0.05
Chlamydia trachomatis n=50	0.44±0.09 (0.34-0.57)	< 0.05
Haemophilus ducreyi n=50	0.62±0.04 (0.52-0.74)	> 0.05
Control n=50	0.64±0.18 (0.43-0.93)	> 0.05

Analysis of the result showed that thirty-five (35) or 70% of the patients were below the control value. For Heamophilus ducreyi - see Fig 7.

4.06 Bacterial Killing Index (BKI) or Polymorph Function in Neisseria gonorrhoea, CHLAMYDIA TRACHOMATIS, HAEMOPHILUS ducreyi genital infections.

Table 8 showed the mean ± SD and the range for the control subjects. The mean ± SD and the range are 0.04 ± 0.03 and 0.01-0.1 respectively. There was no patient whose BKI was above 0.1. Therefore, the BKI in gonococcal urethritis/cervicitis Chlamydia trachomatis, Haemophilus ducreyi genital infections were not depressed. Twenty-three (23) or 46% of the Neisseria gonorrhoea patients' BKI were below the mean value but within the control of 0.1. The difference between the means was statistically not significant, $p > 0.05$.

Table 8 showed the mean ± SD and range obtained. The mean ± SD and the range are 0.04 ± 0.03 and 0.01-0.1 respectively. Ten (10) or 20% of the chlamyeha traclomatis patients' BKI were below the mean value 0.04 but none was above the maximum 0.1. This indicated that there is no bacterial killing defect in chlamydia genital infection. The difference between the two means was not significant, $p > 0.05$.

TABLE 8 **Bacterial Killing Index (BKI) in STD's Patients and Control Subjects in Zaria**

STD	BKI mean±SD (range)	p value
Neisseria gonorrhoea n=50	0.05±0.03 (0.01-0.10)	> 0.05
Chlamydia trachomatis n=50	0.05±0.03 (0.01-0.10)	> 0.05
Haemophilus ducreyi n=50	0.05±0.03 (0.01-0.10)	> 0.05

14% of the chancroid ulcer patients were also below the mean control value of 0.04. These patients warrent further investigation. This will ascertain if the polymorph function in these studied STD's is reduced or unchanged.

CHAPTER FIVE

5.00 DISCUSSION

Lymphocyte sub-populations CD3, CD4, CD8, CD19, in Neisseria gonorrhoea, Chlamydia trachomatis, Haemophilus ducreyi genital infections.

There is increasing interest in the lymphocyte sub-populations of CD3, CD4, CD8, CD19 in clinical diseases and their role in maintaining the asymptomatic phase of several diseases (187,188). Explanation of asymptomatic phase of some diseases: when the body is invaded by micro-organisms such as bacterial, virus, fungal or parasite the body triggers both the humoral and cellular immune responses to eliminate it. These immune system components are complements antibodies, neutrophils, monocytes, macrophages, lymphocytes, cytokines. Their combine responses lead to elimination of the micro-organism or containment of the infection without clinical signs or symptoms. This absence of clinical signs or symptoms is known as asymptomatic phase (189). This is more apparent in sexually transmitted diseases, which might be associated with immunosuppression. The quantitative assessment of these lymphocyte sub-populations are with the effect of detecting any immunodeficiency state present in the disease.

Neisseria gonorrhoea genital infection, is one of the first traditional sexually transmitted diseases to be studied by lymphocyte proliferation test. Wyle and Rowlett conducted this study by invitro using incorporation of (^{3}HT-UR).(190). Cell mediated Immunity was investigated by these authors. They used 29 men, 21 women control subjects were 10 men and women. Patients with uncomplicated genital gonorrhea were investigated using non-purified gonococal antigen, GG-1, and non-purified meningococeal antigen with phytoheamgullitin-P (PHA) as a T- cell stimulant. Lymphocyte transformation was measured by incorporation of tritiated thymidine 3H - Tdr in to DNA. They concluded that there was cell-mediated immune response in men and women with uncomplicated Neisseria gonorrhoea, compared to controls. Their method did not investigate lymphocyte subset population and non-specific in response. They showed

that there was peripheral blood lymphocyte transformation in uncomplicated genital gonorrhoea and the blastogenic responses of the peripheral blood lymphocyte from men and women were substantially higher than that of control. Early in 1973, Grimble & Mcillmurray (1973), studied cell mediated Immune (CMI) response in Neisseria gonorrhoea patients. They used 14 patients; men and 5 women, with 10 controls to study the response. By the use of lymphocyte transformation technique of labelling with C 14 thymidine which is incorporated into newly formed DNA as measure of blast cell transformation. They used strains of Neisseria gonorrhea CN. 890. CN 100 as antigen from freezed dried old laboratory cultures.

The result obtained were 12\14 or 85% of the patient had positive transformation. They concluded that there was a cell mediated immune respose in uncomplicated acute Nesseria gonococcal gentital infection. This investigation agrees with my studies. It is pertinent to point out that the number used was *small as regard to patients and controls and it did not measure lymphocyte subsets population (158).* Though this is a functional test. These two studies indicated that there is no decrease in the lymphocyte population in Neisseria gonorrhoea and the lymphocyte were capable of responding to stimulation by infection. Kearns, Seibert, O 'Reily et al in 1973, studied anti-gonococcal immune response of thirty-five men with uncomplicated gonorrhea to detect immune lymphocytes, serum and secretory antibodies. He concluded that there was lymphocyte blastogenesis induced by gonococcal antigens which was marginally greater in 11\35 or with first infectlion.

Their investigiation used small population of 35 men only and 24 controls; it used the up-take of I 126 labeled 5- Iodo-2- deoxyuridine (IDU), a thymidine analogue effector, namely lymphocytes, serum antibodies and secretion of IgA antibodies.

The above study is defective as it did not include women and the number of patients was rather small. But the result tend to agree with my study.

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With the availability of monoclonal antibody, this study investigated the lymphocyte sub-population response in Neisseria gonorrhoea genital infection; it arrived at the conclusion that there is increase in the lymphocyte sub-populations of CD3, CD8, CD19 compared to control subjects. The study agrees with the two studies above and also agrees with that of Rosenthal (1974) (191). Rosenthal (1974) investigate cell mediated immune response in gonococcal patients, 34 patients, consisting of 16 patients with post gonococcal arthritis, 4 patients with acute Reiters syndrome, 14 patients with chronic uro-arthritis and 34 patients as controls. Using commensal gonococcal antigen from National Swedish Bacteriological Laboratory, he used modified version of Soeborg-Bendixen for the determination of macrophage Inhibition Factor. For Lymphocyte Transformation (LT), 10 patients with post-gonococcal arthritis and 8 controls were used. Non-Neisseria bacterial antigens were used in these investigation. Neisseria gonorrhea of morphology T2 strains, Neisseria phargitis (APN) was used and purified protein derivative (PPD). Phytoheamagglutin - (PHA) was used a stimulant. Lymphocyte reactivity to different bacterial antigens and PHA was studied by measurement of Thymidine up-take, T2 14 C, and the radioactivity 25mc1\mM in cell cultures.

There was positive MIF in 21 of the 34 patients and in LT, there was 9 out of 10 positive responses. The number used in the study was small, the patients were of mixed infection, it did not investigiate lymphocyte subset populations, further research was neccessary in veiw of these inherent defects. The functional significance of the raised levels of CD3, CD8, CD19 is not clear in this disease. The CD4 cells function on class II MHC restricted T-cells and CD8 cells on class I MHC T-cells. There is increased avidity of cell-cell interactions by binding to monomorphic determinant on the appropriate HLA molecules. Thus CD4 and CD8 can synergize with CD3 in T-cell activation. In the study the CD4/CD8 was 1.3:1 and that of the control subjects was 1.6:1, thus there is tendency to reversal of the CD4/CD8 cell ratio in this disease. In this study there were reduction of CD4 lymphocyte subpopulations

in Neisseria gonorrhea, Chlamydia trachomatis genital infection, and chancroid genital ulcer. This reduction increased the tendency to decrease in the CD4\CD8 ratio, from 1.6;1 for control subjects to 1.3;1 for Neisseria gonorrhea; 1.2;1 for Chlamydia trachomatis genital infection; and 1.5 for chancroid genital ulcer. The reduction in the CD4\CD8 lymphocyte subset in these studied STD'S needs further investigation, to ascertain if they permanent features or temporary immunodeficiency.

In this study the lymphocyte subsets CD3, CD8 and CD19 were increased in genital Chlamydia trachomatis infection. It agrees with the two results mentioned above. This study also discovered that the CD4/CD8 cell ratio in patients with genital chlamydia was 1.2:1, compared to control of 1.6:1. Therefore, there is tendency to reversal of the CD4/CD8 ratio in genital chlamydia infection. In chlamydia genital infection, 12 patients or 24% of the patients CD4 cell fell below the control mean values. This may be due to the fact that chlamydia trachomatis is an intracellular organism. Though there is tendency to reversal of the CD4/CD8 ratio.

There are some studies on Haemophilus ducreyi but no studies have been conducted on the lymphocyte population, therefore, no comparative studies could be made (19,22-24,192). This study has confirmed that there is no depletion of the CD3, CD8, and CD19 lymphocyte subsets and there is tendency to inversion of the CD4/CD8 subsets of the lymphocyte in uncomplicated genital ulcer of Haemophilus ducreyi aetiology.

Brunham, Martin, Wang (1981) also investigated cellula immunity in Chlamydia trachomatis genital infection and ocular disease. They used the method of lymphocyte transformation method (LT). The number of patients include thirty-one men and seventy-six women with uncomplicated Chlamydlia genital infection. Their method consisted of the use of radioactive substances. They concluded that there was positive Lymphocyte Transformation(LT). Though number of patients was adequate, they did not investigate the number of absolute changes in the lymphocyte subsets.

Cell Mediated Immune Response in Neisseria gonorrhoea, Chlamydia trachomatis, Haemophilus ducreyi genital infections.

The broadening knowledge of cell mediated immunity, CMI, in several diseases has led to its importance in re-infection and immunity. Wyle and Rowlett (1977), Grimble and Mcilirray (1973), investigated cell mediated immunity in gonorrhoea (158,190). Wyle and Rowlett concluded that there is cell mediated immune response in gonorrhoea, although it seems to offer little or no protection for the patient. Grimble and Mcillmurray concluded that there is CMI which limited or prevents dissemination of gonococcal infection beyond primary genital mucous membrane sites and that the host defence against uncomplicated acute gonococcal genital infection needs further elucidation (158).

Rosenthal (1974) studied uncomplicated gonorrhoea urethritis and reported that there was positive lymphocyte transformation using lymphocyte stimulation. This is a functional test of cell mediated immunity.

There is a large body of information regarding antibody responses to chlamydia infection in humans (140,167), but apart from skin test results, virtually no data are available on human cell mediated immune reactions. Studies on delayed type hypersensitivity and other types of CMI responses have been reported from animal studies in which the natural course of chlamydia infection appears to be different from that encountered in humans. One such study is that from Pavia and Schacter, their results did not detect cell mediated cytotoxicity response in mice infected with Chlamydia trachomatis (192). Using lymphocyte transformation index (LTSI), Brunham, Martin et al (194), reported positive LTSI which declined significantly 3 to 4 weeks after curative therapy.

Hana and Schmidt (1979) reported positive lymphocyte stimulation by lymphocyte transformation in volunteers infected with genital Chlamydia trachomatis: that there was a narrow range of responses but there was great difference in the mean responses of different individuals (193). Hana & Schmidt (1979)

investigated cell mediayed immune response in Chlamydia traohomatis. Their method consisted of the use of 52 volunteers, the use of radia-active Cr. The method did not specify the number of men or women, even though the number was adequate. They did not investigate absolute number of lymphocyte subpopulations. The use of purified protcm Derivative is more accurate method of asssessment of cell mediated immune response then radio-active method . Hana & Schmidt concluded that there was a narrow range of responses in cell mediated immune responses but there was great difference in the mean responses of different individuals. Brunham and Martin in 1981 in their investigation concluded that there was positive lymphocyte transformation (LT) in thirty-one men and seventy-six women with uncomplicated Chlamydia trachomatis genital infection, their study included six newborn babies (194).

Infection by Chlamydia trachomatis affects the eye, the genitals, newborn babies. Ocular infection with chlamydia occurs in two distinct epidemiological situations. The most common is the classical eye to eye transmission. This infection is caused by chlamydia serotypes A to C, whereas for infection of the genital tract is due to serotypes D to K. Neonates born to mothers with cervical chlamydia infection become infected.

Early in 1979, Hana and Schmidt investigated human cell mediated immune responses in chlamydia trachomatis and concluded that it was a narrow response. When human volunteers were re-infected.with trachoma and inclusion conjunctivitis symptoms were usually less severe than at primary infection. Only partial immunity to homologous chlamydia strains has been observed, thus a high titre inoculum can overcome induced resistance. This study using Macrophage Migration Inhibition Factor test, found out that there is complete cell mediated immune response in chlamydia genital infection.

Schwarz in 1981 concluded in his review of chancroid that there is auto-inoculation in H. ducreyi genital infection and that there is no complete immunity to the disease re-infection being relatively common in the typical patient (196). Another study in primates did not find cell mediated immune

response (192). In the present study, the assessment of cell mediated immunity showed that there is cell mediated immunity in H. ducreyi, because 70% of the patients MIF were below the control mean value. Bacterial Killing Index (BKI) in Neisseria gonorrhoea, Chlamydia trachomatis, Haemophilus ducreyi genital infections.

The measurement of polymorph function or the Bacterial Killing Index in several infections has assumed more prominence in clinical diseases in recent years (196, 197). This is due to the discovery by Pos, Stevenhagen et al in 1992, that Acquired Immunodeficiency disease patients have decreased phagocytosis but normal killing of Staphylococcus aureus (198). This impaired the body defence or intracellular killing of other microorganisms such as Candida albicans. The microbactericidal function of neutrophils was assessed in Neisseria gonorrhoea genital disease, it was demonstrated that there is no decreased intracellular killing of Staphylococcus aureus in gonococcal infection compared with control subjects. This means that gonococcal urethritis/cervicitis patients have intact polymorph bactericidal ability to eliminate invading microorganisms.

King, James et al studied gonococcal interaction with polymorphonuclear neutrophils (PMN) and concluded that there was no decrease, that the percentage of Neisseria gonorrhoea attached to or ingested by neutrophils varies from 30% to 90% (199).

Polymorph function, as assessed by bacterial killing index, in chlamydia genital infection also showed there is no impairment in the polymorph function. Johnson, Osborn et al (1983) investigated the inactivation of Chlamydia trachomatis by normal human serum, by the use of unheated inactivated homologous serum (200). Their results showed that Chlamydia trachomatis was susceptible to inactivation by unheated human serum and that human serum possesses antichlamydial activity which is possibly mediated by antibody and complement. This literature was cited here to show how serum components could affect BKI.

Odumeru, Wiseman et al (1984) investigated the serum bactericidal activity of normal human serum phagocytosis and killing by polymorph leukocytes to strains 409, C149, and 3500 of H. ducreyi (177). They concluded that virulent strains were resistant to complement mediated lethal action of normal human and rabbit sera, whereas avirulent strains were susceptible. Virulent strains were relatively resistant to phagocytosis and killing by human polymorphonuclear leukocytes.

In this study, using the method of Philpls et al (1979), the bacterial killing index of these patients with genital ulcer caused by H. ducreyi was found not to be impaired. Therefore, there is no defect in the killing ability of polymorph leukocytes in these patients. This result agrees with that of Odumeru, Wiseman et al (1983). Resistance to bactericidal action of serum have been associated with virulence in several Gram-negative diseases, most notable as a marker for bacteriaemic spread (201). Haemophilus ducreyi does not produce bacteraemia, but resistance to bacteriolysis by normal serum may allow successful extracellular action of the organism within tissues. The intact bactericidal action of the polymorphonuclear leukocyte in human with genital ulcer due to H. ducreyi may be a factor in the limiting spread.

CONCLUSION:

1. This study showed that patients infected with the following STD'S namely Neisseria gonorrhea, Chlamydia trachomatis, genital infection, Heamophilus ducreyi genital ulcer have reduction in lymphocyte sub-population CD4 cells and no reduction in CD3; CD8; CD19 cells.

2. There is tendency to inversion of the CD4/CD8 ratio of these subsets of lymphocytes in this study.

3. There is tendency towards immunodeficiency associated with these studied STD'S.

4. There is protective acquired cell mediated response associated with chlamydia genital infection; while there is incomplete cell mediated immune response associated with Neisseria gonorrhea and Haemophilus ducreyi genital disease.

5. The bacteria killing index or the Polymorph function is not impaired or compromised in the patient infected by the studied STD'S.

The study illustrated the recognition of prompt treatment/management of the STD'S.

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APPENDIX

TABLE FOR THE DETERMINATION OF MPN

Most probable number table (derived from Halvorson and Ziegler, 1933)

Number of positive tubes at the stated dilution							
10^0	10^{-1}	10^{-2}	MPN	10^0	10^{-1}	10^{-2}	MPN
5	5	5	250	4	2	1	2.66
5	5	4	162	4	2	0	2.17
5	5	3	91.8	4	1	1	2.10
5	5	2	54.2	4	1	0	1.69
5	5	1	34.9	4	0	1	1.66
5	5	0	24.0	4	0	0	1.28
5	4	3	27.8	3	2	1	1.71
5	4	2	22.1	3	2	0	1.39
5	4	1	17.1	3	1	1	1.37
5	4	0	13.0	3	1	0	1.07
5	3	2	14.1	3	0	1	1.06
5	3	1	10.9	3	0	0	0.78
5	3	0	7.92	2	2	0	0.93
5	2	2	9.43	2	1	0	0.68
5	2	1	7.00	2	0	1	0.67
5	2	0	4.93	2	0	0	0.45
5	1	1	4.56	1	1	1	0.41
5	1	0	3.29	1	0	1	0.40
5	0	1	3.14	1	0	0	0.20

FACULTY OF MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA

Our Ref: F-MED/COMM. 19 Date: 16th June, 1995

Dr. R.N. Eni,
Immunology Unit
A.B.U. Teaching Hospital
Zaria

Dear Sir,

DECISION OF THE MEDICAL ETHICAL COMMITTEE

I write to inform you that at its meeting held on 24/5/95 the Medical Ethical Committee approved your research proposal titled: CELL-MEDIATED IMMUNE RESPONSE IN SEXUALLY TRANSMITTED DISEASE.

The following is the extract of the relevant minutes:

Project Number: ESC/95/00076

Yours faithfully,

Sgn

Dr. H.A. Aikhionbare
Chariman,
Medical Ethical Committee

N.B. Members of the Committee would like to have a progress report on your proposals in future.

APPENDIX

LYMPHOYTE SUBPOPULATIONS IN CONTROL SUBJECTS n=30 CD 3% Pan T cells

Patients	Total Wbc x 10^9	Lymphocyte %	Absolute no x 10^9	CD 3%	CD Absolute Nox10
1	4.2	30%	1.260	68	0.952
2	4.0	21%	0.840	61	0.512
3.	3.8	25	0.950	62	0.589
4	2.7	22	0.594	64	0.368
5.	4.2	29	1.218	63	0.767
6	3.8	34	1.292	67	0.865
7	4.0	19	1.517	59	0.448
8	3.1	32	0.992	63	0.624
9	2.3	24	0.552	63	0.347
10	4.3	26	1.118	61	0.681
11	4.2	25	1.050	63	0.661
12	4.3	23	0.989	66	0.652
13	5.4	32	1.728	62	1.071
14	4.7	34	1.598	63	1.006
15	4.7	42	1.974	61	1.204
16	5.2	39	2.028	64	1.297
17	4.2	27	1.134	65	0.737
18	4.4	24	1.056	64	0.675
19	5.1	44	2.244	62	1.391
20	5.7	29	1.653	66	1.090
21	2.9	30	0.870	67	0.582
22	3.1	29	0.899	66	0.593
23	2.1	24	0.504	67	0.337
24	5.1	27	1.377	65	0.895
25	4.1	25	1.025	63	0.645
26	4.5	45	2.085	66	1.336
27	3.7	30	1.110	67	0.743
28	4.4	44	1.936	64	1.239
29	3.7	29	1.073	60	0.643
30	5.8	34	1.972	66	1.301

Range ..... 59% - 68%

Mean .....$\bar{x}$ 63.93%

Standard Deviation SD 2.311

Standard Error Of MEAN ..... sem SEM 0.6526

APPENDIX

LYMPHOCYTE SUBPOPULATIONS IN CONTROL SUBJECTS n=30 CD 4

Patient	Total wbc x 10^9	Lymphocyte %	Absolute No x 10^9	CD 4%	CD Absolute N
1	4.8	34	1.632	35	0.057
2	4.0	21	0.840	37	0.310
3	4.8	34	1.632	35	0.057
4	2.7	22	0.595	40	0.237
5	4.2	29	1.218	40	0.487
6	3.8	34	1.292	39	0.503
7	4.8	34	1.632	35	0.057
8	3.1	32	0.992	37	0.367
9	2.3	24	0.552	36	0.198
10	4.3	23	0.989	38	0.375
11	4.2	25	1.050	40	0.420
12	4.3	23	0.989	38	0.375
13	4.8	34	1.632	35	0.057
14	4.7	34	1.598	40	0.639
15	4.7	42	1.974	36	0.710
16	5.2	39	2.028	41	0.831
17	4.2	27	1.134	36	0.408
18	4.4	24	1.056	40	0.422
19	4.8	34	1.632	35	0.057
20	5.7	29	1.653	39	0.644
21	2.9	30	0.870	38	0.330
22	3.1	29	0.899	36	0.323
23	4.8	34	1.632	35	0.052
24	5.1	27	1.377	36	0.495
25	4.1	25	1.025	39	0.399
26	4.5	45	2.025	36	0.729
27	3.7	30	1.110	40	0.444
28	5.1	34	1.734	37	0.641
29	3.7	29	1.073	36	0.386
30	5.8	34	1.972	39	0.769

Range ..... 35% - 41%

Mean ....$\bar{x}$ 37.43%

Standard Deviation 2.08

Standard Error Of Mean 0.38

APPENDIX

LYMPHOCYTE SUBPOPULATIONS IN CONTROL SUBJECTS r = 30 CD 8

Patients	Total Whc x 10^9	Lymphocyte %	Absolute No x 10^9	CD d %	Absolute No x 10^9
1.	4.4	45	2.025.	27	0.541
2.	4.1	37	1.517	19	0.288
3.	3.8	25	0.950	26	0.247
4.	2.7	22	0.594	23	0.136
5.	4.1	37	1.517	19	0.288
6.	2.8	34	1.4	23	0.297
7.	4.1	37	1.517	19	0.288
8.	3.1	32	0.992	21	0.208
9.	2.3	24	0.552	23	0.33
10.	4.3	26	1.4	21	0.32
11.	4.1	25	1.517	19	0.288
12.	4.3	23	0.989	24	0.237
13.	5.4	32	1.728	21	0.362
14.	4.1	25	1.517	19	0.288
15.	4.3	32	1.376	23	0.33
16.	4.1	25	1.517	19	0.288
17.	4.2	27	1.134	25	0.283
18.	4.1	25	1.517	19	0.288
19.	5.1	44	2.244	26	0.583
20.	5.7	29	1.653	23	0.380
21.	2.9	30	0.870	25	0.217
22.	3.1	29	0.899	23	0.206
23.	2.1	24	0.504	25	0.126
24.	5.1	27	1.377.	21	0.289
25.	4.1	25	1.512	19	0.288
26.	5.5	45	2.025	27	0.546
27.	3.7	30	1.11	23	0.255
28.	4.4	44	1.936	25	0.484
29.	4.1	25	1.517	19	0.288
30.	4.5	45	2.025	27	0.546

Range ..... 19% - 27%

Mean ..... $\bar{x}$ 22.43%

Standard Deviation SD 2.760%

Standard Error of Mean ... SEM ... 0.5039

APPENDIX

LYMPHOCYTE SUBPOPULATIONS IN CONTROL SUBJECT = CD 19

Patients	Total Wbc x 10^9	Lymphocytes	Absolute No x 10^9	CD 19%	Absolute No x 10^9
1.	4.2	30	1.260	22	0.378
2.	4.1	25	1.025	20	0.205
3.	3.8	25	0.950	18	0.171
4.	4.1	25	1.025	20	0.205
5.	4.4	32	1.376	23	0.33
6.	3.8	34	1.292	19	0.245
7.	4.1	37	1.025	20	0.205
8.	3.1	32	0.992	17	0.168
9.	2.3	24	0.552	18	0.099
10.	4.3	32	1.376	23	0.33
11.	4.2	25	1.050	22	0.231
12.	4.3	23	0.989	17	0.168
13.	4.3	32	1.376	23	0.33
14.	4.7	34	1.598	21	0.071
15	4.7	42	1.974	22	0.432
16.	5.2	39	2.028	18	0.238
17.	4.2	27	1.134	21	0.238
18.	4.4	26	1.376	23	0.33
19.	5.1	44	2.244	19	0.426
20.	5.7	29	1.653	21	0.347
21.	4.4	26	1.376	23	0.33
22.	4.1	25	1.025	20	0.205
23	2.1	24	0.504	19	0.507
24.	4.9	27	1.377	18	0.247
25.	3.7	25	1.025	20	0.205
26	1.4	45	2.025	19	0.387
27.	4.4	30	1.110	21	0.233
28.	5.8	44	1.936	18	0.348
29.	4.4	26	1.376	23	0.33
30.	5.8	34	1.972	21	0.414

Range ..... 17% - 23%

Mean ...$\bar{x}$ 20.30%

Standard Deviation SD..... 1.93

Standard Error of Mean SEM 0.0335

Patients	Total Wbc x 10^3/l	Lymphocyte %	Absolute No x 10^9	CD 3 %CD	Absolute No x 10^9
1	5.5	42	2.310	69	1.707
2	5.7	43	2.451	73	1.789
3	5.1	38	1.938	71	1.375
4	5.2	44	2.288	68	1.855
5	5.0	43	2.150	71	1.410
6	6.5	38	2.470	66	1.630
7	5.2	39	2.028	73	1.651
8	5.3	40	2.120	71	1.730
9	5.4	38	2.052	72	1.399
10	6.5	38	2.470	70	2.261
11	7.4	37	2.738	68	1.509
12	8.4	31	2.604	73	1.900
13	9.1	37	3.367	71	2.390
14	6.0	26	1.560	69	1.076
15	5.8	41	2.378	68	1.617
16	7.4	30	2.220	69	1.531
17	7.9	40	3.160	66	0.924
18	5.9	47	2.350	73	1.022
19	5.1	34	1.734	69	1.196
20.	5.9	37	2.183	73	1.593
21	6.1	30	1.830	70	1.281
22	7.9	42	3.180	72	2.388
23	5.8	36	2.088	67	2.3986
24	7.8	40	3.120	66	2.0592
25	6.2	44	2.728	73	1.991
26	7.7	42	3.234	69	2.231
27	6.1	39	2.379	67	1.593
28	7.9	31	2.449	71	1.738
29	5.5	35	1.970	72	1.346
30	4.8	34	1632	69	1.126
31	6.5	36	2.320	72	1.684
32	7.5	37	2.775	67	1.859
33	7.1	42	2.982	71	2.117
34	7.9	37	3.081	70	2.156
35	6.1	32	1.952	68	1.327
36	6.4	34	2.176	70	1.523
37	6.7	31	2.077	72	1.495
38	5.6	42	2.352	69	1.622
39	7.8	37	2.886	72	2.077

Patients	Total Wbc x 10^9	Lymphocyte %	Absolute No x 10^9	CD %	CD Absolute No x 10^9
40	7.4	39	2.886	71	2.049
41	6.9	41	2.829	73	2.065
42	5.9	35	2.065	69	1.342
43	6.5	36	2.340	68	1.591
44	7.4	32	2.360	71	1.681
45	6.3	41	2.658	68	1.812
46	6.7	39	2.613	69	1.802
47	7.1	36	2.556	72	1.840
48	6.9	65	4.485	70	3.139
49	6.5	36	2.340	66	1.544
50	6.4	42	2.688	70	1.881

Range ..... 66% - 73%

Mean ....$\bar{x}$..... 69.86%

Standard Deviation ..SD... 1.81

Standard Error Of Mean ...SEM.... ...0.60

LYMPHOCYTE SUBPOPULATIONS IN C. TRACHOMATIS URETHRITIS/

Patients	CERVICITIS n=38 Total Lymphocytes $\times 10^9/l$	Lymphocyte %	Absolute No $\times 10^9$	CD3%	Absolute No $\times 10^9$
1	5.2	38	1.9	65	1.15
2	6.1	38	2.470	70	1.722
3	4.2	41	2.173	72	1.564
4	6.1	44	2.684	66	1.771
5	5.4	32	1.728	72	1.244
6	5.0	40	2.000	69	1.750
7	7.3	46	3.628	71	1.865
8	6.8	49	3.344	70	2.340
9	6.2	51	3.160	65	1.305
10	4.2	57	2.723	71	1.983
11	7.1	30	2.130	66	1.405
12	7.5	36	2.700	72	1.944
13	7.3	37	1.271	70	1.379
14	4.1	31	1.324	67	0.933
15	6.1	42	2.562	71	1.819
16	6.7	37	2.472	69	1.710
17	7.1	40	2.840	71	2.0164
18	6.3	42	2.648	73	1.931
19	5.4	42	2.322	72	1.671
20	6.9	42	2.898	71	2.057
21	5.8	39	2.262	70	1.583
22	5.4	42	2.322	71	1.648
23	5.1	46	2.346	67	1.571
24	5.9	42	2.537	69	1.750
25	6.2	28	2.666	73	1.719
26	6.7	42	2.881	71	2.045
27	5.8	37	2.146	73	1.566
28	6.0	40	2.360	71	2.556
29	2.4	54	5.076	70	3.553
30	3.7	40	1.480	72	1.065
31	7.1	65	4.615	73	3.368
32	5.7	37	2.109	71	1.497
33	6.5	43	2.795	73	2.040
34	5.4	40	2.160	71	1.533
35	6.4	63	4.032	71	2.862
36	5.8	42	2.436	73	1.778
37	7.8	50	3.900	66	0.81
38	5.9	39	2.301	73	1.679

Patients	Total WBC x 10^3/l	Lymphocyte %	Absolute No x 10^9	CD3 %	Absolute No x 10^9
6.4	[illegible]	[illegible]	2.624	72	1.889
38	[illegible]	[illegible]	2.532	69	1.749
41	[illegible]	[illegible]	3.213	72	2.313
42	[illegible]	[illegible]	2.684	71	1.905
43	[illegible]	[illegible]	2.268	72	1.651
[illegible]	[illegible]	[illegible]	2.226	66	1.469
[illegible]	[illegible]	[illegible]	2.412	70	1.692
46	[illegible]	[illegible]	2.372	69	1.641
47	[illegible]	[illegible]	2.520	70	1.764
48	[illegible]	[illegible]	1.764	69	1.217
[illegible]	[illegible]	[illegible]	2.090	73	1.518
[illegible]	[illegible]	[illegible]	1.947	72	1.401

Range [illegible]

Mean [illegible]

Standard deviation [illegible]

Standard error of mean [illegible]0.324

ANNEXE

LYMPHOCYTE SUBPOPULATIONS CD 3 IN GENITAL ULCER DUE TO HEAMOPHILUS DUCREYI n=50

Patients	Total Wbc x 10^9/l	Lymphocyte %	Absolute No x 10^9	CD3 %	Absolute No x 10^9
1	3.1	30	0.930	67	0.623
2	4.5	37	1.665	68	1.132
3	5.4	41	2.214	69	1.527
4	5.1	35	1.785	66	1.178
5	6.5	46	2.990	71	2.122
6	7.2	39	2.808	67	1.881
7	6,8	41	2.788	70	1.951
8	6.1	40	2.440	60	1.464
9	7.4	36	2.664	69	1.838
10	6.4	32	2.048	72	1.474
11	5.-	43	2.322	68	1.578
12	6.9	36	2.196	61	1.515
13	6.7	38	2.546	71	1.807
14	7.1	34	2.414	68	1.641
15	6.0	32	1.920	60	1.152
16	7.3	41	2.993	70	2.095
17	6.9	40	2.760	72	1.987
18	7.2	45	3.240	71	2.300
19	6.5	43	2.795	67	1.872
20	5.7	30	1.710	60	1.026
21	4.8	46	1.748	66	1.153
22	8.3	37	3.071	68	2.088
23	5.3	38	2.014	65	1.309
24	6.8	40	2.720	61	1.659
25	5.9	34	2.006	66	1.323
26	4.2	36	1.548	65	0.070
27	6.1	40	2.440	60	1.464
28	6.5	39	2.535	67	1.698
29	5.4	29	1.566	63	0.986
30	5.6	31	1.736	71	1.232
31	6.1	33	2.013	68	1.368
32	3.4	35	1.190	71	0.844
33	4.9	37	1.813	61	1.105
34	6.0	35	2.100	64	1.344
35	6.2	41	2.542	70	1.779

APPENDIX

ients	Total Wbc x 10^9/l	Lymphocyte %	Absolute No x 10^9	CD/3 %	Absolute No x 10^9
36	6.7	37	2.479	62	1.536
37	5.9	36	2.124	65	1.380
38	5.2	38	1.976	66	1.304
39	6.5	41	2.665	69	1.838
40	6.1	34	2.074	65	1.348
41	4.1	41	1.681	62	1.042
42	4.7	29	1.363	67	0.913
43	6.7	37	2.479	69	1.710
44	6.1	27	1.647	65	1.070
45	7.2	38	2.736	69	1.887
46	5.9	29	1.711	66	1.129
47	5.0	31	1.550	69	1.069
48	5.4	30	1.62	70	1.134
49	6.1	33	2.013	70	1.429
50	7.1	34	2.414	66	1.593

Range ....60% — 72%

MEAN ..$\bar{x}$...66.68%

Standard Deviation SD 3.54

Standard Error Of Mean .. ...SEM....0.501

Patients	Total abs. x 10^9	Lymph cyte	Absolute No x 10^9	CD4%	Absolute No x 10^9
1	4.6	34	1.632	35	0.571
2	6.7	37	2.451	39	0.558
3	5.1	34	1.734	37	0.641
4	6.6	41	2.728	35	0.956
5	5.0	40	2.000	39	0.780
6	5.1	34	1.734	37	0.641
7	5.1	34	1.734	37	0.641
8	6.6	36	2.438	36	0.877
9	5.5	41	1.810	41	0.766
10	4.8	34	1.632	35	0.571
11	7.4	30	2.220	36	0.799
12	8.1	34	1.870	41	0.766
13	9.1	37	3.367	39	1.313
14	6.9	26	1.560	39	0.608
15	5.8	41	2.378	35	0.832
16	7.4	30	2.220	37	0.821
17	7.9	40	3.100	39	1.232
18	5.0	47	2.250	40	0.940
19	5.1	34	1.734	37	0.641
20	5.2	37	2.183	39	0.851
21	6.1	32	1.800	36	0.658
22	4.8	34	1.632	35	0.571
23	5.8	36	2.080	38	0.793
24	7.6	40	2.120	37	1.154
25	6.2	44	2.728	39	1.063
26	7.2	42	3.024	40	1.209
27	6.1	39	2.379	37	0.880
28	7.2	31	2.449	38	0.930
29	5.5	34	1.870	41	0.766
30	4.8	34	1.632	35	0.571
31	6.5	36	2.340	40	0.936
32	7.5	37	2.775	38	1.054
33	7.1	42	2.982	40	2.84
34	7.9	37	2.923	39	1.139
35	6.1	32	1.952	36	0.702
36	4.8	34	1.632	35	0.571
37	6.7	31	2.077	39	0.810
38	5.6	42	2.352	38	1.881
39	7.8	37	2.886	39	1.125

Patients	Total Lymphocyte x 10^9	Lymphocyte %	Absolute No x 10^9	CD 4%	Absolute NO x 10^9
40	7.4	38	2.886	37	1.067
41	6.9	41	2.829	39	0.560
43	5.7	41	2.065	36	0.743
43	6.5	36	2.340	35	0.819
44	7.4	32	2.368	39	0.923
45	5.2	34	1.810	41	0.766
46	6.7	32	2.613	37	0.966
47	7.1	36	2.556	39	0.996
48	6.9	65	4.485	37	1.659
49	6.5	36	2.340	40	0.936
50	6.4	42	2.688	38	1.021

Range.....35% - 65%

Mean.....x.....37.5%

Standard Deviation .....SD.....7.1

Standard Error of Mean.....S.E.M ...0.358

APPENDIX

[illegible] CHLAMYDIA TRACHOMATIS URETHRITIS/
[illegible] = 50

Patients	[illegible]	[illegible]	Absolute no x 10^2	CD4 %	Absolute No x 10^2
1	[illegible]	46	2.232	41	0.915
2	[illegible]	39	2.47	39	0.691
3	[illegible]	41	2.173	37	0.804
4	[illegible]	44	2.684	35	0.939
5	[illegible]	38	2.562	38	0.973
6	[illegible]	35	3.115	37	1.152
7	[illegible]	46	2.628	39	1.024
8	[illegible]	38	3.344	35	1.170
9	7.9	39	3.160	41	1.295
10	[illegible]	37	2.793	41	1.145
11	7.1	39	2.130	35	0.745
12	[illegible]	36	2.700	37	0.999
13	[illegible]	37	1.971	36	0.709
14	[illegible]	34	1.394	35	0.487
15	6.1	40	2.562	38	0.973
16	6.7	37	2.472	36	0.892
17	7.1	40	2.840	40	1.136
18	[illegible]	32	2.148	43	1.056
19	[illegible]	43	2.322	36	1.671
20	[illegible]	42	2.898	38	1.101
21	[illegible]	42	2.262	36	0.814
22	[illegible]	43	2.322	40	0.928
23	[illegible]	46	2.346	36	0.844
24	[illegible]	42	2.537	40	1.014
25	[illegible]	38	2.6660	38	0.895
26	[illegible]	43	2.881	37	2.479
27	[illegible]	37	2.146	36	0.772
28	[illegible]	40	2.369	35	0.826
29	[illegible]	64	4.0	31	0.443
30	[illegible]	40	1.48	39	0.577
31	[illegible]	65	4.615	35	1.661
32	[illegible]	37	2.1090	37	0.780
33	[illegible]	43	2.795	41	1.145
34	[illegible]	40	2.16	38	0.820
35	[illegible]	64	4.032	35	1.411

Patients	Total T cells /l	Eosinophils	Absolute Eo	ESR 1st hr	Absolute Eo x 10^9
36	[illegible]	4	[illegible]	30	0.050
37	7.2	18	[illegible]	38	1.326
38	5.0	18	[illegible]	30	0.897
39	5.4	41	.604	39	1.023
40	[illegible]	20	[illegible]	35	0.887
41	[illegible]	31	[illegible]	39	1.253
42	.1	11	[illegible]	36	0.966
43	[illegible]	20	[illegible]	40	0.904
44	[illegible]	42	[illegible]	30	0.869
45	[illegible]	41	[illegible]	41	0.991
46	[illegible]	39	[illegible]	37	0.880
47	[illegible]	45	[illegible]	35	0.882
48	4.0	30	1.764	37	0.652
49	[illegible]	40	[illegible]	39	0.811
50	[illegible]	34	1.037	34	0.661

Range .... 31'-45'

Mean .... $\bar{x}$ 37.14'

Standard Deviation (SD. [illegible]

Standard Error of Mean (SE) [illegible]

LYMPHOCYTE SUBPOPULATIONS CD4 IN GENITAL ULCER DUE TO HEAMOPHILUS DUCREYI n=50

Patients	Total Wbc x 10^9/l	Lymphocyte %	Absolute No x 10^9	CD 4 %	Absolute No x 10^9
1	5.1	30	0.930	37	0.344
2	4.5	37	1.665	39	0.649
3	5.4	41	2.214	36	0.797
4	5.1	35	1.785	41	0.731
5	6.5	46	2.990	35	0.570
6	7.2	39	2.808	37	1.038
7	6.6	43	2.788	39	1.087
8	6.1	40	2.440	40	0.976
9	7.4	36	2.664	37	0.985
10	6.4	32	2.048	42	0.860
11	5.4	43	2.322	37	0.859
12	6.9	36	2.196	40	0.993
13	6.7	38	2.546	43	1.094
14	7.1	34	2.414	36	0.869
15	6.0	32	1.920	35	0.072
16	7.3	41	2.993	39	1.167
17	6.9	40	2.760	41	1.131
18	6.1	37	2.489	38	0.913
19	6.5	43	2.795	42	1.173
20	5.7	30	1.710	38	0.649
21	3.8	46	1.748	43	0.751
22	8.3	37	3.071	38	1.166
23	5.3	38	2.014	39	0.785
24	6.8	40	2.72	35	1.088
25	5.3	34	2.000	43	0.862
26	4.3	36	1.548	36	0.557
27	6.1	40	2.440	39	0.951
28	6.5	39	2.535	40	1.014
29	5.4	29	1.566	36	0.563
30	5.6	31	1.736	35	0.6076
31	6.1	33	2.013	37	0.744
32	3.4	35	1.190	40	0.476
33	4.9	37	1.813	36	0.652
34	6.0	35	2.100	41	0.861
35	6.2	41	2.542	40	1.016

atients	Total Wbc x 10^9/l	Lymphocyte %	Absolute No x 10^9	CD4	% Absolute NO x 10^9
36	6.7	37	2.479	38	0.942
37	5.4	36	1.944	36	0.764
38	5.2	36	1.976	41	0.810
39	6.5	41	2.665	37	0.986
40	6.1	36	2.074	39	0.808
41	4.1	41	1.681	35	0.588
42	4.7	29	1.363	41	0.558
43	6.7	27	1.809	42	1.041
44	6.7	37	2.479	38	0.973
45	7.2	36	2.736	36	0.984
46	5.9	29	1.711	41	0.701
47	5.0	31	1.550	40	0.620
48	5.4	30	1.620	36	0.583
49	6.1	33	2.013	42	0.845
50	7.1	34	2.414	35	0.844

Range..... 35 -43

Mean ....$\bar{x}$....38.52

Standard Deviation ..SD....2.369

Standard Error Of MeAN ..SEM.... ...0.3350

APPENDIX

LYMPHOCYTE SUBPOPULATIONS CD8 IN GONOCOCCAL URETHRITIS/CERVICITIS (n=50)

Patients	Total Wbc x 10^9	Lymphocyte %	Absolute No x 10^9	CD 8%	Absolute N
1	5.5	45	2.475	28	0.693
2	5.7	43	2.451	30	0.735
3	5.1	38	1.938	30	0.581
4	6.2	44	2.728	27	0.736
5	5.0	40	2.000	28	0.560
6	6.5	38	2.470	27	0.666
7	5.8	39	2.262	33	0.746
8	5.3	46	2.438	30	0.731
9	5.4	36	1.944	31	0.948
10	8.5	38	3.230	31	1.001
11	7.4	30	2.220	28	0.621
12	8.4	31	2.604	32	0.833
13	9.1	37	3.367	28	0.942
14	6.0	26	1.560	26	0.405
15	7.8	37	2.088	29	0.836
16	7.4	30	2.220	27	0.599
17	7.9	40	3.160	28	0.884
18	5.0	47	2.035	32	0.752
19	5.1	34	1.734	27	0.468
20	5.9	37	2.183	30	0.654
21	6.1	30	1.830	32	0.585
22	7.9	42	3.318	33	1.094
23	7.8	37	2.088	29	0.836
24	7.8	40	3.120	27	0.842
25	6.2	44	2.728	30	0.818
26	7.8	37	2.088	29	1.836
27	6.1	39	2.379	27	0.642
28	7.9	31	2.449	30	0.734
29	5.5	34	1.870	31	0.797
30	7.8	37	2.088	29	0.836
31	6.5	36	2.340	31	0.725
32	7.3	37	2.775	30	0.832
33	7.1	42	2.982	27	0.805
34	7.9	37	2.923	27	0.818
35	6.1	32	1.952	31	0.605
36	6.4	34	2.176	30	0.652
37	7.8	37	2.886	29	0.836

38	5.6	42	2.356	27	0.635
39	7.8	37	2.886	29	0.836
40	6.0	26	1.560	26	0.405
41	6.9	41	2.829	30	0.848
42	7.8	37	2.088	29	0.836
43	6.5	36	2.340	32	0.748
44	7.8	37	2.088	29	0.836
45	6.5	41	2.665	27	0.719
46	6.0	36	1.560	26	0.405
47	6.0	36	1.560	26	0.405
48	6.9	65	4.485	28	0.720
49	6.5	36	2.340	27	0.613
50	6.4	42	2.688	28	0.652

Range ..... 26% - 33%

Mean ... $\bar{x}$ 29.04%

Standard Deviation SD 1.564

Standard Error Of Mean ..... S E M 0.22

ANEXO 4

LYMPHOCITS SUBPOPULATIONS CD 8 IN CHLAMYDIA TRACHOMATIS URETHRITIS/CERVICITIS n=50

Patients	Total Wbc x 10^9/l	Lymphocyte %	Absolute No x 10^9	CD 8 %	Absolute No x 10^9
1	6.2	36	2.232	33	0.736
2	6.5	38	2.470	28	0.691
3	5.3	41	2.173	31	0.673
4	6.7	44	2.684	29	0.854
5	5.4	32	1.728	30	0.518
6	8.9	35	3.115	32	0.996
7	7.3	36	2.628	30	0.832
8	8.8	38	3.344	32	1.070
9	6.8	49	3.332	34	1.132
10	4.9	57	2.793	26	0.726
11	6.7	44	2.948	29	0.854
12	7.5	36	2.700	26	0.512
13	7.3	27	1.971	33	0.650
14	4.1	34	1.394	32	0.446
15	6.7	44	2.562	29	0.854
16	6.7	44	2.479	29	0.854
17	7.1	40	2.840	27	0.766
18	6.3	42	2.648	26	0.687
19	5.4	43	2.322	32	0.743
20	6.9	42	2.898	30	0.869
21	5.8	39	2.262	32	0.723
22	5.4	43	2.322	28	0.650
23	5.1	46	2.346	27	0.633
24	6.7	44	2.537	29	0.854
25	6.2	38	2.537	33	0.777
26	6.7	43	2.881	30	0.864
27	5.8	37	2.146	28	0.600
28	5.9	40	2.036	27	0.637
29	9.4	54	5.076	32	1.624
30.	6.7	44	1.480	29	0.854
31.	7.1	65	4.615	28	1.292
32	5.7	37	2.109	30	0.632
33	6.5	43	2.795	28	0.782
34	6.7	44	2.160	29	0.854
35	6.4	63	4.032	32	1.290
36	5.8	42	2.436	31	0.755
37	7.8	50	3.900	28	1.092
38	5.9	39	2.301	32	0.736
39.	6.7	44	2.624	29	0.854

40	6.5	30	2.535	28	0.709
41	6.7	44	3.213	29	0.854
42	6.1	44	3.684	31	0.912
43	6.8	40	2.262	34	1.132
44	5.3	42	2.226	32	0.712
45	5.9	41	2.419	28	0.677
46	6.1	39	2.329	32	0.761
47	5.6	45	2.520	31	0.781
48	4.9	36	1.764	28	0.493
49	5.2	40	2.090	30	0.624
50	5.9	33	1.947	27	0.525

Range ..... 26% - 34%

Mean ..... $\bar{x}$ 29.90%

Standard Deviation 2.16

Standard Error Of Mean ... SEM ..... .3057

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ANNEXES

LYMPHOCYTE SUBPOPULATIONS CD 8 IN GENITAL ULCER DUE TO HEAMOPHILUS DUCREYI n=50

Patients	Total Wbc x 10^9/l	Lymphocyte %	Absolute No x 10^9	CD 8 %	Absolute No
1	3.1	30	0.930	27	0.251
2	4.5	37	1.665	25	0.649
3	5.4	41	2.214	29	0.416
4	5.1	35	1.785	22	0.392
5	6.5	46	2.990	32	0.956
6	7.2	39	2.808	26	0.730
7	6.8	41	2.788	27	0.752
8	4.1	25	1.517	19	0.280
9	7.4	36	2.664	28	0.745
10	6.4	32	2.048	23	0.471
11	5.4	43	2.322	27	0.626
12	6.9	36	2.196	20	0.496
13	6.7	38	2.546	24	0.600
14	7.1	34	2.414	28	0.675
15	6.0	32	1.902	21	0.403
16	7.3	41	2.993	29	0.867
17	6.9	40	2.760	27	0.745
18	7.2	45	3.024	29	0.939
19	6.5	43	2.795	23	0.642
20	5.7	30	1.710	20	0.342
21	3.8	46	1.748	21	0.367
22	8.3	37	3.071	30	0.921
23	5.3	38	2.014	23	0.463
24	6.8	40	2.072	24	0.610
25	4.1	25	1.517	19	0.288
26	4.3	36	1.548	27	0.417
27	6.1	40	2.440	19	0.463
28	6.5	39	2.535	22	0.557
29	5.4	29	1.566	23	0.360
30	5.6	31	1.736	28	0.486
31	6.1	33	2.013	25	0.503
32	3.4	35	1.190	27	0.321
33	4.9	37	1.813	20	0.321
34	6.0	35	2.100	21	0.441
35	6.2	41	2.542	24	0.503
36	6.7	37	2.479	20	0.495

37	5.2	36	2.124	25	0.764
38	5.2	38	1.976	21	0.414
39	6.5	41	2.665	28	0.746
40	6.1	34	2.074	22	0.456
41	4.1	36	1.476	24	0.354
42	4.7	29	1.363	21	0.286
43	4.1	36	1.476	24	0.495
44	6.1	27	1.647	23	0.378
45	7.2	38	2.736	31	0.848
46	5.2	29	1.711	21	0.359
47	5.0	31	1.550	25	0.387
48	5.4	30	1.620	29	0.469
49	6.1	33	2.013	25	0.503
50	7.1	34	2.414	29	0.700

Range ..... 19% - 42%

Mean .....$\bar{x}$ 24.38%

Standard Deviation SD 5.80

Standard Error Of Mean ... SEM..... 0.538

APPENDIX

LYMPHOCYTE SUBPOPULATIONS CD 19 IN GONOCOCCAL URETHRITIS/CERVICITIS n=50

Patients	Total Wbc x 10^9	Lymphocyte %	Absolute No x 10^9	CD19 %	Absolute No x 10^9
1	5.5	45	2.475	30	0.742
2	5.7	43	2.451	25	0.612
3	5.1	38	1.938	29	0.562
4	6.2	44	2.728	26	0.709
5	5.0	40	2.000	23	0.460
6	5.5	30	1.65	31	0.700
7	5.8	39	2.262	24	0.543
8	5.3	46	2.438	25	0.609
9	5.4	36	1.944	26	0.505
10	8.5	38	3.230	28	0.904
11	7.4	30	2.220	24	0.532
12	8.4	31	2.604	27	0.703
13	9.1	37	3.367	23	0.774
14	5.5	30	1.65	31	0.5115
15	5.8	41	2.378	29	0.689
16	7.4	30	2.320	28	0.616
17	7.9	40	3.160	27	0.853
18	5.0	47	2.350	30	0.705
19	5.1	34	1.734	29	0.502
20	4.5	31	1.395	22	0.600
21	6.1	30	1.830	26	0.475
22	7.9	42	3.318	24	0.796
23	5.8	36	2.088	27	0.561
24	7.8	40	3.120	30	0.936
25	6.2	44	2.728	25	0.682
26	7.2	42	3.024	23	0.695
27	6.1	39	2.379	27	0.642
28	7.9	31	2.449	28	0.685
29	5.5	30	1.65	31	0.5115
30	4.8	34	1.632	24	0.391
31	6.5	36	2.340	25	0.585
32	7.5	37	2.775	28	0.777
33	7.1	42	2.982	27	0.461
34	4.5	31	1.395	22	0.3069

Patients	Total Wbc x 10^9/l	Lymphocyte %	Absolute No x 10^9	CD1?%	Absolute No x 10^9
35	6.1	[illegible]	1.952	24	0.466
36	6.4	34	2.176	30	0.652
37	6.7	31	2.077	29	0.602
38	5.6	40	2.352	25	0.588
39	7.8	37	2.886	33	0.95[illegible]
40	7.4	29	2.086	26	0.[illegible]
41	6.5	40	2.829	26	0.735
42	5.2	32	2.065	25	0.516
43	6.5	36	2,340	32	0.5388
44	7.4	32	2.368	24	0.568
45	6.5	41	2.665	23	0.613
46	6.7	39	2.613	28	0.731
47	4.5	31	1.395	22	0.3069
48	6.9	65	4.485	27	1.210
49	6.5	36	2.340	30	0.702
50	4.5	31	1.65	31	0.511[illegible]

Range.....[illegible] - 31%

Mean.....$\bar{X}$ 26.52%

Standard Deviation ..SD..?.857

Standard Error of Mean...S.E.M....0.40.

[illegible] OF [illegible] TRACHOMATIS URETHRITIS/

[illegible] 6-50

Patients	Total WBC x 10^3/-	Lymphocyte	Absolute No x 10^2	CD12%	Absolute No x 10^9
1	5.2	40	2.898	29	0.840
2	6.1	39	2.470	34	1.832
3	5.2	33	2.173	33	1.717
4	6.1	41	2.684	30	1.505
5	7.8	44	3.308	46	1.219
6	5.3	22	3.115	30	1.148
7	7.1	36	2.628	28	1.128
8	6.8	38	3.344	32	1.070
9	7.2	40	3.160	30	1.148
10	4.3	57	2.793	27	0.754
11	7.1	30	2.130	30	0.639
12	7.5	36	2.700	28	0.755
13	7.1	27	1.971	27	0.532
14	4.1	34	1.394	26	0.362
15	6.1	42	2.562	32	0.819
16	6.7	37	2.476	33	0.866
17	6.9	42	2.898	29	0.840
18	6.7	42	2.648	30	1.128
19	5.4	43	2.322	32	1.248
20	6.9	42	2.898	28	1.028
21	5.8	39	2.262	31	0.701
22	5.4	43	2.322	28	1.128
23	5.1	46	2.346	32	1.550
24	5.9	43	2.537	27	0.684
25	6.3	28	2.666	24	0.565
26	6.7	43	2.881	25	0.720
27	5.8	37	2.146	26	0.557
28	5.9	40	2.898	29	0.840
29	9.4	54	5.076	34	1.725
30	3.7	40	1.480	27	0.399
31	6.9	42	1.615	29	0.840
32	5.7	37	2.109	33	1.695
33	6.5	43	2.795	28	1.028
34	5.4	40	2.160	25	0.540
35	6.4	63	4.032	28	1.128
36	5.8	42	2.436	27	0.657
37	7.8	50	3.900	32	1.248

Patients	Total Wbc x 10^3/*	Lymphocyte %	Absolute No x 10^9	CD19%	Absolute No x 10^9/*
30	5.2	39	2.301	31	0.713
32	6.4	41	2.624	33	0.865
39	6.5	39	2.535	30	0.760
41	6.3	51	3.213	32	1.028
42	6.1	44	2.684	26	0.697
43	6.2	47	2.828	29	0.840
34	2.2	42	2.226	31	0.690
45	5.2	41	2.419	34	0.822
46	6.1	39	2.379	25	0.594
47	5.6	45	2.520	34	0.856
48	4.2	36	1.764	27	0.476
49	6.2	47	2.898	29	0.84
50	5.9	33	1.947	33	0.642

Range.....32 - 51

Mean..$\bar{x}$.....3.487

Standard Deviation .....σ.....1.924

Standard Error of Mean.....S.E.M. ....0.4136.

APPENDIX

LYMPHOCYTE SUBPOPULATIONS IN GENITAL ULCER DUE TO HEAMOPHILUS

SUDREY I n=50

Patients	Total WBC x 10^9/l	Lymphocyte %	Absolute No x 10^9	CD19 %	Absolute No x 10^9
1	5.1	30	0.930	27	0.251
2	4.5	37	1.665	25	0.416
3	5.1	11	2.214	22	0.642
4	5.1	22	1.785	24	0.428
5	7.2	31	2.220	26	0.777
6	7.2	22	2.847	30	0.854
7	6.8	41	2.788	29	0.808
8	6.1	40	2.440	22	0.536
9	4.5	37	1.665	25	0.416
10	5.9	28	1.652	21	0.416
11	5.4	43	2.322	26	0.603
12	6.2	36	2.196	23	0.571
13	6.7	38	2.546	26	0.661
14	7.1	34	2.414	28	0.675
15	6.2	32	1.920	26	0.499
16	7.2	41	2.293	24	0.718
17	6.2	40	2.760	22	0.607
18	5.2	28	1.652	21	0.348
19	6.5	43	2.795	24	0.670
20	5.7	30	1.710	29	0.495
21	6.5	32	2.842	30	0.854
22	5.9	28	1.652	21	0.346
23	5.3	28	2.014	29	0.540
24	7.3	39	2.847	30	0.854
25	5.2	24	2.006	26	0.521
26	4.3	36	1.548	23	0.356
27	6.1	40	2.440	22	0.530
28	6.5	32	2.535	26	0.659
29	5.4	22	1.566	21	0.600
30	4.5	37	1.665	25	0.416
31	6.1	33	2.013	24	0.483
32	3.4	35	1.190	25	0.297
33	4.9	37	1.813	23	0.416
34	5.2	28	1.652	21	0.346
35	6.2	41	2.542	30	0.662

APPENDIX

Patients	Total Abs. x 10^9/l	Lymphocytes%	Absolute No. x 10^9	CD19 %	Absolute No x 10^9
36	6.3	37	2.472	24	0.524
37	7.3	42	3.124	28	0.594
38	4.7	28	1.276	27	0.533
39	6.1	41	2.602	26	0.622
40	6.1	34	2.074	22	0.456
41	4.1	41	1.681	24	0.403
42	4.5	37	1.665	30	0.854
43	6.7	37	2.479	29	0.718
44	6.1	27	1.647	27	0.444
45	7.2	38	2.736	28	0.766
46	5.2	28	1.652	21	0.34692
47	5.0	31	1.550	28	0.434
48	4.5	37	1.665	25	0.416
49	6.1	33	2.013	29	0.583
50	7.1	34	2.414	28	0.678

Range.....20 - 36

Mean...$\bar{x}$.....25.50

Standard Deviation.....7.2

Standard Error of Mean.....[illegible]

APPENDIX

MACROPHAGE MIGRATION INHIBITION FACTOR (MIF) TEST IN CONTROL SUBJECTS n=30

Control Subjects	M I F Test
1	0.81
2	0.62
3	0.67
4	0.72
5	0.72
6	0.81
7	0.81
8	0.85
9	0.79
10	0.60
11	0.58
12	0.58
13	0.69
14	0.50
15	0.75
16	0.51
17	0.48
18	0.65
19	0.57
20	0.81
21	0.44
22	0.90
23	0.43
24	0.64
25	0.75

APPENDIX

Control Subjects	M I F Test
26	0.84
27	0.56
28	0.93
29	0.52
30	0.79

Range ..... 0.61 — 0.93

Mean ..$\overline{x}$ 0.67

Standard Deviation Sb 0.181

Standard Error Mean S e M .. 0.0335

APPENDIX

MACROPHAGE MIGRATION INHIBITION FACTOR (MIF) TEST IN GONOCOCCAL PATIENTS n=50

Patients	M I F Tests
1	0.92
2	0.89
3	0.84
4	0.79
5	0.69
6	0.77
7	0.90
8	0.76
9	0.67
10	0.81
11	0.72
12	0.88
13	0.77
14	0.79
15	0.80
16	0.75
17	0.76
18	0.81
19	0.57
20	0.71
21	0.56
22	0.76
23	0.81
24	0.75
25.	0.61
26	0.60
27	0.64
28	0.69
29	0.71

APPENDIX

Patients	M I F Test
30	0.71
31	0.66
32	0.72
33	0.70
34	0.77
35	0.59
36	0.76
37	0.59
38	0.65
39	0.76
40	0.73
41	0.54
42	0.52
43	0.63
44	0.59
45	0.57
46	0.63
47	0.87
48	0.91
49	0.71
50	0.63

Range ... ... 0.52 — 0.92

Mean $\bar{x}$ 0.71

Standard Deviation 0.117

Standard Error Of MEAN ...(SEM) ...0.0107

APPENDIX

MACROPHAGE MIGRATION INHIBITION FACTOR (MIF) TEST IN CHLAMYDIA PATIENTS - n=50

Patients	M I F Test
1	0.54
2	0.43
3	0.37
4	0.39
5	0.42
6	0.36
7	0.41
8	0.57
9	0.57
10	0.36
11	0.52
12	0.47
13	0.36
14	0.39
15	0.49
16	0.41
17	0.50
18	0.53
19	0.47
20	0.35
21	0.56
22	0.45
23	0.49
24	0.39
25	0.36
26	0.51
27	0.54
28	0.50
29	0.34

Patients	M I F Tests
30	0.46
31	0.56
32	0.48
33	0.45
34	0.58
35	0.55
36	0.50
37	0.47
38	0.42
39	0.37
40	0.51
41	0.53
42	0.47
43	0.49
44	0.35
45	0.39
46	0.56
47	0.51
48	0.45
49	0.39
50	0.41

Range .....0.34 — 0.57

Mean $\bar{x}$ 0.4476

Standand Deviation 0.08617

Standard Error Of Mean 0.01218

CTERIAL KILLING INDEX (POLYMORPH FUNCTION) BKI IN CONTROL SUBJECTS n=39

Control Subjects	B K I Mean
1	0.04
2	0.02
3	0.04
4	0.05
5	0.02
6	0.01
7	0.07
8	0.09
9	0.07
10	0.10
11	0.06
12	0.07
13	0.04
14	0.04
15	0.09
16	0.05
17	0.07
18	0.03
19	0.01
20	0.06
21	0.08
22	0.05
23	0.07
24	0.05
25	0.08

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Control Subjects	B K I Test
26	0.06
27	0.08
28	0.07
29	0.04
30	0.05

Range ..... 0.01 —— 0.10

Mean $\bar{x}$ 0.04

Standard Deviation SD 0.029

Standard Error Of Mean S E M 0.0053

BACTERIAL KILLING INDEX (POLYMORPH FUNCTION) BKI IN CONTROL SUBJECTS n=30

Control Subjects	BKI Test
1	0.03
2	0.02
3	0.04
4	0.05
5	0.09
6	0.04
7	0.07
8	0.09
9	0.07
10	0.10
11	0.06
12	0.07
13	0.04
14	0.04
15	0.09
16	0.05
17	0.07
18	0.04
19	0.01
20	0.06
21	0.08
22	0.05
23	0.07
24	0.05
25	0.08

Control Subjects	B K I Test
26	0.06
27	0.06
28	0.07
29	0.01
30	0.05

Range ..... 0.01 — 0.10

Mean $\bar{x}$ 0.04

Standard Deviation SD 0.029

Standard Error Of Mean S E M 0.0053

APPENDIX

BACTERIAL KILLING INDEX (POLYMORPH FUNCTION) BKI IN GONOCOCCAL PATIENTS n=50

Patients	B K I Test
1	0.01
2	0.07
3	0.04
4	0.01
5	0.10
6	0.02
7	0.03
8	0.07
9	0.03
10	0.04
11	0.03
12	0.05
13	0.07
14	0.01
15	0.08
16	0.05
17	0.10
18	0.06
19	0.07
20	0.09
21	0.01
22	0.10
23	0.07
24	0.06
25	0.04
26	0.10
27	0.09
28	0.01
29	0.05
30	0.04

Patients	B K I Test
31	0.05
32	0.06
33	0.06
34	0.01
35	0.04
36	0.03
37	0.03
38	0.03
39	0.05
40	0.06
41	0.07
42	0.04
43	0.07
44	0.06
45	0.10
46	0.08
47	0.01
48	0.03
49	0.10
50	0.04

Range..... 0.01— 0.10

Mean $\bar{x}$ 0.05

Standard Deviation S D 0.025

Standard Error Of Mean.....0.0038

APPENDIX

BACTERIAL KILLING INDEX (POLYMORPH FUNCTION) BKI IN CHLAMYDIA PATIENTS n=50

Patients	B K I Tests
1	0.01
2	0.07
3	0.05
4	0.10
5	0.05
6	0.07
7	0.01
8	0.04
9	0.07
10	0.01
11	0.06
12	0.03
13	0.09
14	0.01
15	0.06
16	0.07
17	0.05
18	0.06
19	0.07
20	0.06
21	0.05
22	0.07
23	0.01
24	0.04
25	0.10
26	0.01

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Patients	S K I Test
27	0.06
28	0.07
29	0.05
30	0.06
31	0.01
32	0.0[illegible]
33	0.0[illegible]
34	0.05
35	0.04
36	0.04
37	0.09
38	0.08
39	0.07
40	0.05
41	0.07
42	0.05
43	0.10
44	0.04
45	0.04
46	0.06
47	0.08
48	0.09
49	0.02
50	0.04

nge.....0.01-0.10

Mean $\bar{x}$ 0.0558

tandard Deviation SD ... 0.029

ndard Error Of Mean SEM ...0.0042

RIAL KILLING INDEX (POLYMORPH REACTION) PRI IN GENITAL ULCER

ES TO HEAMOPHILUS DUCREYI n = 50

Patients	b & 1 Test
1	0.04
2	0.02
3	0.15
4	0.01
5	0.05
6	0.10
7	0.07
8	0.03
9	0.05
10	0.07
11	0.05
12	0.03
13	0.02
14	0.01
15	0.02
16	0.05
17	0.08
18	0.06
19	0.10
20	0.07
21	0.03
22	0.05
23	0.09
24	0.10
25	0.01

Patients	B K I Test
26	0.07
27	0.05
28	0.07
29	0.01
30	0.09
31	0.07
32	0.08
33	0.07
34	0.06
35	0.01
36	0.09
37	0.07
38	0.08
39	0.01
40	0.09
41	0.05
42	0.07
43	0.01
44	0.07
45	0.09
46	0.04
47	0.01
48	0.07
49	0.09
50	0.08

Range .....0.01—0.10

Mean .....$\bar{x}$....0.0576

Standard Deviation SD....0.0288

Standard Error Of Mean ....SEM ...0.04076