NEUTROPHIL TOXIC GRANULATION IN PREGNANCY IN ONITSHA

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APRIL 2010
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IN ONITSHA

A DISSERTATION PRESENTED TO THE UNIVERSITY OF NIGERIA, FOR THE DEGREE OF MASTER OF SCIENCE

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To my husband, Mr Benjamin, Mootol Ezigbo and our children - Amarchukwu, Oluoma, Toochukwu and Chukwuagoziem
ACKNOWLEDGEMENT

I wish to acknowledge my sincerest indebtedness and gratitude to all whom in one way or the other contributed to the completion of this work.

First my appreciation goes to God, who in His infinite mercy made His grace abundant for me.

My profound appreciation and gratitude go to Ven.Prof. E.O.Ukaejiofo, my able supervisor, for showing me the way forward, I am also grateful to all my lecturers in the Department for their advice.

I want to thank Dr P.N.Nwagbara Consultant Obstetrician and Gynaecologist/Chief Medical Director Shalom Foundation Specialist Hospital and Maternity Onitsha and the then HOD Department of Obstetrics and Gynaecology, General Hospital Onitsha, for allowing his patients to participate in this research, providing his ideas and personal journals. Dr Valentine Obidi, of the Department of Obstetrics and Gynaecology, General Hospital, Onitsha for his assistance in providing the patients files.

This acknowledgement will not be complete without placing on record the generous assistance received from my colleagues Mrs. Nwagu N. of General Hospital, Onitsha, Mr. Festus C. Emengaha, Chemical Pathology Department, St. Charles Borromeo Hospital and Mr. Akagozirim Murphy then Chief Scientist New Hope Medical Diagnostic Centre, Onitsha.

For the enthusiastic support in providing some of the materials I used, I wish to thank Mrs. Nosike, O.K, then Circulation Librarian Medical Library University of Nigeria Enugu Campus (UNEC) and all the staff of Nnamdi Azikiwe University Teaching Hospital (NAUTH) Library Nnewi. I am indebted to Mrs.Akanya Nkoyo.B, of the Department of Pharmacology and Therapeutics UNEC, for the statistical analysis of this work.
My warmest appreciation to my very dear husband Mr. Benjamin M. Ezigbo, you pushed me to start, to continue and provided for everything I needed to accomplish this work, favour will always go with you.

A million thanks goes to my lovely parents Mr. and Mrs. Christian A. Anyadike for the foundation they laid.

May God bless you all Amen.

EZIGBO, EYIUCHE DORIS.

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ABSTRACT
Screening for asymptomatic bacteriuria does remain relevant in improving pregnancy outcome but inexpensive and effective techniques are required. The objective of this
study was to evaluate the white cell morphology in pregnant women with urinary tract infection (UTI) and study their diagnostic utility. Three hundred and eighty one (381) subjects were studied. This comprised: Three hundred and twenty three (323) pregnant subjects and Fifty eight (58) fibroid cases. One hundred (100) subjects matched for age were used as control. The pregnant women were those attending General Hospital Onitsha and Shalom Foundation Specialist Hospital Onitsha for routine antenatal serological testing. Toxic neutrophil count (TNC), Neutrophil alkaline phosphatase assay, total white blood cell count (TWBC) and urine cultures were carried out. Conventional microscopy was used for TNC and TWBC. Neutrophil alkaline phosphatase activity was analysed using Refloctron Plus System by Roche. Statistical analysis was performed with SPSS software using student’s t-test and the sensitivity and specificity of various TNC cut offs were computed. In the presence of pregnancy and infection NAP activity correlated positively with TNC. At a cut off of 10 in TNC a specificity of 79% and a sensitivity of 18% were achieved. With a cut off of 70 specificity was 97% and sensitivity was 0%. TNC although a specific indicator for infection does not serve as a sensitive screening procedure in pregnant women with UTI.
CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY:

Several of the changes seen in neutrophils from pregnant women, including neutrophilia, increased staining of granules and alteration of neutrophil alkaline phosphatase activity are similar to those seen in infections in pregnancy and in patients with myeloid metaplasia (EL-Maalem and Fletcher, 1976). Thus the same pattern of changes occurs in a number of different conditions suggesting a common underlying mechanism.

In infections the circulating neutrophils are already partially exhausted by previous ingestion of opsonised particles (antigen-antibody complexes). In normal pregnancy there are circulating immune complexes of a type which should be ingested by neutrophils (EL-Maalem and Fletcher, 1980).
1.2 Research problem

Despite numerous studies over many years, the issues relating to the prevention and management of asymptomatic bacteriuria and UTI during pregnancy remain unresolved.

Routine cultures for the diagnosis of asymptomatic bacteriuria and UTI are expensive and both false positive and false negative results can occur.

Screening of asymptomatic bacteriuria does remain relevant in improving pregnancy outcome (Akerele, et al 2001), however inexpensive and effective techniques are required.

1.3 Hypothesis:

Neutrophil toxic granulation is the prominent staining by Romanowsky dyes of cytoplasmic granules –as a result of the abnormal maturation of azurophilic granules with persistence of acid mucosubstance.

In the normal uninfected neutrophil these granules are fine and evenly distributed. During infections and pregnancy the granules become more prominent and are unevenly distributed.
1.4 **Significance of study**

The incidence of UTI in pregnancy can be as high as 6.2% in pregnant women (Ezechi, *et al* 2003).

Acute pylonephritis occurs during pregnancy and more commonly in women, who have had asymptomatic bacteriuria.

Prematurity and low birth weights are common conditions associated with untreated asymptomatic bacteriuria.

Some pregnant women with symptoms of UTI who had negative culture results still respond to antibiotic therapy.
1.5 AIMS OF STUDY

This project is aimed at developing a cost effective and sensitive method of screening for UTI in pregnancy, and to compare toxic granulation in pregnancy with those in other disease conditions. Hence, attempt would be made in this research work to:

- Carry out toxic Neutrophil count in pregnant women in Onitsha
- Assay Neutrophil Alkaline phosphatase activity in pregnant women in Onitsha
- Correlate toxic Neutrophil count with Neutrophil alkaline phosphatase activity.
- Use toxic Neutrophil count as an index in screening for UTI in pregnancy.
CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 The Pregnancy State: Pregnancy constitutes a special condition in a woman’s life that affects various physiologic and endocrinologic systems.

2.1.2 Physiological Changes during Pregnancy: The physiologic, biochemical and anatomic changes that occur during pregnancy are extensive and may be systemic or local.

Hormonal changes: The large amounts of oestrogen, progesterone, human placental lactogen, and corticosteroids produced during pregnancy affect various metabolic, physiologic and endocrine systems. The secretion of estrogens and progesterone throughout pregnancy assures appropriate development of the endometrium, uterine growth, adequate uterine blood supply, and preparation of uterus for onset of labour.

2.1.3 The Kidneys and Urinary Tract

Renal Dilatation: During pregnancy, each kidney increases in length by 1-1.5cm with a concomitant increase in weight (Delzell et al, 2000). The renal pelvis is dilated. The ureters are dilated above the brain of the bony pelvis. The
ureters also elongate, widen and become more curved. Thus there is an increase in urinary stasis. This may lead to infection.

The absolute cause of hydronephrosis and hydroureter in pregnancy is unknown and there may be several contributing factors:

- Elevated progesterone levels may contribute to hypotonia of the smooth muscles in the ureter.
- The ovarian vein complex in the suspensory ligament of the ovary may enlarge enough to compress the ureter at the brim of the bony pelvis, thus causing dilation above the level.
- Dextorotation of the uretus during pregnancy may explain why the right ureter is usually more dilated than the left.
- Hyperplasia of smooth muscles in distal one-third of the ureter may cause reduction in the luminal size (Wing et al, 2000).

**Renal Function:** The glomerular filtration rate (GFR) increases during pregnancy by about 50%. The renal plasma flow rate increases by as much as 25-50%. Urinary flow and sodium excretion rate in late pregnancy can be altered by posture, being twice as great in the lateral recumbent position as in the supine position. Even though the GFR increased dramatically during pregnancy, the volume of urine passed each day is not increased. Thus, the urinary system appears to be even more efficient during pregnancy. With the increase in GFR, there is an increase in endogenous clearance of creatinine.
The concentration of creatinine in serum is reduced in proportion to increase in GFR, and concentration of blood urea nitrogen is similarly reduced.

Glucosuria during pregnancy is not necessarily abnormal, and may be explained by the increase in GFR with impairment of tubular reabsorption capacity for filtered glucose. Increased levels of urinary glucose also contribute to increased susceptibility of pregnant women to urinary tract infection.

Proteinuria changes little during pregnancy and if more than 500mg/24 hr is lost, a decrease process should be suspected.

Levels of the enzyme rennin, which is produced in the kidney, increase early in the first trimester, and continue to rise until term. This enzyme acts on its substrate angiotensinogen, to first form angiotensin 1 and then angiotensin 2, which acts as a vasoconstrictor.

**The Bladder:** As the uterus enlarges, the urinary bladder is displaced upward and flattened in the anterior-posterior or diameter. Pressure from the uterus leads to increase in urinary frequency. Bladder vascularity increase and muscle tone decreases, increase in capacity up to 1500ml (Babior and Golde, 2001).

**2.1.4 Haematologic Changes:** Maternal blood volume increases during pregnancy by an average of 45%. Plasma volume increases more rapidly than red cell mass, therefore, in spite of augmented erythropoiesis, the
concentration of haemoglobin, the erythrocyte count, and the haematocrit commonly decrease during normal pregnancy. Haemoglobin concentration at term average 12.1 g/dl compared with 12.8 g/dl (Ukaejiofo et al, 1999), for the non pregnant state. The total white blood cell count increases during pregnancy from a pre-pregnancy level of $4.3 - 4.5 \times 10^9/L$ to $5 - 12 \times 10^9/L$ in the last trimester (Bainton, 2001) although counts as high as $16 \times 10^9/L$ have been observed in the last trimester. Lymphocyte and monocyte numbers stay essentially the same throughout pregnancy (Brambald et al, 1997). Polymorphonuclaeer leucocytes are the primary contributors to the increase (Brambald et al, 1997).

2.2 THE NEUTROPHILS

Neutrophils are granulocytes, which form an essential component of the cellular innate system involved in killing bacteria and fungi. They play critical role in host defence by phagocytising and digesting micro-organisms. Neutrophils are so named because of their neutral staining with Wright’ Stain. They are also known as PMNs or Polys or microphages. They are round cells approximately 12-14µm in diameter. The multilobed nucleus contributes to the extreme elasticity of the cell, which is of importance for the cell to make rapid transit from the blood through tight gaps in the endothelium. In the resting uninfected host, the production and elimination of neutrophils are
balanced, resulting in fairly constant concentration of neutrophils in peripheral blood. When infection occurs, chemotactic agents are generated, that result in migration of neutrophils to the site of the infection and activation of neutrophil defensive function. Cytoplasmic modifications that occur include:

- Alteration of the staining character of the cytoplasm.
- Abnormal granulation.
- Vacuolization (Meranze et al, 1935).

### 2.2.1 Life History

The normal human neutrophil production rate is 0.85 to 1.6 x 10^{9/L} cells per kilogram per day (Babior et al, 2001). Neutrophils are abundant in the circulation, present at concentration of 2 x 10^{9/L} to 7 x 10^{9/L} and equal numbers are margined on vessel walls or sequestered in closed capillaries. The half-life in the blood is 6-7 hrs and in the tissue is about 1-4 days. The blood and bone marrow form an abundant pool of cells. In the bone marrow, the myeloid precursor cells mature to segmented neutrophils in about 9 days.
Neutrophils are activated by numerous stimuli; some of the most described molecules involved include the complement component C5a, LTB₄, FMLP, and interleukin-8 (IL-8). As many as 25-50 particles (bacteria) can be engulfed by a single cell. After emigration to the tissues, they never return to the bloodstream. They are probably disposed off internally by cells of the reticuloendothelial system or externally e.g. loss into the gastrointestinal tract through mucosal surfaces (Babior et al, 2001).

**2.2.2 SUBCELLULAR STRUCTURE OF NEUTROPHILS**

Four well defined types of granules have been defined in neutrophils, which are azurophilic (primary) granules, specific (secondary) granules, gelatinase (Tertiary) granules, and secretory vesicles. Under the electron microscope, the granules are seen to consist of a finely granular matrix bounded by a typical membrane. The granules have been shown to be quite heterogeneous with some rounded forms, some in the shape of grain of rice or small dumbles sand their
number has been shown to vary from 500 – 1500/granulocyte (Rashmi et al, 2006).

Granules contain large amount of protein and traces of lipids and nucleic acids. These granules are essential for post phagocytic function of PMN leucocytes. Their constituents are essential in inflammation and are determinants of intraleucocytic antimicrobial events.

Among the azurophilic contents are myeloperoxidase, defensins lysozyme, azurocidin, etc. that have antibacterial function. These granules fuse with phagocytes vesicles, resulting in the delivery of their contents to the ingested organism. The greenish coloration of pus is imparted by myeloperoxidase (Rashmi et al, 2006). Specific granules are three times more common in the cytoplasm. Release of specific granule contents like collagenase, apolactoferrin lysozyme, histaminase, etc may modify inflammatory process. Collagenase and elastase breakdown fibrous structures in the extracellular matrix, facilitating progress of the neutrophil through tissue. The tertiary granules content include gelatinase, alkaline phosphatase and CD11b/CD18 (Smolen and Boxer, 2001).

2.2.3 Neutrophil Alkaline Phosphatase (NAP)

The NAP is an enzyme expressed on the external aspect of the neutrophilic granulocyte plasma membrane, and represents a specific marker for the fully differentiated granulocyte (Rambaldi et al, 1997). It is Zinc – containing
phosphomonoesterase with a pH optimum near 10 (Beutler, 2001). The activity of NAP is limited to the neutrophilic series; it appears first in the myelocyte and rapidly increases with maturation of the cell to the segmented polymorphonuclear neutrophil.

NAP is used in the investigation of Neutrophil leucocytosis and Erythrocytosis. It differentiates chronic myeloid leukaemia (Low) from reactive leucocytosis (high) e.g. bacterial infection. It may assist in the differentiation of Polycytemia rubra vera (PRV) (high) from other causes of erythrocytosis (normal). Used in the diagnosis of Paroxysmal nocturnal haemoglobinuria (very low); (Normal to high) in other haemolytic and/or hypoplastic anaemia. The NAP is moderately elevated in pregnancy, with oestrogen therapy (e.g. Oral Contraception) and corticosteroid therapy.

2.3 COMPLICATIONS IN PREGNANCY

2.3.1 Urinary tract infections in pregnancy

Urinary tract infections (UTI) are the most common bacterial infections during pregnancy. The incidence of UTI in pregnancy can be as high as 6.2% (Ezechi et al, 2003)

Pathogenesis

Pregnant women are at increased risk for UTIs. Beginning in week 6 and peaking during weeks 22 to 24. Approximately 90% of pregnant women
develop ureteral dilatation, which will remain until delivery (hydronephrosis of pregnancy). Increase bladder volume and decrease bladder tone, along with decreased ureteral tone, contribute to increased urinary stasis and uretero vesical reflux (Delzell and Lefevre 2000). Additionally, the physiologic increase in plasma volume during pregnancy decreases urine concentration. Up to 70% pregnant women develop glycosuria, which encourage bacteria growth in the urine. Increase in urinary progestin and estrogens may lead to a decreased ability of the lower urinary tract to resist invading bacteria. This decreased ability may be caused by decreased ureteral tone or possibly by allowing some strains of bacteria to selectively grow. (Lucas and Cunningham, 1993).

UTI have three principle presentations: asymptomatic bacteriuria acute cystitis and pyelonephritis.

**Asymptomatic Bacteriuria:**

Significant bacteriuria may exist in asymptomatic patient with a prevalence of 10% during pregnancy (Delzell and Lefevre, 2000). Untreated asymptomatic bacteriuria leads to the development of symptomatic cystitis (infection confined to the bladder) in approximately 30% of patients and can lead to the development of pyelonephritis (infection involving the kidney) in up to 50%. Asymptomatic bacteriuria is associated with an increased risk of intrauterine growth retardation and low-birth-weight infants (Harris et al, 1976). The relatively high prevalence of asymptomatic bacteriuria during pregnancy, the
significant consequence for women and for the pregnancy plus the ability to avoid squeal with treatment, justify screening pregnant women for bacteriuria.

**Acute Cystitis:**
Acute cystitis is distinguished from asymptomatic bacteriuria by the presence of symptoms such as dysuria, urgency and frequency in a febrile patient with no evidence of systemic illness. Up to 30% of patients with untreated asymptomatic bacteriuria latter develop symptomatic cystitis.

**Pyelonephritis:**
Acute pyelonephritis during pregnancy is a serious systemic illness that can progress to maternal sepsis, preterm labour and premature delivery. The presence of bacteriuria is accompanied by systemic symptoms such as fever, chills, nausea, vomiting and loin pain. Symptoms of lower tract infection urethritis and cystitis (i.e. frequency and dysuria) may or may not be present. Pyelonephritis occurs in 2% of pregnant women (Gilstrap et al, 1981).

### 2.3.2 HIV Infections

The human immunodeficiency virus (HIV) was discovered in 1983 (two years after the diseases AIDS was described) when Barresinouisi Montagnier and colleagues at the Institute Pasteur, Paris, France, isolated the virus from the T cells of a patient with generalised lymphadenopathy and gave it the name
Lymphadenopathy associated virus (LAV, now HIV 1). In the same year, Robert Gallo and colleagues, working at the National Cancer Institute (NCI), USA made a similar discovery while in their quest to find cancer-causing viruses. In 1986 a second closely related virus, termed HIV 2 was isolated from a patient from West Africa suffering from acquired immune deficiency syndrome (AIDS). The first case of HIV/AIDS in Nigeria was reported in 1986 and by 2002, with a national sero-prevalence of 5.8%, 14 million people have died and 3.43million are living with disease. (Sagay et al, 2004).

HIV is one of the several complex retroviruses in the genus lentivirus. The virus contains three genes required for a replicating retrovirus - *gag*, *pol* and *env*. About three additional genes regulate virus expression and are important in disease pathogenesis *in vivo*. These other gene products include the *Tat, Rev* and *Nef* regulatory proteins that are translated from spliced mRNA species. The HIV structure consists of a lipoprotein surface studded by about 72 envelop knobs consisting of the glycoprotein *gp* 120, the surface (SU) protein and *gp*41, the transmembrane (TM) protein. Inside this lipid bilayer is a matrix (MA) protein (P17). Below the matrix is the nucleoprotein made up of a capsid (CA) protein (P24, P25). Inside the core are various cone shaped nucleocapsid protein (P9, P7), as well as polymerase enzyme containing reverse transcriptase (RT, P63), the Protease (PR, P15) and the integrase (IN), P11 (Levy, 1993).
HIV is transmitted through blood products (including blood transfusions, intravenous drug abuse in sharing of needles, health care workers in needle stick injuries and mucocutaneous exposures), organ transplants, sexual intercourse (both homosexual and heterosexual exposures) and vertical transmission (10-40% of babies born of infected mothers will be infected). Infection may occur in utero, during birth, postnatally or through breast feeding, (Noel, 2005). Virus enters the cell by fusing with the cell membrane. This event involves an interaction with a cellular receptor (the major one being CD4) followed by conformational changes in the viral envelop to permit viral-cell fusion (Hardie, 1999).

HIV is one of the major causes of infant and maternal mortality in resource-poor settings with more than 600,000 children infected each year or 1700 day, with most of the infections occurring in Africa (UNAIDS 2004). Of 815 pregnant women studied in South-eastern Nigeria 31 were HIV +. Severe anaemia has a significant correlation with HIV infections in these women (Uneke et al, 2007). HIV infections have also been associated with neutropenia. HIV can cause decreased growth of progenitor cell, CFU-GM, decreased endogenous G-CSF (Moore, 2008). The patient is exposed to increased risk of bacterial and fungal infection.
2.3.3 MALARIA

Malaria in pregnancy is frequently under-estimated, both as a public health problem and by clinicians who treat individual cases. It is one of the major causes of maternal, foetal and neonatal morbidity and mortality worldwide (Whitty et al, 2005). A woman who is pregnant is at significantly greater risk from malaria than one who is not, irrespective of the setting and malaria poses a severe threat to the pregnancy of any woman. Non-immune pregnant women are at high risk of complicated malaria and foetal loss. Women with previously reasonable immunity to malaria lose part of that protection in pregnancy, especially in first pregnancies, in which there is appreciable mortality. Miscarriages, stillbirths and preterm births are common in pregnant women with malaria. In addition to the huge burden of maternal anaemia caused by malaria, low birth weights are common in infants born to women who had malaria in pregnancy, with over 5% of prenatal deaths in many low resources setting thought to be caused by this mechanism. In our environment malaria has a prevalence of 19.7% in pregnant women, with primigravidae being significantly more infected with malaria. (Uneke et al, 2008)

2.4 UTERINE FIBROIDS

Fibroids are growths of the uterus, or womb. They are also called uterine leimyomas or myomas. They grow from the muscle cells of the uterus and may
protrude from the inside or outside surface of the uterus. Fibroids may also be found within the muscular wall. Fibroids are very common. A study carried out at the University of Ilorin Teaching Hospital, Ilorin, Nigeria shows that of five hundred and sixty-nine cases of confirmed uterine fibroid, uterine fibromyoma constituted 10% of gynaecological admission and were responsible for 26.2% of major gynaecological surgery. (Aboyeji and Ijaiya, 2005).

**Causes** - Although the exact cause of fibroid is unknown, their growth seems to be related to the hormones oestrogen and progesterone. When these hormone levels decrease at menopause, many of the symptoms of fibroid begin to resolve. However, it is not clear that hormones actually cause the fibroids. As an example, women who have had high levels of both of these hormones as a result of pregnancy or birth control pills have lower incidence of fibroids later in life.

**Risk Factors**

A number of factors influence the risk of developing fibroids. These include:

**Ethnic Background** – Fibroids are three times more common in black women as compared with white. In studies of women undergoing hysterectomy (removal of the uterus), black women were significantly more likely to have fibroids, were younger at the time of diagnosis hysterectomy, and had more severe problems associated with fibroids as compared to white women. (Mohammed et al, 2005)
Number of pregnancies – Women with one or more pregnancies that extended beyond 5 months have a decreased risk of fibroid formation.

Use of birth control - Women who use birth control pills have lower risk of developing fibroids, although women who use the pill at an early age (between age 13 and 16) may have an increased risk.

Diet – Significant consumption of beef, ham, or other red meats is associated with increased risks of fibroids, while consumption of green vegetables decreased risks. However, no study has shown that changes in diet influence changes in the incidence or symptoms of fibroids. Women who consume alcohol, especially beer, have an increased risk of developing fibroids. (Lee, 2003).

Clinical presentation

Many women with fibroids have significant bleeding and/or pain that interfere with one aspect of their lives. The severity of symptoms is related to the number, size, and location of the fibroids, and fall into three main groups: increased uterine bleeding, pelvic pressure and pain, and problems related to pregnancy and fertility.

Increased Uterine Bleeding – Fibroids can cause an increase in the amount of blood flow and length of a women’s menstrual period. The presence and amount of uterine bleeding is determined mainly by the location and size of the fibroids. Women with fibroids that protrude into the uterus are more likely to
have significant increases in bleeding, although women with all types of fibroids can have this problem. If the bleeding is very heavy, anaemia can occur. (Lee, 2003).

Jadhav et al (2003) studied the prognostic implications of white cell differential count in malaria; Birdi et al (1990) studied the early diagnosis of Kawasaki disease using toxic neutrophil count as an index. The prognostic significance of a left shift at the time of diagnosis of acute lymphocytic leukaemia was investigated by Shen et al (1984). Al Gwaiz et al (2007) studied the diagnostic value of Absolute Neutrophil count, band count etc of neutrophils in predicting bacterial infections. In 2005 Chaves et al studied the use of neutrophil mean channels of cell volume, conductivity, and light scatter (VCS parameters) using coulter automated haematology analyzer, as an indicator for bacterial infection. In this study, we investigated the significance of toxic neutrophil count in pregnant women.
CHAPTER THREE

3.0 STUDY DESIGN

3.1 Population

**Pregnant Women:** Three Hundred and Twenty-Three (323) pregnant women attending General Hospital, Onitsha and Shalom Foundation Specialist Hospital, Onitsha for routine antenatal serological testing were enrolled for this study. Information was available concerning gestational age, and pregnancy complications. This study lasted from February 2007 - March 2008.

**Normal Controls:** The normal controls were mostly patient’s relatives, blood donors, matched for age.

**Women with Fibroids:** The 58 women were those confirmed for intrauterine or extramural fibroids after ultrasonography by a physician.
3.2 Sampling technique

Blood and urine samples were collected from the pregnant and non-pregnant women who fulfil the inclusion criteria

3.2.1 Inclusion and exclusion criteria

The following categories of subjects were included after oral interviews and study of individual case files.

For the pregnant women:

- Pregnant women on antiretroviral therapy.
- Pregnant women with clinical symptoms of UTI e.g. dysuria, urgency and frequency of urination in a febrile patient with no evidence of systemic illness.

Those excluded were:

For the pregnant women

- Those with gestational hypertension.
- Diabetic pregnant women.

For the control

- Menstruating women
- Women taking oral contraceptives.
3.3.0 RESEARCH METHODOLOGY

3.3.1 NEUTROPHIL ALKALINE PHOSPHATASE ACTIVITY

3.3.1.2 ISOLATION OF NEUTROPHILS:

METHOD:

(Percy and Brandy (1968) modified (Brien, 1986).

PRINCIPLE:

Whole blood is allowed to stand at room temperature for 30 – 60mins. Erythrocytes settle first, then leucocytes, whose buoyant density is less than that of red blood cells (rbc) sediment more slowly and form a “buffy coat” between red blood cells (rbc) and the supernatant plasma. If the density of the medium through which sedimentation occurs is increased e.g. by addition of dextran, leucocytes remain suspended. Recovery of the supernatant and centrifugation at low centrifugal force results in a pellet made up largely of leucocytes. Contaminating erythrocytes can be eliminated by selective shock treatment, since they are less resistant than white cells to lysis in hypotonic solutions.

Procedure:

1. 5ml of whole blood was collected in acid – citrate phosphate dextrose adenine solution in a 15ml centrifuge tube.

2. 1ml of a freshly prepared solution of dextran 5g/dl, in Sodium Chloride (NaCl) 0.7g/dl was added and mixed gently by inversion.

3. Mixture was allowed to stand at room temperature for
35mins.

4. Supernatant was drawn-off with plastic disposable pipette and discharged into another centrifuge tube.

5. Centrifugation at 500xg for 10mins was done and the supernatant discarded.

6. White blood cells at the bottom of the tube were resuspended in 1.0ml of cold Sodium Chloride (Nacl) 0.9g/dl.

For Shock Treatment:

7. 3.0ml of ice-cold distilled water was added and mixed gently for 45 sec.

8. Immediately 3.0ml of cold Sodium Chloride (Nacl) (1.8g/dl) was added and mixed.

9. Centrifugation was done at 500xg for 10 mins and supernatant discarded.

10. Steps 7 through 9 were repeated for a second shock treatment.

3.3.1.3 NEUTROPHIL ALKALINE PHOSPHATASE (NAP) ASSAY

(El – Maallem and Fletcher, 1980)

Neutrophils suspended in saline were disrupted by forceful ejection through 22-G needles three burst of 10s each, 10µl of the disrupted cells were then resuspended in 90µl of Saline. Alkaline phosphatase in the homogenate (50µl) was then measured using reflotron machine. The Reflotron plus is an
invitrodiagnostic device designed for the quantitative determination of clinical chemistry parameters using reflotron reagent strips. It works on the principle of reflectance photometry and ensures rapid and reliable results while being simple to use.

**Measuring Principle:** Measuring the reflectance with the aid of an Ulbricht sphere using a reference beam for compensation.

**Procedure:** 50µl of the sample was placed on the Reflotron strip, which was then inserted into the machine and the result read out from the screen. This enzyme is present mainly in neutrophils and therefore neutrophils were not separated from other white cells. Enzyme activity was expressed as units per 10⁶ neutrophils.

### 3.3.2 TOXIC NEUTROPHIL COUNT

**Specimen:** The thin films were made immediately the blood was collected (without anticoagulant) using the two-slide method and air dried. Staining was done using Leishman’s stain.

**Principle:** With Romanowsky dyes, the acid groupings of the nucleic acids, proteins of the cell nuclei and primitive cytoplasm will determine the uptake of the basic groupings on the Haemoglobin (Hb) molecule results in its affinity for acidic dye and its staining by Eosin (Baker and Silverton, 1985).

The toxic granules stains blue black and are larger in size (500nm) than the normal granules (200-300nm) (Bainton et al, 2001).
Staining Thin Films (Bain and Lewis, 2003)

1. 1.5g of Leishman’s powder was added to 500mls of methanol.
2. After shaking the mixture was incubated at 37°C and left overnight. Filtration was done the following day.
3. The dried slides were flooded with the stain for 2mins, after which double the volume of buffered distilled water was added and staining done for 8mins.
4. The slides were washed with buffered distilled water until it acquires a pinkish tinge (up to 2mins). The back of the slides were cleaned and the slides set up right to air dry.

Toxic Neutrophil Count (TNC), defined as the sum of the number of peripheral blood neutrophils with vacuoles plus the number with toxic granulation per 100 neutrophils examined (Birdi et al, 1999) was then carried out.

3.3.3 MALARIA PARASITE EXAMINATION

Making Thick Film

- The thick films were made by placing a small drop of blood on the slide.
- This was then spread out with the corner of another slide so as to cover an area about four times its original area.
- The films were allowed to air dry thoroughly for at least 30mins at 37°C
Staining the Blood Films using Giemsa’s Stain (Dacie and Lewis, 1994)

- The Giemsa stain was diluted with 20 volumes of buffered water (pH 7.2).
- The dried slides were then immersed into the freshly diluted Giemsa stain without fixing.
- After 20 – 30 mins. The slides were washed with buffered water (pH 7.2) for 3 mins.
- The slides were then stood upright to air dry. They were then viewed under the microscope using the oil immersion objective, for the ring forms of malaria parasite.

3.3.4 TOTAL LEUCOCYTE COUNT

**Principle:** When anticoagulated blood is mixed with Turk’s solution, the red cells are lysed by the diluting fluid but the leucocytes remain intact, their nuclei staining deep violet black.

**Procedure:**

i) To 0.38ml of Turk’s solution in a glass tube, 0.02ml of EDTA anticoagulated blood was added.

ii) The mixture was thoroughly mixed by rotation for 1min.
iii) The Improved Neubauer counting chamber was cleaned and charged with the dilution and was allowed to stand for 1 min. for proper settling of the cells.

iv) The count was done using the x10 eyepiece lens. The value obtained was multiplied by 50 to obtain the total leucocytes count.

White cell count: \[ \frac{N \times DF \times 10^6}{A \times D} \] per litre

Where:
- \( N \): Number of cells
- \( DF \): Dilution factor
- \( 10^6 \): Converts to cell per litre.
- \( A \): Area of chamber counted
- \( DF \): Depth of chamber

3.3.5 URINE CULTURES

Preparation of Culture Plates (Cheesbrough, 1984)

**Blood Agar Plates:** Nutrient agar was prepared from the dehydrated media. 2.8g was dissolved in 100ml of distilled water. Sterilization was done by autoclaving at 121\(^\circ\)C for 15mins.

- The agar was transferred to a 50\(^\circ\)C water bath
- 5mls of sterile blood was aseptically added and mixed gently with the agar.
- 15ml of the media were dispensed aseptically into sterile Petri dishes.
MacConkey Plates
This was prepared using the dehydrated media 5.5g of the agar was dissolved in 100ml of distilled water. Sterilization was done by autoclaving at 121°C for 15mins. The media was cooled to 45 – 50°C and poured aseptically into sterile Petri dishes (15ml each). Mid – stream urine collected in a sterile universal bottles form the pregnant women were cultured on both culture plates and incubated at 37°C for 24hrs.

Results: Those with significant bacterial growth (presence of $>10^5$ cfu/ml) of single bacterial specie in one specimen of voided urine (Cheesbrough, 1984) were grouped as having urinary tract infection.

3.3.6 Statistical Analysis
The statistical analysis was performed with SPSS soft ware using student’s t-test. Specificity and sensitivity of various TNC cut offs were computed from:

Sensitivity=TP÷TP+FN
Specificity=TN÷FP+TN

Were; TP=true positive
TN=true negative
FP=false positive
FN=false negative
CHAPTER FOUR

4.0 RESULTS

The result of the present study demonstrated the mean TNC and NAP (table 4.1A) activity for the control subjects to be $0.003 \times 10^9/L \pm 0.001$ and $30.40 \pm 0.608 (\mu/x10^6 pmn)$ respectively. For normal pregnant women it was $0.22 \pm 0.028 \times 10^9/L$ and $70.07 \pm 2.498 (\mu/x10^6 pmn)$. Pregnant women with UTI it was $0.43 \times 10^9/L \pm 0.067$ and $58.14 \pm 2.271 (\mu/x10^6 pmn)$, those with HIV $1.13 \times 10^9/L \pm 0.103$ and $73.10 \pm 3.877 (\mu/x10^6 pmn)$ respectively. For pregnant women with malaria $0.23 \times 10^9/L \pm 0.034$ and $48.77 \pm 1.834 (\mu/x10^6 pmn)$ respectively, and for the fibroid subjects it is $0.01 \pm 0.003 \times 10^9/L$ and $30.90 \pm 0.766 (\mu/x10^6 pmn)$. Pregnant women with HIV had the highest number of Neutrophils with toxic granules, followed by those with UTI then those with malaria. This may reflect the relative risk of infection in these subjects. Normal pregnant women had higher values than the control and women with fibroid.

For NAP activity subjects with HIV also show the highest value for mean NAP activity, this finding has not been reported earlier.

Significant difference exists (table 4.1B) between the control subjects and normal pregnant women, mean TNC (P<0.0001) from the 9th week, for NAP activity (P<0.0001) from 13th week and at 9 – 12 week (P<0.001). There was also significant differences in both parameters (P<0.0001) between the control and subjects with
complications in pregnancy (HIV (table 4.4), UTI (table 4.2) and Malaria (table 4.3)) and also between the control and subjects with fibroids (P<0.0001). This may reflect the different states of the patients – pregnancy and infection.

Between the normal pregnant subjects and those with complications in pregnancy there was no significant difference (P>0.05) in NAP activity (table 4.5), except at 5 – 8 weeks where (P< 0.05) in UTI and malaria subjects. TNC shows significant differences (table 4.6) in UTI subjects (P<0.0001) at weeks 5 – 8, 25 – 28 and 37 – 40 weeks, at 9 – 12 weeks (P<0.001) and (P<0.05) at 21 – 24 and 33 -36 weeks. In HIV subjects (P<0.0001) across the gestational period, in subjects with malaria (P<0.001) at weeks 5 – 8, 9 – 12, 21 – 24 and 25 – 28. At 13 – 16 and 17 – 20 weeks there was no significant difference. This indicates a response in TNC in the presence of infection.

Subjects with HIV showed no significant difference in TWBC (table 4.7) when compared with the normal pregnant subjects except at week 13 – 16 weeks (P<0.05). Those with malaria show significant difference (P<0.001) at 5 – 8 weeks and 21 – 24 (P<0.0001) at 25 – 28 weeks and at 9 – 12 weeks (P<0.05)

In subjects with UTI there was significant difference in TWBC (P<0.0001) at 21 – 24 and 25 – 28 weeks, (P<0.001) at 9 – 12 weeks and 33 – 36 weeks and (P<0.05) at 5 – 8 weeks. This also signifies response in TWBC to infection.

Between HIV subjects and the control group there was no significant difference in TWBC except at 13 – 16 weeks were (P<0.05). The same pattern of difference
occurred between HIV subjects and normal pregnant subjects. This is not as expected and may be attributed to:

1. The prevention of mother-to-child transmission of HIV (PMTCT) antiretroviral regimens being administered to the patients – Zidovudine.

2. Pregnancy - which causes mild leucocytosis 6.3 – 16.0 x 10^9/L.

   El-maalem et al 1980).

TABLE 4.1A Means±SE in TNC and NAP for the control, normal pregnancy, women with fibroid and pregnant women with UTI, HIV and malaria

<table>
<thead>
<tr>
<th></th>
<th>TNC (%)</th>
<th>S.E</th>
<th>R</th>
<th>R²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=100)</td>
<td>0.003</td>
<td>0.001</td>
<td>0.051</td>
<td>0.003</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>NAP(μ/10⁶)pmn</td>
<td>30.40</td>
<td>0.608</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (n=100)</td>
<td>0.22</td>
<td>0.028</td>
<td>0.2005</td>
<td>0.4477</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>TNC (%)</td>
<td>70.07</td>
<td>2.498</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAP(μ/10⁶)pmn</td>
<td>0.43</td>
<td>0.067</td>
<td>0.3059</td>
<td>0.5531</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>NAP(μ/10⁶)pmn</td>
<td>58.14</td>
<td>2.271</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTI (n=75)</td>
<td>1.13</td>
<td>0.103</td>
<td>0.7325</td>
<td>0.5365</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>TNC (%)</td>
<td>73.10</td>
<td>3.877</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAP(μ/10⁶)pmn</td>
<td>0.24</td>
<td>0.034</td>
<td>0.3958</td>
<td>0.1567</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>NAP(μ/10⁶)pmn</td>
<td>48.77</td>
<td>1.834</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV (n=53)</td>
<td>0.01</td>
<td>0.003</td>
<td>-0.0630</td>
<td>0.004</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>NAP(μ/10⁶)pmn</td>
<td>30.90</td>
<td>0.766</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaria (n=95)</td>
<td>0.01</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNC (%)</td>
<td>48.77</td>
<td>1.834</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAP(μ/10⁶)pmn</td>
<td>30.90</td>
<td>0.766</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroid (n=58)</td>
<td>0.01</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.1 B Mean ± SE TWBC, TNC and NAP activity for control (non-pregnant) and normal pregnant subjects

<table>
<thead>
<tr>
<th></th>
<th>WBC Total (X10^9/L) Mean ± SE</th>
<th>TNC (%) Mean ± SE</th>
<th>NAP (µ/ 10^6 PMN) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (non-pregnant) n = 100</td>
<td>2.82 ± 0.07</td>
<td>0.003 ± 0.00</td>
<td>30.4 ± 0.61</td>
</tr>
<tr>
<td>Normal Pregnant Subjects Gestational Age in weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 – 8 (n = 10)</td>
<td>3.01 ± 0.24</td>
<td>0.01 ± 0.01</td>
<td>30.5 ± 2.05</td>
</tr>
<tr>
<td>9 – 12 (n = 10)</td>
<td>2.85 ± 0.29</td>
<td>0.02 ± 0.01***</td>
<td>36.4 ± 2.17**</td>
</tr>
<tr>
<td>13 – 16 (n = 8)</td>
<td>3.21 ± 0.26</td>
<td>0.23 ± 0.13***</td>
<td>50.2 ± 3.05***</td>
</tr>
<tr>
<td>17 – 20 (n = 10)</td>
<td>3.33 ± 0.24*</td>
<td>0.28 ± 0.06***</td>
<td>71.4 ± 2.65***</td>
</tr>
<tr>
<td>21 – 24 (n = 10)</td>
<td>2.61 ± 0.25</td>
<td>0.16 ± 0.02***</td>
<td>59.1 ± 2.05***</td>
</tr>
<tr>
<td>25 – 28 (n = 11)</td>
<td>2.16 ± 0.09**</td>
<td>0.13 ± 0.01***</td>
<td>74.8 ± 2.27***</td>
</tr>
<tr>
<td>29 – 32 (n = 11)</td>
<td>3.06 ± 0.23</td>
<td>0.20 ± 0.05***</td>
<td>80.0 ± 3.34***</td>
</tr>
<tr>
<td>33 – 36 (n = 10)</td>
<td>2.47 ± 0.24</td>
<td>0.23 ± 0.22***</td>
<td>99.6 ± 2.19***</td>
</tr>
<tr>
<td>37 – 40 (n = 20)</td>
<td>2.74 ± 0.10</td>
<td>0.47 ± 0.10***</td>
<td>96.6 ± 2.14***</td>
</tr>
</tbody>
</table>

**Key:**
- F – Distribution (ANOVA)*P<0.05, **P<0.001, ***P<0.0001

There were significant differences in the mean value of TNC (P<0.0001) and NAP activity (P<0.0001) from the 9th week of pregnancy to 40th week.
### Table 4.2 Mean ± SE TWBC, TNC and NAP activity for control (non-pregnant) and pregnant women with UTI

<table>
<thead>
<tr>
<th></th>
<th>WBC Total (X10⁹/L) Mean ± SE</th>
<th>TNC (%) Mean ± SE</th>
<th>NAP (µ/ 10⁶ PMN) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Non-Pregnant)</td>
<td>2.82 ± 0.07</td>
<td>0.003 ± 0.00</td>
<td>30.4 ± 0.61</td>
</tr>
<tr>
<td>Pregnant women UTI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Gestational Age in weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-8 (n= 18)</td>
<td>3.95 ± 0.22 ***</td>
<td>0.17 ± 0.03 ***</td>
<td>36.4 ± 1.54 ***</td>
</tr>
<tr>
<td>9 -12 (n = 17)</td>
<td>3.94 ± 0.29 ***</td>
<td>0.14 ± 0.03 ***</td>
<td>36.4 ± 1.69 ***</td>
</tr>
<tr>
<td>13 – 16 (n = 16)</td>
<td>3.42 ±0.34 *</td>
<td>0.19 ± 0.07 ***</td>
<td>50.4 ± 2.03 ***</td>
</tr>
<tr>
<td>17 – 20 (n = 14)</td>
<td>3.64 ± 0.12***</td>
<td>0.33 ± 0.76 ***</td>
<td>71.0 ± 2.37 ***</td>
</tr>
<tr>
<td>21 – 24 (n = 10)</td>
<td>5.15 ± 0.43***</td>
<td>0.60 ± 0.18 ***</td>
<td>59.4 ± 2.06 ***</td>
</tr>
<tr>
<td>25 – 28 (n = 6)</td>
<td>5.28 ± 0.76 ***</td>
<td>0.37 ± 0.05 ***</td>
<td>75.6 ± 2.88 ***</td>
</tr>
<tr>
<td>29 -32 (n = 6)</td>
<td>4.30 ± 0.73 ***</td>
<td>0.43 ± 0.07 ***</td>
<td>80.3 ± 4.89 ***</td>
</tr>
<tr>
<td>33 – 36 (n = 6)</td>
<td>6.93 ± 1.77 ***</td>
<td>1.18 ± 0.42 ***</td>
<td>10.2 ± 2.31 ***</td>
</tr>
<tr>
<td>37 – 40 (n = 5)</td>
<td>9.98 ± 1.44 ***</td>
<td>2.44 ± 0.53 ***</td>
<td>10.2 ± 3.75 ***</td>
</tr>
</tbody>
</table>

**Key:**

* P < 0.05, ** P < 0.001, *** P < 0.0001

Significant differences exist in TWBC, TNC and NAP activity (P<0.0001) in all the gestational ages
Table 4.3 Mean ± SE TWBC, TNC and NAP activity for control (non-pregnant) and pregnant women with malaria

<table>
<thead>
<tr>
<th></th>
<th>WBC Total (X10⁹/L) Mean ± SE</th>
<th>TNC (%) Mean ± SE</th>
<th>NAP (µ/ 10⁶ PMN) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control (Non-Pregnant)</strong></td>
<td>2.82 ± 0.07</td>
<td>0.003 ± 0.00</td>
<td>30.4 ± 0.61</td>
</tr>
<tr>
<td><strong>Pregnant Subject with Malaria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age in weeks 5 - 8 n= 20</td>
<td>4.05 ± 0.23***</td>
<td>0.13 ± 0.03 ***</td>
<td>36.4 ± 1.49 ***</td>
</tr>
<tr>
<td>9 - 12 (n = 16)</td>
<td>4.01 ± 0.27 ***</td>
<td>0.17 ± 0.03 ***</td>
<td>35.4 ± 1.84 ***</td>
</tr>
<tr>
<td>13 – 16 (n =15)</td>
<td>3.43 ± 0.40 *</td>
<td>0.18 ± 0.05 ***</td>
<td>50.9 ± 2.11 ***</td>
</tr>
<tr>
<td>17 – 20 (n = 10)</td>
<td>3.44 ± 1.49 *</td>
<td>0.33 ± 0.18 ***</td>
<td>71.4 ± 2.65 ***</td>
</tr>
<tr>
<td>21 – 24 (n = 8)</td>
<td>3.88 ± 0.48 ***</td>
<td>0.37 ± 0.08 ***</td>
<td>60.9 ± 2.52 ***</td>
</tr>
<tr>
<td>25 – 28 (n = 6)</td>
<td>4.67 ± 0.59 ***</td>
<td>0.55 ± 0.16 ***</td>
<td>70.9 ± 1.74 ***</td>
</tr>
</tbody>
</table>

**Key:**

P < 0.05, ** P < 0.001, *** P <0.0001

Significant differences exist in TNC and NAP activity (P<0.0001) for all the gestational age.
Table 4.4 Mean + SE TWBC, TNC and NAP activity for control (non-pregnant) and pregnant women with HIV and patients with Fibroid.

<table>
<thead>
<tr>
<th></th>
<th>WBC Total (X10^9/L) Mean ± SE</th>
<th>TNC (%) Mean ± SE</th>
<th>NAP (µ/ 10^6 PMN) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Non-Pregnant)</td>
<td>2.82 ± 0.07</td>
<td>0.003 ± 0.00</td>
<td>30.4 ± 0.61</td>
</tr>
<tr>
<td>Women with Fibroid (n = 18)</td>
<td>2.71 ± 0.09</td>
<td>0.01 ± 0.00</td>
<td>30.9 ± 0.78</td>
</tr>
<tr>
<td>Pregnant women with HIV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Gestational Age in weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 - 12 (n = 10)</td>
<td>2.86 ± 0.25</td>
<td>0.23 ± 0.03***</td>
<td>36.4 ± 2.17**</td>
</tr>
<tr>
<td>13 - 16 (n = 9)</td>
<td>2.26 ± 0.08*</td>
<td>0.97 ± 0.06 ***</td>
<td>53.4 ± 3.32***</td>
</tr>
<tr>
<td>29 - 32 (n = 6)</td>
<td>2.70 ± 0.22</td>
<td>0.96 ± 0.01 ***</td>
<td>80.6 ± 3.37 ***</td>
</tr>
<tr>
<td>37 - 40 (n = 11)</td>
<td>3.12 ± 0.20 ***</td>
<td>1.80 ± 0.14 ***</td>
<td>98.8 ± 2.58 ***</td>
</tr>
</tbody>
</table>

Key:
* P < 0.05, ** P < 0.001, *** P < 0.0001

Significant difference (P<0.0001) exist in the mean, TNC and NAP activity in the control subject and pregnant subjects with HIV in all the gestational weeks studied.
Table 4.5 Mean +SE NAP activity (µ/l x 10^6 PMN) for normal pregnant subjects and pregnant subjects with UTI, HIV and Malaria.

<table>
<thead>
<tr>
<th>Gestational Age in weeks</th>
<th>Normal pregnant subjects</th>
<th>Pregnant subjects with UTI</th>
<th>Pregnant subjects with HIV</th>
<th>Pregnant subjects with malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 – 8(n=48)</td>
<td>30.5 ± 2.05</td>
<td>36.4 ± 1.54*</td>
<td></td>
<td>36.4 ± 1.49</td>
</tr>
<tr>
<td>9 – 12(n=53)</td>
<td>36.4 ± 2.17</td>
<td>36.4 ± 1.69</td>
<td>36.4 ± 2.17</td>
<td>35.4 ± 1.84</td>
</tr>
<tr>
<td>13 – 16(n=48)</td>
<td>50.2 ± 3.05</td>
<td>50.4 ± 2.03</td>
<td>53.4 ± 3.32</td>
<td>50.9 ± 2.11</td>
</tr>
<tr>
<td>17 – 20(n=34)</td>
<td>71.4 ± 2.65</td>
<td>71.0 ± 2.37</td>
<td></td>
<td>71.4 ± 2.65</td>
</tr>
<tr>
<td>21 – 24(n=28)</td>
<td>59.1 ± 2.05</td>
<td>59.4 ± 2.06</td>
<td></td>
<td>60.9 ± 2.52</td>
</tr>
<tr>
<td>25 – 28(n=23)</td>
<td>74.8 ± 2.27</td>
<td>75.6 ± 2.88</td>
<td></td>
<td>70.9 ± 1.74</td>
</tr>
<tr>
<td>29 – 32(n=23)</td>
<td>80.0 ± 3.34</td>
<td>80.3 ± 4.89</td>
<td>80.6 ± 3.37</td>
<td></td>
</tr>
<tr>
<td>33 – 36(n=16)</td>
<td>99.6 ± 2.19</td>
<td>10.2 ± 2.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 – 40(n=36)</td>
<td>96.6 ± 2.14</td>
<td>10.2 ± 3.75</td>
<td>98.8 ± 2.58</td>
<td></td>
</tr>
</tbody>
</table>

Key: *P<0.05. **P<0.001, ***P<0.0001

Shows comparison of mean NAP activity in normal pregnant subjects and pregnant subjects with UTI, HIV and Malaria. There were no significant differences (P>0.05) except in pregnant subject with malaria and those with UTI at 5 – 8 weeks (P<0.05).
Table 4.6 Mean ± SE Toxic Neutrophil count (TNC %) in normal pregnant subjects and pregnant subjects with UTI, HIV and Malaria.

<table>
<thead>
<tr>
<th>Gestational Age in weeks</th>
<th>Normal pregnant subjects</th>
<th>Pregnant subjects with UTI</th>
<th>Pregnant subjects with HIV</th>
<th>Pregnant subjects with malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 – 8 (n=48)</td>
<td>0.01 ± 0.01</td>
<td>0.17 ± 0.03***</td>
<td></td>
<td>0.13 ± 0.03**</td>
</tr>
<tr>
<td>9 - 12 (n=53)</td>
<td>0.02 ± 0.02</td>
<td>0.14 ± 0.03**</td>
<td>0.23 ± 0.03***</td>
<td>0.17 ± 0.03**</td>
</tr>
<tr>
<td>13 – 16 (n=48)</td>
<td>0.23 ± 0.13</td>
<td>0.19 ± 0.07</td>
<td>0.97 ± 0.06***</td>
<td>0.18 ± 0.05</td>
</tr>
<tr>
<td>17 - 20 (n=34)</td>
<td>0.28 ± 0.06</td>
<td>0.33 ± 0.08</td>
<td></td>
<td>0.33 ± 0.18</td>
</tr>
<tr>
<td>21 - 24 (n=28)</td>
<td>0.16 ± 0.02</td>
<td>0.60 ± 0.18*</td>
<td></td>
<td>0.37 ± 0.08**</td>
</tr>
<tr>
<td>25 - 28 (n=23)</td>
<td>0.13 ± 0.01</td>
<td>0.37 ± 0.05***</td>
<td></td>
<td>0.55 ± 0.16**</td>
</tr>
<tr>
<td>29 - 32 (n=23)</td>
<td>0.20 ± 0.05</td>
<td>0.43 ± 0.07</td>
<td>0.96 ± 0.01***</td>
<td></td>
</tr>
<tr>
<td>33 - 36 (n=16)</td>
<td>0.23 ± 0.02</td>
<td>1.18 ± 0.42*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 - 40 (n=36)</td>
<td>0.47 ± 0.10</td>
<td>2.40 ± 0.53***</td>
<td>1.80 ± 0.14***</td>
<td></td>
</tr>
</tbody>
</table>

**Key:** * P<0.05, ** P<0.001, *** P<0.0001

In pregnant subjects with UTI there were significant differences (P<0.0001) at weeks 5 – 8, 25 – 28, 37 – 40, (P<0.001) at week 9 – 12 and P<0.05 at 21 – 24 week. In pregnant subjects with HIV there were significant difference in mean TNC (P<0.0001) across the gestational age. Significant difference exists (P<0.001) in TNC in pregnant subjects with malaria and normal pregnant subjects for weeks 5 – 8, 9 – 12, 21 – 24 and 25 – 28.
Table 4.7 Mean ± SE Total white blood cell count (TWBC) \times 10^9/L for Normal Pregnant Subjects and Pregnant subjects with UTI, HIV and Malaria

<table>
<thead>
<tr>
<th>Gestational Age in weeks</th>
<th>Normal pregnant subjects</th>
<th>Pregnant subjects with UTI</th>
<th>Pregnant subjects with HIV</th>
<th>Pregnant subjects with malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 – 8 (n=48)</td>
<td>3.01 ± 0.24</td>
<td>3.95 ± 0.22*</td>
<td></td>
<td>4.05 ± 0.23**</td>
</tr>
<tr>
<td>9 - 12 (n=53)</td>
<td>2.85 ± 0.29</td>
<td>3.94 ± 0.29**</td>
<td>2.86 ± 0.25</td>
<td>4.01 ± 0.27*</td>
</tr>
<tr>
<td>13 - 16 (n=48)</td>
<td>3.21 ± 0.26</td>
<td>3.42 ± 0.34</td>
<td>2.26 ± 0.08*</td>
<td>3.43 ± 0.40</td>
</tr>
<tr>
<td>17 - 20 (n=34)</td>
<td>3.33 ± 0.24</td>
<td>3.64 ± 0.12</td>
<td></td>
<td>3.44 ± 1.49</td>
</tr>
<tr>
<td>21 - 24 (n=28)</td>
<td>2.61 ± 0.25</td>
<td>5.15 ± 0.43***</td>
<td></td>
<td>3.88 ± 0.48**</td>
</tr>
<tr>
<td>25 - 28 (n=23)</td>
<td>2.16 ± 0.09</td>
<td>5.28 ± 0.76***</td>
<td></td>
<td>4.67 ± 0.59***</td>
</tr>
<tr>
<td>29 - 32 (n=23)</td>
<td>3.06 ± 0.23</td>
<td>4.30 ± 0.73</td>
<td>2.70 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>33 - 36 (n=16)</td>
<td>2.47 ± 0.24</td>
<td>6.93 ± 1.77**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 - 40 (n=36)</td>
<td>2.74 ± 0.10</td>
<td>9.98 ± 1.44</td>
<td>3.12 ± 0.20</td>
<td></td>
</tr>
</tbody>
</table>

**Key:** * P<0.05, ** P<0.001, *** P<0.0001

Shows a comparison of mean TWBC in normal pregnant subjects and pregnant subjects with UTI, HIV and Malaria. In pregnant subjects with UTI there were significant differences at 5 – 8 weeks (P<0.05) at 9 – 12, and 33 – 36 weeks (P<0.001), at 21 – 24 and 25 – 28 weeks (P<0.0001). In pregnant subjects with HIV there were no significant difference except at 13 – 16 weeks (P<0.05). Pregnant subjects with malaria show significant differences at 5 – 8, 21 – 24 weeks (P<0.001), 9- 12 weeks (P<0.05) and at 25 – 28 weeks (P<0.0001).
TABLE 4.8 TNC COUNT AND URINE CULTURE RESULT

<table>
<thead>
<tr>
<th>TNC cut off point</th>
<th>True Positive (TP)</th>
<th>True Negative (TN)</th>
<th>False Positive (FP)</th>
<th>False Negative (FN)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>18</td>
<td>306</td>
<td>77</td>
<td>80</td>
<td>18.4</td>
<td>79.9</td>
</tr>
<tr>
<td>70</td>
<td>0</td>
<td>373</td>
<td>10</td>
<td>98</td>
<td>0</td>
<td>97.4</td>
</tr>
</tbody>
</table>
Significant relationship exists between NAP activity and TNC in pregnant subjects with HIV (P<0.0001, r = 0.7325, $r^2 = 0.5365$). Pregnant subjects with UTI (P<0.0001, r = 0.3059, $r^2 = 0.5531$), Pregnant subjects with malaria (P<0.0005, r = 0.3958, $r^2 = 0.1567$) and also for normal pregnant subjects (P<0.0001, r = 0.2005, $r^2 = 0.4477$) Fig. 4.2DBC, B, C and Fig 4.1B There was no relationship between NAP activity and TNC for the control fig.4.1A (P>0.05, r = 0.051, $r^2 = 0.003$) and fibroid subjects fig.4.2A (P>0.05, r = 0.3013, $r^2 = 0.091$). These points to a relationship between NAP activity and TNC in the presence of a common factor – pregnancy and infection. These results could not be readily compared with any, as none, to the best of our knowledge existed.
Figure 4.1

(A): Relationship between NAP activity and TNC in control subjects. NAP activity exhibits no significant relationship with TNC. (P > 0.05, r = -0.051, r² = 0.003)

(B): Relationship between NAP activity and TNC for normal pregnant subjects. NAP activity exhibited a positive relationship with TNC. (P < 0.0001, r = 0.2005, r² = 0.4477).
(A): Relationship between NAP activity and TNC for fibroid subjects. NAP activity exhibits no significant relationship with TNC. (P>0.05, r =-0.0630, r² =0.004).

(B): Relationship between NAP activity and TNC for pregnant subjects with UTI. NAP activity exhibits a positive relationship with TNC. (P<0.0001, r =0.3059, r² =0.5531).

(C): Relationship between NAP activity and TNC for pregnant subjects with malaria. NAP activity exhibits a positive relationship with TNC. (P<0.0005, r =0.3958, r² =0.1567).

(D): Relationship between NAP activity and TNC for pregnant subjects with HIV. NAP activity exhibits a positive relationship with TNC. (P<0.0001, r =0.7325, r² =0.5365).
Figure 4.3  
**e:** Normal peripheral blood film

Peripheral blood film of normal pregnant subjects:

- **a:** At 5-8 weeks of Gestation, shows scarce granulation.
- **c:** At 21-24 weeks of Gestation, shows moderate toxic granulation
- **h:** At 36-40 weeks of Gestation, shows prominent granulation of the neutrophil
Figure 4.4

d: Peripheral blood film of pregnant women with UTI shows the presence of toxic granules.
g: Peripheral blood film of pregnant women with Malaria, toxic granules are present.
b: Peripheral blood film of pregnant women with HIV shows prominent granulation.
f: Peripheral blood film of women with Fibroid, toxic granules are scarce.
CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

Toxic granules are dark blue or purple cytoplasmic granules in the band/polymorph cells. Toxic granules are azurophilic that retain basophilic staining reaction by lack of maturation or increased basophilia. The granules in the neutrophil represent packages of enzymes, which are involved in the killing of ingested microbes and the digestion of phagocytised material. Based on differences in enzyme content, the granules can be classified into two different subtypes. The primary lightly basophilic staining granules contain, for example, peroxidase plus acid hydrolytic enzymes, while secondary granules contain alkaline phosphatase and certain other enzymes (Jadhav et al (2003)).

There was a gradual rise in NAP activity across the gestational age. This agrees with an earlier report by EL –Maalem et al (1980), however contradicts with the cytochemical study of Beal et al (1967) who reported no differences between the means for tests done in early pregnancy and those performed later in pregnancy.

Hence, the relationship between the quantitative biochemical measurements of NAP by direct assay of leucocytes and the NAP cytochemical score need to be established.

Several studies have evaluated the complete blood count and leukocyte differential counts for disease detection and found the utility to be very low.
The result of our study showed TNC to have a specificity of 79% at a cut off of 10 and 97% at a cut off of 70 and a sensitivity of 18% at a cut off of 10 and 0% at a cut off of 70.

Our present study of the significance of the morphological changes in neutrophil (toxic granulation) has also followed the same pattern of low utility, probably due to biological variability. When expressed as coefficient of variance it was found that the CV for granulocytes in the elderly was 17.3-19.3% within subjects and 21.2-29% between subjects. (Gwendolyn, 1991).

The within individual values may provide a much more sensitive index to changes with disease.
5.1 RECOMMENDATIONS

There appears to be no previous study reporting about the significance of Neutrophil toxic granulation in pregnancy. The present study should evoke interest amongst researchers spurring them to undertake larger studies on the subject.
REFERENCES


www.hopkins-hivguide.org/diagnosis/organ 

Mohammed, A., Sheu S.M., Ahmed, A.A., Mayun, A.A, Tiffin, I.U., 
year clinicopathological review in Zaria Nigeria. 

www.umm.ed.

Rambaldi, A., Masuhara, K., Borleri, G.M., Amaru, R., Gianni, M., 
of leucyte alkaline phosphatase in normal and pathologic 


Sagay, A.S., Imade, G.E., Kapiga, S., Omoregie, .R. Egah, D.Z., Falusi, 

Peripheral blood count at diagnosis in acute Lymphocytic 
Leukaemia is significantly correlated with duration of complete 


Prevalence of public – health significance of HIV infection 
and anaemia among pregnant women in South – Eastern Nigeria. 

