THE ROLE OF VITAMIN A (RETINOL PALMITATE) AND BETA–CAROTENE ON B-LYMPHOCYTES AND NATURAL KILLER CELLS IN HIV-1 SEROPOSITIVE PREGNANT WOMEN

BY

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PREFACE

The majority of the work reported in this thesis was performed in the Department of Haematology, University of Natal, Durban, under the supervision of Professor H.M. Coovadia and Dr. D.G. Kenoyer. The High Pressure Liquid Chromatography work was performed in the Analytical Unit, Department of Physiology, University of Natal, Durban, by Mrs. Inga Elson. This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

DEDICATION

This thesis is dedicated to my grandmother Mrs Mangama Padavettan,

whose sage words were "Knowledge is Power"

She made sacrifices to make my life extremely comfortable and pleasant for which I am eternally grateful.

Within the context of my family she is the greatest person who walked the face of the earth.

I shall continue to pay tribute to this great soul until my very last breath.

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ABSTRACT

Objective:

To evaluate the role of vitamin A on B lymphocytes, natural killer cells and haematological parameters including the activation marker(I2) in HIV-1 seropositive pregnant women.

Patients and Methods:

Baseline, delivery or 6 weeks post supplementation, 1 week post- partum and 3 month post-partum samples were collected from subjects. Patients were treated with vitamin A and beta-carotene or placebo after the baseline bloods were collected and were supplemented with a bolus dose one day post delivery. For the purposes of this study the delivery and 6 weeks post supplementation samples were grouped as delivery samples. Quantification of B lymphocytes, the activation marker (I2) and natural killer cells were measured by flow cytometry, using CD19, I2(HLA-DR) and CD56 monoclonal antibodies respectively in a random subsample of 208 patients drawn from a cohort of 400 HIV-1 positive subjects.
Vitamin A levels at baseline and complete blood cell counts were also evaluated. In this cohort a vitamin A level of <40ug/dl was considered low normal and <20ug/dl was considered deficient.

Results:

B lymphocyte counts were lower during pregnancy and recovered between 1 week and 3 months post delivery. While natural killer cell counts were comparable at baseline, the placebo group did not change significantly at any of the intervals studied, but the vitamin A group showed a rise at 1 week post delivery and a subsequent fall. The activation marker (I2) did not show any significant change in both groups. In the vitamin A group 37.5% (15 out 40) and in the placebo group 38.5% (20 out of 52) had retinol levels of <20ug/dL. Fifty percent of the patients in the vitamin A group and 55.7% in the placebo group had low normal retinol levels. The mean retinol levels prior to intervention were 25.65 ug/dL (95% CI =21.71→29.60) in the vitamin A group and 24.38 ug/dL (CI=21.80→26.97) in the placebo group; both groups were comparable at 3 months post partum. At baseline 65.4% (136 out of 208) patients were anaemic; this corrected at 3 months post partum. Six patients had thrombocytopenia at baseline. At 3 months the two groups were comparable with platelet counts within expected normals in the post partum period. Leucopenia was noted in

5.2% (11 out of 208) patients at baseline with counts <5.0 X 10^9/l. At 3 months the two groups were comparable with a mean white cell count of 5.62 X 10^9/l in the vitamin A group and 5.56 X 10^9/l in the placebo group which was significantly lower than the baseline levels(vitamin A -7.80 X10^9/l and placebo-7.92 X 10^9/l). Lymphopenia was noted in 9.1% (19 out of 208) patients at baseline. A significant increase was noted at 3 months post partum in the two groups.

Conclusion:

There was no significant effect of vitamin A on B lymphocytes. Natural killer cells in the vitamin A group increased more than in the placebo group but this "booster" effect by vitamin A was very transient. No difference was noted in the activation marker between the 2 groups at any point. The cellular changes with pregnancy, in HIV+ patients in this study are the same as in HIV- patients. No beneficial or detrimental effect was noted on natural killer cells, B lymphocytes and the activation marker for vitamin A .

CHAPTER 1

BACKGROUND

1.1 Vitamin A:

During the 1920s and 1930s, vitamin A became known as the "anti-infective" vitamin, and the first attempts were made then to use it therapeutically during infectious illnesses. Vitamin A deficiency is the leading cause of xerophthalmia, visual impairment and nutritional blindness in children (1,2), and has emerged as a major cause of childhood mortality and morbidity in the developing world(3). Pregnancy and HIV infection are risk factors for vitamin A deficiency(4). As a single intervention vitiamin A therapy may alter disease morbidity even when other concurrent macro- and micronutrient deficiencies are not corrected(5). It may also have significant immune effects in hosts who are not deficient in this micronutrient. Vitamin A when administered, may also have protective effects in various other disease states such as bronchopulmonary dysplasia, alcoholism and cancer. Vitamin A is an important modulator of the immune system being responsible for growth, cellular differentiation, maintenance of epithelial surfaces, reproduction and vision(6,7), as well as cytokine production.(8). Vitamin A deficiency and hypovitaminosis have been shown to impair immune responses(8). Vitamin A is bound to its physiological carriers, retinol binding protein (RBP), cytoplasmic retinol binding protein (CRBP), retinoic acid receptor (RAR) and retinoic X receptor (RXR) which is essential for the growth of activated human B cells(9).

Hypovitaminosis is associated with compromised B-cell haematopoiesis and impaired humoral immunity. Vitamin A deficiency and hypovitaminosis have been shown to impair normal immune cytokine production which enhance B-cell activation. HIV infection is associated with multiple nutritional deficiencies, including Vitamin A(10). Vitamin A deficiency has also been implicated as a risk factor for increased mortality during infancy and in the perinatal period(11), and as a risk factor for mother to infant transmission of HIV in developing countries(12). Vitamin A influences the numbers and nature of white cells involved in immune, inflammatory and wound healing processes(13).
Liver, cod liver oil, and dairy products are good sources of preformed vitamin A alcohol (retinol). Green leafy vegetables, carrots, and fruits such as mangoes are sources of carotenoids such as β-carotene, which are converted to retinol in the intestine. Many plant carotenoids, such as β-carotene and β-cryptoxanthin can be converted to vitamin A by an oxygenase present in the intestine and elsewhere. Carotenoids are detectable in various tissues, but unlike vitamin A they do not combine with specific transport proteins and are transported mainly as non-polar lipids(14).

Retinol receptors are present on cells of many different body tissues. In intracytoplasmic spaces retinol is converted to its active metabolites, retinoic acid and retinal. These metabolites in turn bind to specific intranuclear receptors that modulate cellular activities. Decreased serum vitamin A content is noted in a wide variety of infective and febrile conditions. This is probably a manifestation of the acute phase response. Large oral doses of vitamin A have reduced

measles morbidity and mortality(15). Vitamin A supplementation has improved immune function as shown by speedier reversal of measles induced lymphopenia and by improvement of the measles IgG antibody response during the acute phase of the disorder(16). Retinoic acid is probably the physiologically important metabolite for sustaining IgG immune responses in vivo(17). Reduced liver stores of vitamin A are noted following infections and the low levels may be due to anorexia, decreased absorption, increased metabolic requirements, or increased catabolism. Plasma and liver levels of vitamin A are often used for investigational purposes and in the absence of clinical manifestations plasma levels below 0.7 umol/l (20 ug/dl) reflect vitamin A deficiency. Levels below 1,05 umol/l (30 ug/dl) may be associated with compromised biological action of retinol. A single high oral dose of the vitamin may replenish liver stores. Excessive vitamin A administration to pregnant women during the first trimester may be teratogenic(5), and extremely high intake may lead to signs of chronic toxicity. This is manifested by bone pain, dry skin and anorexia. Beta-carotene supplementation improves some indices of immune response, including increasing numbers of circulating Natural Killer cells in humans with inadequate vitamin A intake or with a previously stressed immune response(18). Low vitamin A levels have also been reported in many other infectious conditions, eg. tuberculosis, schistosomiasis, malaria, leprosy, rheumatic fever, otitis media, gastrointestinal and respiratory infections(5). Vitamin A is vital for maintenance of mucosal integrity and immunity. In both humans and animals who are vitamin A deficient lymphocytes have collectively expressed fewer activation markers

and at times displayed reduced mitogen-induced proliferation(19). Abnormal specific IgG subclass proportions have also been described in vitamin A deficiency, and this may also relate to reduced clonal expansion of specific B cell subsets in response to alterations in T cell cytokine production. Abnormal peripheral blood lymphocyte proportions in vitamin A deficient rats were corrected by retinoic acid supplementation (19). Rats with induced vitamin A deficiency showed decrease in the proliferative response of B cells but no decrease in T cell response was observed.

Beta-carotene is a non-toxic compound whose main side-effect, when taken in high doses, is a benign and reversible yellowing of the skin (hypercarotenaemia). Mechanisms of action of beta-carotene include quenching of oxygen free radicals, improved cell to cell communication, modulation of lipo-oxygenase activity, increased cytokine release and stabilization of lysosomal membranes(20). Beta-carotene may play a more important role than retinol in HIV-1 infected patients(21). Vitamin A together with beta-carotene in HIV-positive women could greatly reduce viraemia(22); and an increased viral load has been previously shown to increase the risk of vertical transmission(23).

Altered functional capacity of virtually all types of immune cells has been described in vitamin A deficiency. With HIV infection there is alteration in T-cell subsets and dysregulation of B lymphocytes. This study will assess the prevalence of vitamin A deficiency and the impact of vitamin A supplementation in HIV infected pregnant females.

1.2 HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME :

The impact that human immunodeficiency virus (HIV) and AIDS have had on the world is unparalleled in modern times. AIDS was first diagnosed in mid-1981 when an unusual form of pneumocystis carinii pneumonia (PCP) and Kaposi sarcoma were reported in young, previously healthy homosexual men. The pandemic is particularly devastating in sub-Saharan Africa. Over five million people in South Africa are expected to be HIV positive by the turn of the century and the epidemic is expected to peak in seven years time. HIV-1 infection in Kwa-Zulu Natal is increasing rapidly with 14.4%(1997) of child bearing women being seropositive(24).

The World Health Organization (WHO) estimates that at least 30 million people are infected worldwide. There have been more than 1.4 million new infections in Africa every year since 1991. An estimated 90% of the world's HIV/AIDS burden is in resource poor settings and more than 70% of people requiring care are in sub-Saharan Africa(25).

An estimated 26.8 million adults and 2.6 million children had become infected with HIV throughout the world since the start of the pandemic; of these, an estimated 5 million adults and 1.4 million children had died(26).

Recent studies have addressed the timing of HIV transmission in neonates and suggest that the infection is more likely to occur as a peripartum event(27).

HIV-1 is a member of the lentivirus subfamily of human retroviruses and the

etiologic agent of AIDS in the vast majority of cases throughout the world. HIV-2 is another human retrovirus that causes AIDS and is endemic in parts of West Africa. It is closely related to simian immunodeficiency virus (SIV) and may be less pathogenic than HIV-1. HTLV-1 and HTLV-II are also found with increased frequency in HIV-1 infected populations, particularly intravenous drug users(27). CD4+ lymphocytes and viral load have been unequivocally established as having clinical utility in management of HIV infected patients(28). Primary infection with HIV is followed by the development of detectable humoral and cellular immune responses to the virus and a prolonged period of clinical latency. Associated with the progression to AIDS is the rapid decline in normal T-cell function (switch to the T-helper type-2 response)(29). The polyclonal hypergammopathy, associated with B-cell responses, are attributed to the T-helper type-2 responses (CD4+/CD29+ memory phenotype)(29,30). HIV activates resting B-cells to produce various cytokines viz. TNF-alpha and IL-6. These cytokines have been shown to increase viral replication by acting on the Nf-KB-like LTR (long terminal repeats) sequences of the viral genome (30,31,32). Apoptosis appears to account for the progressive loss of T cells in infected individuals who develop AIDS irrespective of whether they bear the integrated proviral genome. Cells from long-term nonprogressors undergo a decreased level of apoptosis compared with those from patients with AIDS(33).

Patients with HIV infection have been documented to have symptoms compatible with adrenal insufficiency(34) and symptoms occur in this setting with multiple opportunistic infections and profound wasting. Weight loss, often profound in

magnitude, is an almost universal feature of HIV infection, and patients may lose 30 to 50% of their body mass before succumbing to the disease. Weight loss can be caused by five mechanisms: inadequate dietary intake, reduced intestinal absorption, abnormal utilization, increased excretion of nutrients, and increased host requirements. Weight loss contributes to an accelerated, downhill course; it can also be used to predict the time of death in patients with HIV infection. It has been suggested that reversal of weight loss in HIV-infected patients could repair the immunologic abnormalities caused by the wasting process, independent of those induced by the viral infection(35).

Infection with the human immunodeficiency virus has been associated with the dysfunction of both T-and B-cell activities soon after the primary viraemia. A slow replicating virus can be the more pathogenic type(36). Most paediatric HIV infection occurs in the perinatal period, when immunologic immaturity and antigenic naivete is well recognised(37).

CD30 triggering may play an important role in promoting HIV replication(38). A rise in virus production then occurs concomitant with the onset of symptoms and eventually AIDS. Characteristics of long-term survivors include low infectious virus load, a less cytopathic HIV strain, no enhancing antibodies, TH1 cell response greater than TH2 cell response and strong CD8+ cell antiviral response(39).

The social and biologic consequences of HIV disease are greater in females. Women are more likely than men to rely on clinic care or to need drug

rehabilitation services. Several gynaecologic infections have been reported to have their natural course modified by HIV infection. Pelvic inflammatory disease(PID) has been noted to appear with less leucocytosis, but with more abscesses and a greater need for surgical intervention. Vaginal candidiasis is a common gynaecologic infection in HIV-infected women, and it has been commonly noted in those with normal CD4 counts, prior to the appearance of either oral or oesophageal candidiasis. HIV-1 seropositive pregnant women develop high rates of genital ulcer disease, genital warts, positive syphilis serology and stillbirths(40). HIV-1 seropositive women had significantly lower birthweight and with premature neonates . Factors that have been identified as possible risk factors for HIV mother-to-child transmission include impaired maternal clinical and immunological status, HIV seroconversion during pregnancy, shortened duration of pregnancy, vaginal delivery, prolonged or complicated labour and breast-feeding(41). Pregnant women with CD4 counts < 30% have higher risk of preterm delivery and postpartum endometritis is more common in HIV-1 infected women. More HIV seropositive pregnant women have raised temperatures on admission to the labour ward compared to the HIV negative women(42). Haematological parameters were abnormal in seropositive women and these did not progress over the course of pregnancy. At delivery seropositive women were more likely to receive antibiotics and have episiotomy although the obstetric outcome was unaffected. Seropositive women admitting to drug use had a significant increase in absolute lymphocyte count at delivery. Seropositive mothers were more likely to have sexually transmitted disease and

medical complications during pregnancy(43). In asymptomatic HIV infection, changes in the absolute levels of CD4 and CD8 lymphocyte counts were primarily related to changes in the other components of the white cell count. Pregnancy itself had no adverse effect on immunological markers in HIV infected individuals(44).

In newborns the absence of the env-specific TH activity places them at risk for infection(45).The presence of immunologic abnormalities shortly after birth, confirms that HIV-1 may affect the immune system even during intrauterine life(46). Most of the HIV-1 infected babies showed early abnormalities in humoral and cellular immunity, hypergammaglobulinaemia, low percentage of CD4 circulating lymphocytes and increased spontaneous in vitro immunoglobulin production. These changes were persistent in the HIV-1 infected children, but sporadic in those uninfected and immunological abnormalities were frequently found before clinical symptoms occurred(47).

1.3 B LYMPHOCYTES / B CELLS

Lymphocytes are generated throughout human life though in gradually easing numbers. They play a dynamic role in the inflammatory response and the production of antibodies (48). In birds, B cells originate in a specialized organ called the Bursa of Fabricius, while in mammals B-cell development occurs in the bone marrow. Each B cell bears a unique receptor and undergoes gene rearrangement which leads to the production of immunoglobulin. Each mammalian B cell produces only one heavy chain and one light chain, and thus bears receptors of a single specificity. A large and diverse repertoire of B-cell receptors is generated during the early phases of B-cell development. The expression of antigen receptors on the surface of the B lymphocyte marks a major watershed in its differentiation(48). The binding of antigen to surface immunoglobulin early in development leads to inactivation or loss of the B cell. B cells develop from progenitor cells in the bone marrow and rearrange their immunoglobulin genes to produce a receptor with unique antigen specificity. No surface immunoglobulin can be expressed until gene rearrangement is completed. This process is independent of the antigens in the environment, but it is however dependent on interactions with bone marrow stromal cells. Immature B cells expressing surface immunoglobulin interact with antigens in their environment; if they encounter antigen they are rendered tolerant. These changes occur in the bone marrow, from which immature B cells emerge into the peripheral lymphocyte pool. Immature B cells differentiate within few days into mature B cells expressing surface IgM and IgD(48). The B cell can be

activated by encounter with antigen in a lymphoid organ and then respond by proliferating and differentiating into the last phase of the B-cell life span. This is the antibody-secreting plasma cell, which may remain in the lymphoid organ but more usually migrates to the bone marrow. The successive stages of B-cell differentiation are marked by the successive steps in the rearrangement of the immunoglobulin genes. Differentiation can be assessed on the basis of CD5 expression and production of polyspecific Ig(49). CD5+ B cells are strongly associated with autoimmune disorders and also in the rejection of HLA-DR and DQ compatible bone marrow graft. CD5+ B cells play a role in natural immunity in humans(50).

CD23 is also involved in B-cell growth, pro-thymocyte maturation, myeloid precursor proliferation, inhibition of macrophage migration and antigen presentation(51).

Tallon *et al* reported that haemodilution in pregnancy could be the cause for decrease in absolute lymphocyte counts of T and B cells in the third trimester of pregnancy(52). As the plasma volume returns to normal post partum these cells return to normal numbers.

Maclean *et al* reported that immunoglobulin production from peripheral blood was elevated in normal pregnancy and spontaneous abortion(53). This group of workers were not certain as to whether the increase was as a result of increase in B cell numbers or increased production of immunoglobulin. B cell activation was also noted in some cases of spontaneous abortion.

Maclean *et al* found in normal pregnancy there is an increase in immunological potential of T and B lymphocytes without an increase in activity(54).

Dodson *et al* found that T and B cell numbers were well within normal limits throughout gestation and the values were not significantly different from the normal non pregnant control women(55).
It was also confirmed in another study that B cell counts did not alter in early pregnancy(unpublished).

Strelkauskas *et al* reported altered blood levels of T and B lymphocytes in the first half of human pregnancy(56). All their subjects showed an inversion of T and B cell levels in early pregnancy. They concluded that B-cell levels (measured by surface immunoglobulin or surface antigen) were increased.

Baines *et al* reported that gravid females show no increase or decrease in circulating thymus-derived or bone-marrow derived lymphocyte levels during the first eight months of pregnancy(57).

Scott *et al* reported that the mean number of B cells is increased during gestation(58).

HIV infected individuals, in addition to the depletion of CD4+ T cells, exhibit abnormalities in the B-cell limb of the immune response and some are secondary to T-cell deficiency, while others are T-cell independent. B lymphocytes are not directly infected by HIV(59), although their function in HIV infection is severely impaired. The majority of AIDS patients exhibit polyclonal B-cell activation with

spontaneous B-cell proliferation, increased Ig secretion, and hypergammaglobulinaemia, suggesting chronic activation. These activated B cells are specific for HIV epitopes. In addition to spontaneous B-cell hyperactivity, B cells from infected patients exhibit an intrinsic defect in antigen- and mitogen-induced responses at all stages of infection(27). IL-6 is a critical factor in the terminal differentiation of activated B cells. It has been shown that exposure of normal PBL to HIV whole-virus preparation induces IL-6 production in vitro, predominantly by monocytes and that IL-6 induces HIV expression in infected monocytes, in synergy with other cytokines. Monocytes and T cells contribute to the secretion of IL-6, which plays an important role in the pathogenesis of B-cell activation in HIV infection. B cells themselves secrete TNF-α and IL-6 when activated and spontaneously activated B cells from HIV-infected individuals secrete TNF-α and IL-6 which can induce HIV expression in chronically infected monocyte lines. The high frequency of B-cell lymphomas in patients with AIDS is another manifestation of B-cell dysregulation(27). CD30+ CD4+ T cells exhibit significantly greater helper activity of B cell Ig production than CD30-CD4+ T cells. CD30+ T cells exhibit potent helper activity for PWM-driven B cell differentiation(60). B cell activation by most antigens requires binding of the antigen by the B-cell surface immunoglobulin and interaction with antigen-specific helper T cells(61). These helper T cells induce a phase of vigorous B-cell proliferation, after which the clonally-expanded progeny of naive B cells differentiate into either antibody-secreting or memory B cells. During the differentiation of activated B cells, several changes occur in the antibody

molecule. The number of B cells that express CD23 nearly doubles between infancy and adulthood(62). Retroviruses can induce autoimmunity, including polyclonal B-cell activation, cytokine dysregulation, and molecular mimicry(59).

1.4 HLA-DR (I2) ANTIGENS

The highly polymorphic cell surface structures involved in this system were initially identified in mice where they became known as the major histocompatibility complex (MHC) . Later, the human equivalent of the MHC, known as the human leucocyte antigen (HLA) system, was identified and found on chromosome 6 (63). Three classes of molecule, denoted I, II, and III, have been identified in the MHCs of both mouse and man. There are multiple Class I loci but the classical transplantation antigens fall into three positions termed H-2K, H-2D and H-2L in the mouse and HLA-A, HLA-B and HLA-C in man. The Class II genes, encoded in the A and E regions of the mouse MHC and the HLA-D region of man, are now known to correspond directly to the immune response(Ir) genes known to control responses to different antigens(63). Class I gene products are primarily recognized by cytotoxic T cells. Class II gene products, often called Ia (immune associated antigens), are primarily involved in the activation of helper T cells. Human HLA-DR (Ia) antigens were initially detected and studied using alloantisera, then heteroantisera and finally monoclonal antibodies. Originally, HLA –DR antigens were believed to be present exclusively on B cells but they were subsequently discovered on antigen presenting cells (monocytes, macrophages). HLA-DR antigens are widely distributed on haematopoietic cells and are even expressed on some non-haematopoietic cells, especially in certain pathologic conditions(63). HLA-DR antigens are expressed in the earliest stages of haematolymphoid cell development. They are expressed throughout B cell ontogeny until the plasma

cell (secretory) stage when they are lost. They are also present on haematopoietic progenitors and granulocyte, monocyte, erythroid and megakaryocytic precursors. HLA-DR antigens are expressed on myeloblasts but lost during maturation to the promyelocyte stage(64). Similarly, they are expressed on proerythroblasts and megakaryocytes but are absent from erythrocytes beyond the basophilic normoblast stage and platelets. HLA-DR antigens are present throughout monocyte/macrophage differentiation. HLA-DR are expressed at the earliest stages of T cell ontogeny but are quickly lost. The vast majority of thymocytes and mature peripheral and circulating lymphoid tissue T cells are HLA-DR negative. In vitro and in vivo activated T cells express HLA-DR antigens. HLA-DR antigens are expressed on the vast majority of B cell neoplasms(64). B cell neoplasms that are HLA-DR negative are those undergoing plasma cell differentiation. HLA-DR antigens have also been described in a variety of nonhaematopoietic neoplasms such as malignant melanomas.

Maclean *et al* reported in normal pregnancy there was no activation of the immune system as noted by the activation marker (Ia)(54) . Moore *et al* found the activation marker (Ia) percentages and absolute numbers were similar in normal and pre-eclamptic pregnancies(65).

Haynes *et al* reported that MHC class I and class II genes play a major role in determining the specificity of T and B cell antiviral immune responses(66). There is considerable interest in searching for an HLA association with long-term

survivors of HIV infection(67).

1.5 NATURAL KILLER CELLS

A great deal of work regarding the study of Natural Killer (NK) activity has occurred over the last decade. The cell type identified as being responsible for this activity was a large granular lymphocyte (LGL). This form of lymphocyte has a high cytoplasm to nuclear ratio and displays distinct azurophilic granules. It should be noted that, as with other cell types, heterogeneity exists amongst the NK cell population with some NK cells not displaying the distinct LGL morphology during certain states of differentiation and/or activation. In studies performed over the last decade on spontaneous cell-mediated cytotoxicity, it has become clear that natural effectors comprise a variety of cell types which mediate both distinct cytolytic capacities as well as non-cytolytic functions. These capacities include not only NK activity, but also lymphokine-activated killer (LAK) activity and antibody-dependent cellular cytotoxicity (ADCC). Natural Killer cells work synergistically with B cells. This interaction has been demonstrated to decrease viral load in severe combined immunodeficiency (SCID) in mice(68). Natural Killer cells are effectors of the natural immune system and provide the first line of defense against infection(69). Spontaneous non-major histocompatibility complex (MHC) restricted killing can be mediated by NK cells(70), by certain activated T-cells, and by cells in the monocyte/macrophage series. NK cells are present in high numbers at birth, when they constitute about 20% of circulating lymphocytes. After an initial decline during the first year of life, the percentage of NK cells slowly increases to adult levels. NK cells in cord blood are less active than those in adults; this may reflect their immaturity or the absence

of cytokines necessary for full NK activation(62). HIV positive children may have increased natural killer cells which is associated with a decreased CD4 count. Unlike adults an increase in these cells might be noted in early stages of HIV infection(71). Extensive research is under way worldwide to determine the role of interferons and interleukins in the regulation of the NK cell. They mediate cytolytic reactions that do not require expression of class II MHC molecules on the target cells. Certain T lymphocytes which are either α/β positive or γ/δ positive may express, particularly upon activation, a cytolytic activity that resembles that of NK cells. These T lymphocytes should not be termed NK cells. They could be termed either T lymphocytes displaying "NK-like" activity or "non-MHC requiring" cytolysis. It is known that the recognition of target cells by NK cells will lead to target cell destruction. Propodium iodide was used to stain target cells killed by NK cells and it was found that NK cell cytotoxicity was not cell cycle specific(72).

The activity of NK cells against infectious diseases has received less attention, although in recent years increasing evidence has emerged that NK cells are involved in defence against such forms of disease(70). Cells with NK activity have been able to inhibit microbial colonization and growth, including intracellular and extracellular parasites, fungi, and a wide variety of viral infections. The role played by NKcells in host defences against bacterial infections has remained unclear. NK cells may be of major significance in viral infections because of the ability of viruses to alter cell metabolism and architecture, and also because viruses are potent inducers of interferons (IFNs), which augment NK cell

cytotoxicity and proliferation(70). The augmented NK cell response that occurs after viral infection is the result of two phenomena: the activation of NK cells to a higher state of cytotoxicity and the proliferation of NK cells, which results in an increase in NK cell number. Natural killer cells are dependent on a population of HLA-DR+ accessory cells in viral infection(73). Depletion of HLA-DR positive cells from peripheral blood lymphocytes depletes NK activity(74).

The most profound changes in LGL populations in pregnancy occur at the maternal/fetal interface in the uterus. NK activity has been measured and compared to blood samples from pregnant and non-pregnant women and from men. Blood NK activity was found to be lower in women than in men, and to fall significantly in non-pregnant women in the periovulatory period. In pregnancy, NK activity of blood lymphocytes is decreased from 16 weeks of pregnancy onwards. NK activity returns to normal (control) levels between 9 and 40 weeks post-partum. Antibody-dependent cell-mediated cytotoxic (ADCC) activity decreases during pregnancy and returns to slightly above normal (control) levels post-partum(70). The mechanism for the reduction in peripheral NK activity in pregnancy is not clear.

CHAPTER 2

AIMS AND OBJECTIVES

2.1 Purpose of Study

To evaluate the immunological basis for the use of vitamin A as an intervention to modulate B cells, natural killer cell counts and the activation marker (I2) in HIV seropositive pregnant women in developing countries. The effects of vitamin A on the T lymphocytes was the research focus of another researcher.

2.2 Hypothesis

Vitamin A and beta-carotene supplementation will modulate the immune responses in HIV infected pregnant women, either by enhancing B-lymphocyte production and differentiation, by stimulating the production of natural killer cells or by altering immune activation.

2.3 Primary Objective

To assess the effects of Vitamin A and Beta-Carotene on natural killer cells and B cells.

Specific Objectives:-

(a) To evaluate B cell percentages and absolute numbers.

(b) To evaluate natural killer cell percentages and absolute numbers.

2.4 Secondary Objectives

To assess the effects of Vitamin A on haematological parameters and activation status of lymphocytes.

Specific Objectives:-

(a) To evaluate the following parameters (haemoglobin, haematocrit, white cell counts, platelets and lymphocytes).

(b) To evaluate I2 (HLA-DR), the activation marker.

CHAPTER 3

PATIENTS AND METHODS

3.1 PATIENTS

Trial Population

Subjects were recruited from pregnant women presenting to the ante-natal clinic (ANC), King Edward VIII Hospital (KEH). These subjects were part of a randomised, double blind, placebo controlled intervention trial using vitamin A.

Recruitment of Study Subjects

Routine HIV screening was conducted at King Edward VIII Hospital-Ante Natal Clinic (KEH-ANC) and was preceded by pre-test counselling. When women returned for their results those testing positive were given post-test counselling. Those positive women who were between 28-32 weeks gestation had the study objectives and design explained to them and were asked for written informed consent to participate in the trial. Women were then randomly allocated to receive daily vitamin A (retinol palmitate and beta-carotene) or placebo.

Sampling

Sample size was determined at 39 in order to obtain test significance level alpha 0.05 with 80% power in order to detect 5-7% change in numbers of absolute lymphocyte and Natural Killer cells. The larger numbers recruited initially allowed for the subsequent sub-sampling at the various time intervals (not all patients

recruited had the tests repeated at each interval and allowance was made for drop-outs.

For the purposes of this study the first 208 patients recruited to the intervention trial were evaluated. Of the 208 patients intially recruited 103 were in the vitamin A group and 105 were in the placebo group. Sampling was performed on enrollment, at delivery, 1 week, and 3 months post delivery.

Exclusion Criteria

Any woman who did not have a fixed, traceable address at which she would remain for 15 months.

Ethical Considerations

It has been well documented that vitamin A is a potential teratogen at daily doses of 25 000 IU. The Teratology Society has recommended that a daily dose of 8000 IU of vitamin A (as retinol esters) should be considered as the maximum dose to be given during pregnancy. The subjects were given 5 000 IU of retinol palmitate and it was given in the last trimester of pregnancy to minimise any possibility of embryotoxic effects. Since beta-carotene has not been associated with any embryotoxic effects an additional 30 mg of beta-carotene was included together with the 5000 IU of retinyl palmitate.

Ethical Approval

Ethical approval was granted by the Ethics Committee for the application of the randomised trial on vitamin A which was submitted by the Department of Paediatrics and Child Health to the Ethics Committee, University of Natal.

Ethical approval for this study was granted by the Ethics Committee.

The reference number is H192/97.

3.2 METHODS

3.2.1 Vitamin A Concentrations

Vitamin A - a daily tablet containing 5000 IU of retinol palmitate and 30mg of beta-carotene was administered from time of entry to study (28-32 weeks) until onset of labour. This was followed by a further 200,000 IU one day after delivery. Blood was taken for vitamin A concentration on entry to the study and one month later to test the effect of supplementation. The serum was separated and stored at -70 ^{o}C until analysis. Precautions were taken to protect the serum from light during separation, storage and analysis as vitamin A is sensitive to photo degradation. Vitamin A (serum retinol) was measured by normal phase high pressure liquid chromatography using fluorescence detection. The method used was a modification of a previously reported method that had been successfully set up in our analytical unit and had been used in several studies (attached to a programmable fluorescence detector - HP 1046)(75). This method has been validated by using standard reference material for retinol (SAM 968a) from the National Institute for Standards and Technology (Gaithersburg, MD). All samples were analysed in duplicate within six months of collection and the technician was blinded as to the treatment groups.

Retinol levels for the study was done in collaboration with the Analytical Unit, Department of Physiology, by Mrs Inga Elson in the Medical School, University of Natal.

3.2.2 Monoclonal Antibodies

Monoclonal antibodies are produced by clones of plasma cells. Antibodies from a given clone are immunochemically identical and react with a specific epitope on the antigen against which they are raised(76).

In our study CD19 was used as a marker for B lymphocytes, CD56 as the marker for natural killer cells and I2 (HLA-DR) as an activation marker.

CD19 / B4 Monoclonal Antibody

CD19 is a murine monoclonal antibody reagent. In conjunction with a fluorescent label, it is used to identify and enumerate the percentage of CD19 positive B lymphocytes in whole blood or mononuclear cell preparations by flow cytometry or fluorescence microscopy. This antigen is expressed on all normal B lymphocytes, follows the Ia antigen in B cell ontogeny, and is only lost prior to plasma cell differentiation(77). The CD19 antigen is present on all B cells isolated from lymphoid organs and on approximately 5% of normal adult bone marrow cells. The immunoglobulin chain composition of CD19 is mouse IgG1 heavy and kappa light chains.

CD56/NKH-1 Monoclonal Antibody

CD56 (NKH-1) defines a human Natural Killer cell antigen, with a molecular weight of 200-220 Kd(78). It is expressed on a subpopulation of peripheral blood large granular lymphocytes (LGL) which demonstrate Natural Killer activity. More than 95% of cells capable of mediating spontaneous non-MHC restricted

cytotoxicity in peripheral blood are contained within the 10-12% of peripheral blood mononuclear cells (PBMC) that express CD56 (NKH-1)(78). 20-25% of CD56 positive cells co-express CD3 and T cell receptor gene products. CD56 (NKH-1) is not expressed on other T cell populations, B cells, monocytes, granulocytes and erythrocytes. The CD56 (NKH-1) antibody helps detect and enumerate Natural Killer cells in normal and disease states. Natural Killer cells also commonly express the cell surface marker CD16(70). The immunoglobulin chain composition of CD56/NKH-1 is mouse IgG1 heavy and kappa light chains.

HLA-DR/I2 Monoclonal Antibody

The I2 antigen is an HLA-D/DR related Ia-like antigen with a estimated molecular weight of 29-34 Kd. It is present on normal B cells, monocytes, and activated T lymphocytes(64). It is not present on progranulocytes or resting T cells. The immunoglobulin chain composition of HLA-DR/I2 is mouse IgG2a heavy and kappa light chains. Because dual markers were not used to identify subsets, the results of the activation marker I2 reflect both B lymphocyte and T lymphocyte activation.

3.2.3. Flowcytometry

Flowcytometry is a modern method of studying cells. Various techniques could be employed to ascertain multiple physical or biological properties of a cell. Quantitative cell analysis, at the single-cell level, is possible through flow-cytometry. Cell populations and sub-populations in a specimen can be determined by the number and type of cell surface antigens, or by measured internal parameters(79). Samples for flowcytometry were processed on a Coulter Epics Profile II flowcytometer. (Coulter Electronics-Hialeah, Florida, U.S.A.). The instrument was programmed to measure forward scatter (FS), log side scatter (LSS), and log fluorescence.

Alignment and Sample Preparation

Flow Check (Coulter Electronics-Hialeah, Florida, U.S.A.) alignment reagent was used. Immunobrite beads (Coulter Electronics-Hialeah Florida,U.S.A.) were used to calibrate the instrument. A normal control was prepared and analysed (this sample was collected from a normal person who had not been on any medication or suffered from any ailment affecting the immune system fourteen days prior to sample collection). This sample was prepared exactly like the study patients' test samples of the day. The normal control lymphocyte zone was gated to give a yield of at least 96% of total cells analysed. Cursors were set on histograms to differentiate between positive and negative zones. Samples from the study patients were collected and prepared as follows:-

1. 5mls of whole blood was collected in EDTA anticoagulant.
2. All tests were set up within one hour of collection.
3. 100ul of a well mixed sample was aliquoted into 75 x 12mm plastic tubes(polyurethane).
4. Respective monoclonal antibodies and isotypic controls were added.
5. The samples were vortexed and incubated at room temperature in the dark for 30 minutes.

The samples were passed through a Coulter Workstation - Q-Prep.(Coulter Epics Immunology Workstation - Hialeah, Florida, U.S.A.). This instrument has a Lysing (reagent A), stabilising (reagent B) and fixing agent (reagent C).

Reagent A - (formic acid) - 600ul

Reagent B - (sodium chloride, sodium sulphate and sodium carbonate)- 265ul

Reagent C - (paraformaldehyde) - 100ul

The reagents were dispensed automatically (the amounts as indicated above) with vigorous mixing at intervals in a 35 second cycle in the Coulter workstation. After preparation in the Q-Prep workstation the control and test samples were analysed on the flowcytometer. Gated cells were expressed as a percentage on the positive analysis histograms. This was later converted to absolute numbers using the full blood count parameters.

Quality Control

Fluorescent microspheres were used to align the flowcytometer laser and for calibration. To control for non-specific binding isotypic controls were used.

3.3 Data Management and Statistical Analysis

MRC-Biostatistician was consulted. The following software packages were used: EPI-INFO6 for data capture and SAS Version 6.12 was used for statistical analysis. Statistical analysis consisted of univariate analysis. Univariate analysis depended on type of data (eg Chi-square, T-test).

Descriptive statistics consisting of means and confidence intervals were calculated by group and time for each of the variables of interest. Variables were generally normally distributed. Parameters analysed were haemoglobin, platelets, white cell counts, absolute lymphocyte counts, B lymphocyte percentage and absolute counts, Natural Killer cell percentage and absolute counts, activation marker (I2), percentage and absolute counts and retinol levels. Baseline parameters were compared between the placebo and vitamin A groups using Student's paired and unpaired t-test. The difference between the three month measurement and the baseline was calculated to assess the changes over time. The two groups were then compared with respect to this change using a unpaired t-test. The difference from baseline to 3 months post-delivery within each group was assessed using a paired t-test. A significance level of 0.05 was used.

3.2.4 Haematological Parameters

The patients peripheral blood samples were analysed for – haemoglobin, haematocrit, platelets, white cells, and absolute lymphocyte counts. These parameters were evaluated at each sampling interval on the Coulter Stks. Model (Coulter Electronics - Hialeah, Florida, U.S.A.).

CHAPTER 4

Results

The results are presented as follows:-

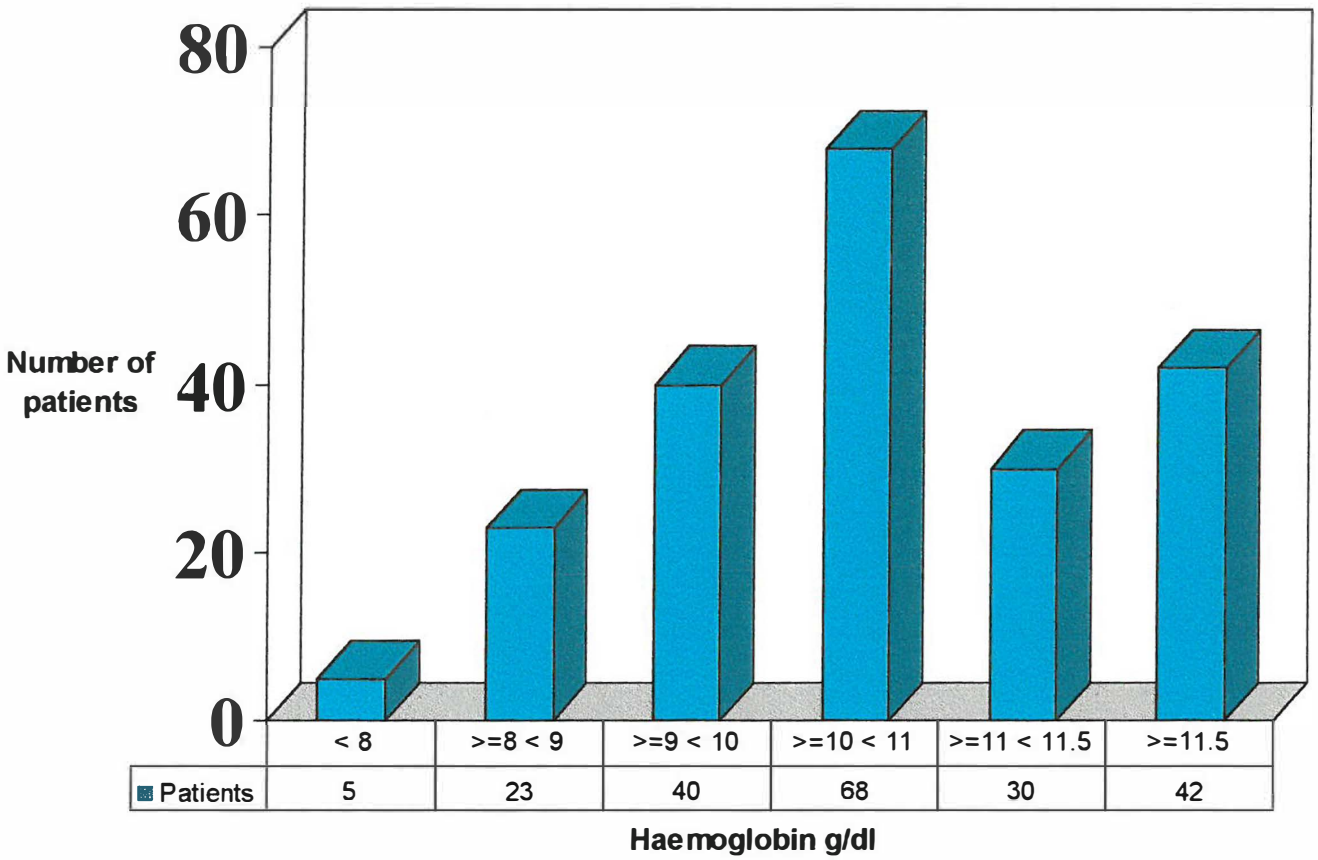
(1) Baseline Values

Figure 1 to Figure 4.

(2) Trends

Figure 5 to Figure 15.

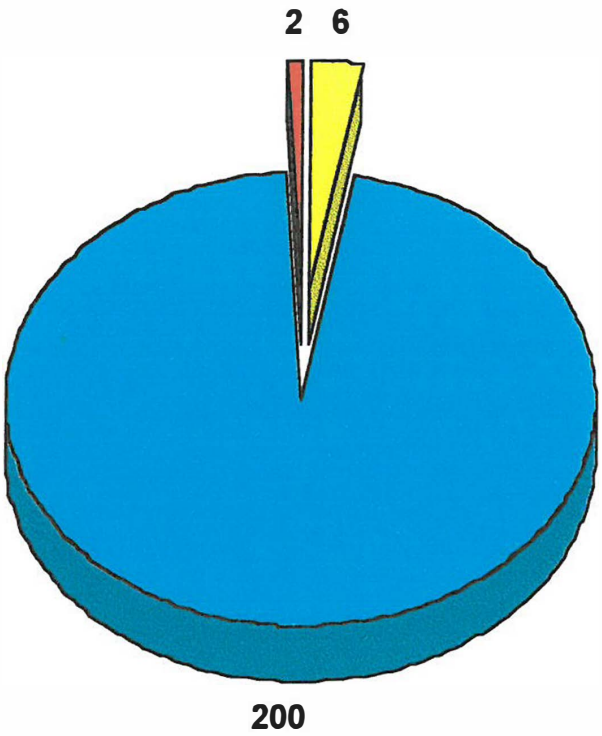
Figure 1
BASELINE HAEMOGLOBIN VALUES



Mean = 10.57g/dl Range = 7.6 - 13.5 (n=208)

Figure 2

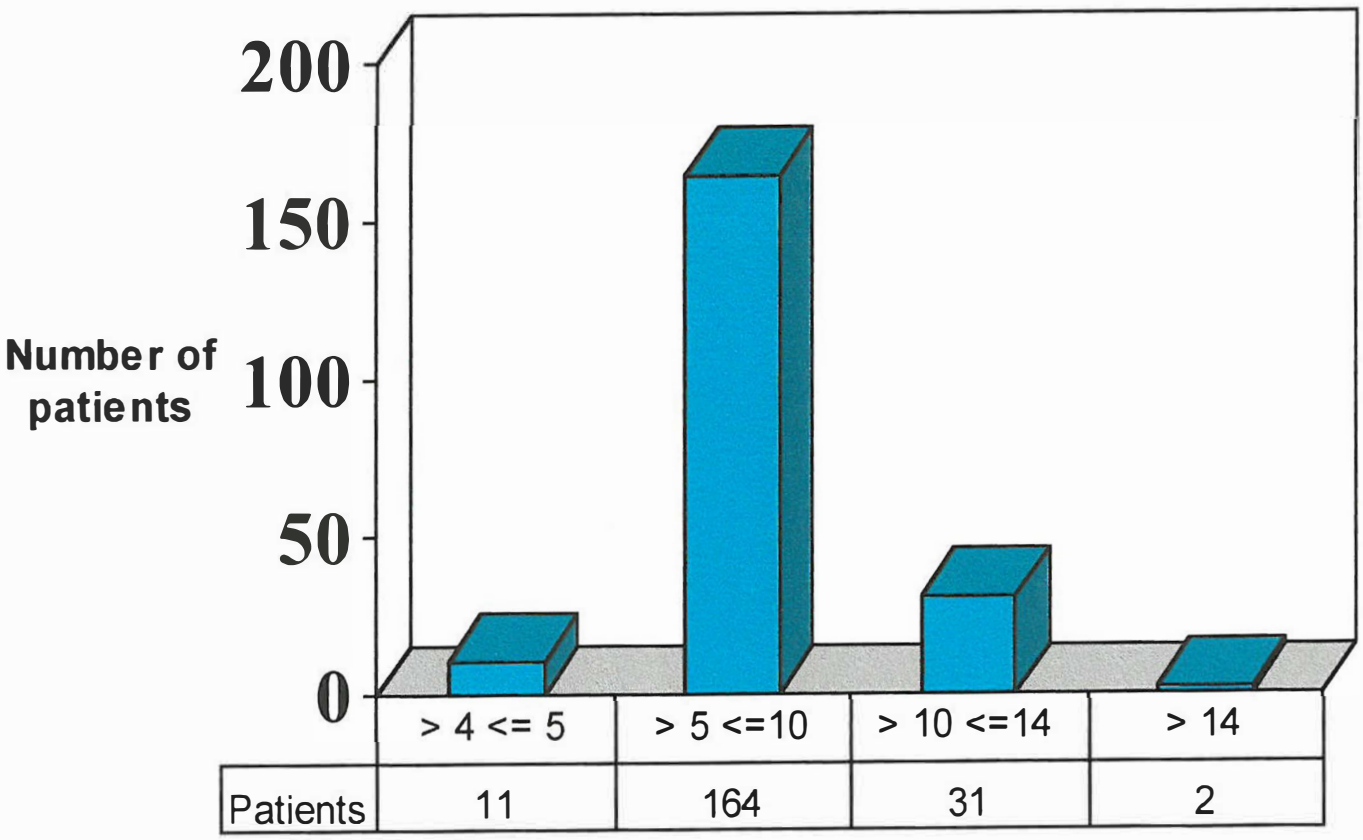
BASELINE PLATELET COUNTS



Mean = 255.82 x 10^{9}/l Range = 65 - 490 (n=208)

Figure 3

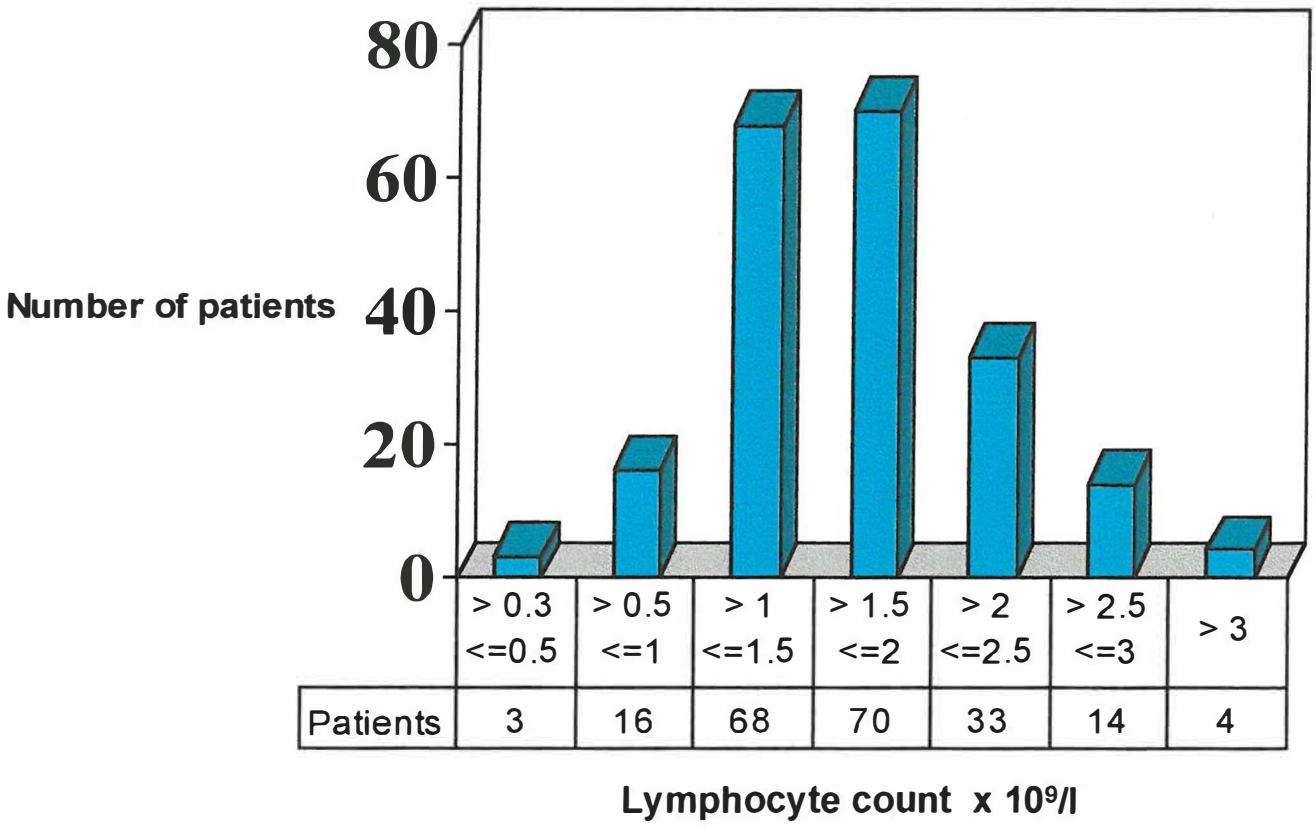
BASELINE WHITE CELL COUNTS



Mean = 7.86 X 10^9 /l Range = 4.0 - 16.7 (n=208)

Figure 4

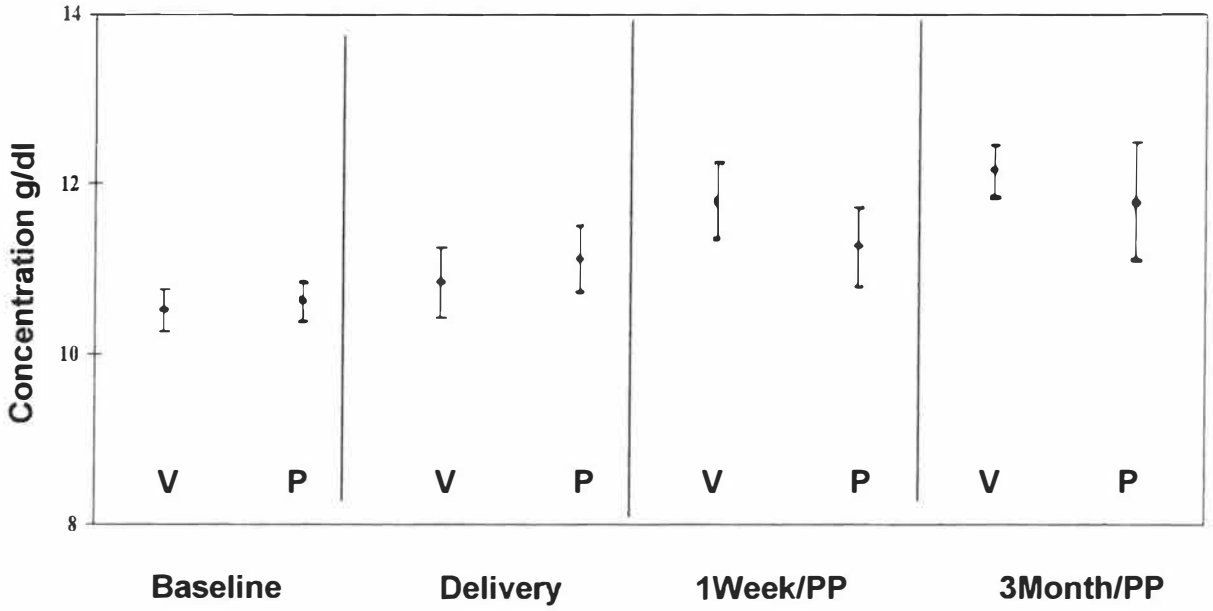
BASELINE LYMPHOCYTE COUNTS



Mean = 1.74 X 10 ^{9} /l Range = 0.4 - 4.9 (n=208)

Figure 5

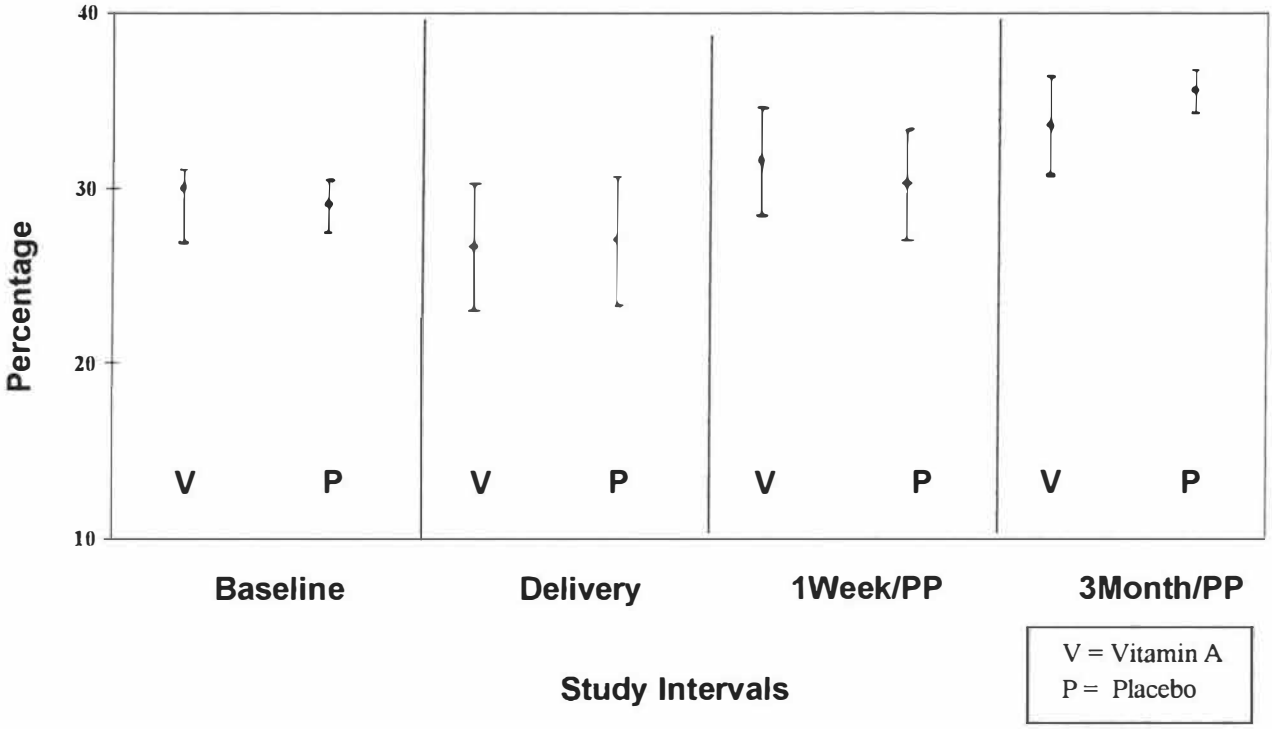
HAEMOGLOBIN - TRENDS



- **Vitamin A - significant increase between baseline and 3 months p < 0.0000**
- **Vitamin A - significant increase between delivery and 1 week p < 0.0017**
- **Vitamin A - significant increase between delivery and 3 months p < 0.0000**
- **Placebo - significant increase between baseline and delivery p < 0.0209**
- **Placebo - significant increase between baseline and 3 months p < 0.0000**
- **Placebo - significant increase between baseline and 1 week p < 0.0057**

Figure 6

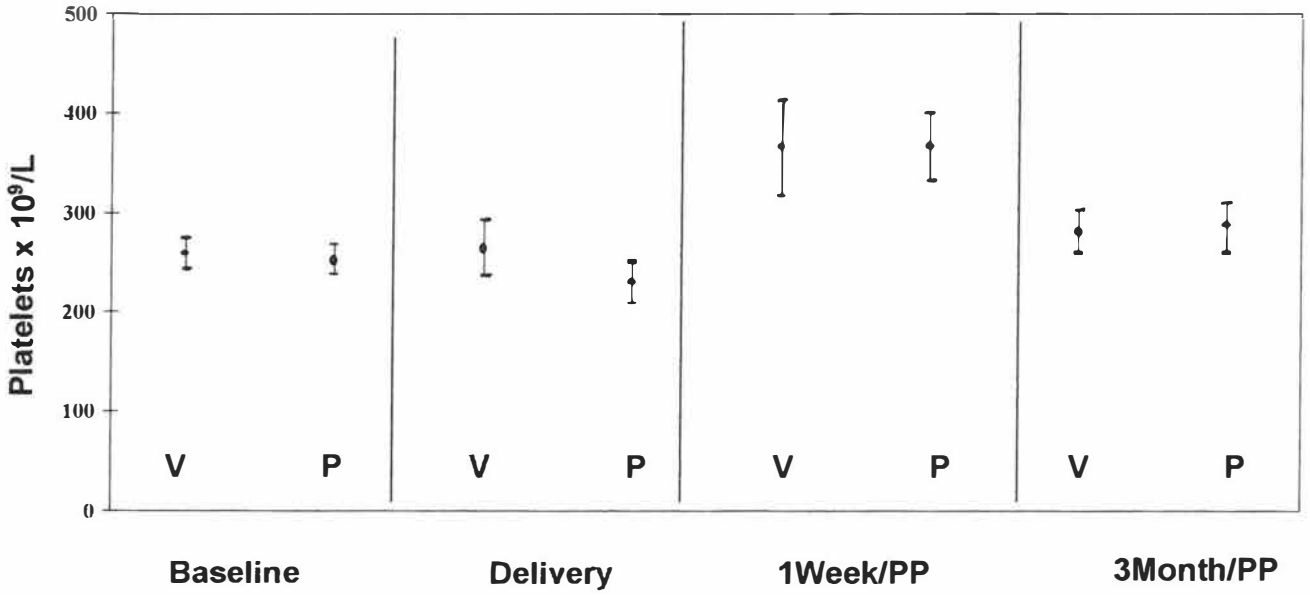
HAEMATOCRIT - TRENDS



- **Vitamin A - significant increase between baseline and 3 months $p < 0.0121$**
- **Vitamin A - significant increase between delivery and 1 week $p < 0.0389$**
- **Vitamin A - significant increase between delivery and 3 months $p < 0.0029$**
- **Placebo - significant increase between baseline and 3 months $p < 0.0000$**
- **Placebo - significant increase between delivery and 3 months $p < 0.0001$**
- **Placebo - significant increase between 1 week and 3 months $p < 0.0099$**

Figure 7

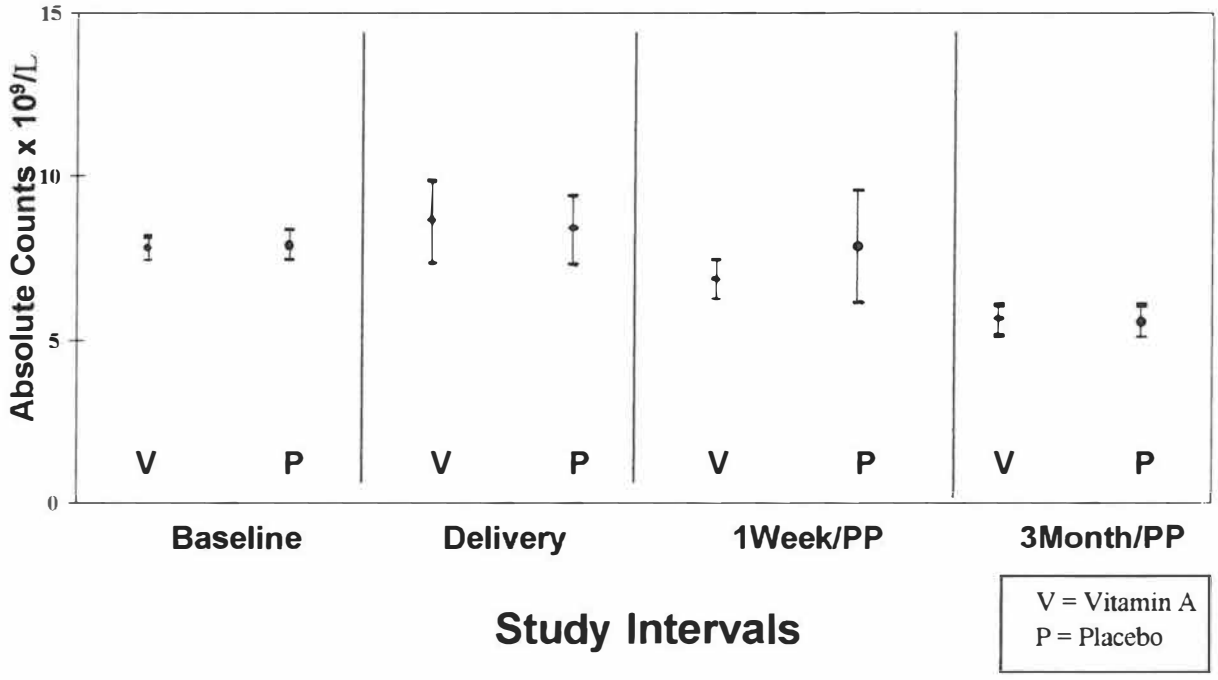
PLATELET COUNTS - TRENDS



- **Vitamin A - significant increase between baseline 1 week p < 0.0000**
- **Vitamin A - significant increase between delivery and 1 week p < 0.0007**
- **Vitamin A - significant decrease between 1 week and 3 months p < 0.0147**
- **Placebo - significant increase between baseline and 1 week p < 0.0000**
- **Placebo - significant increase between baseline and 3 month p < 0.0224**
- **Placebo - significant increase between delivery and 1 week p < 0.0000**
- **Placebo - significant increase delivery and 3 months p < 0.0010**
- **Placebo - significant decrease between 1 week and 3 months p < 0.0027**

Figure 8

WHITE CELL COUNTS - TRENDS



- **Vitamin A - significant decrease between baseline and 1 week $p < 0.0079$**
- **Vitamin A - significant decrease between baseline and 3 months $p < 0.0000$**
- **Vitamin A - significant decrease between delivery and 1 week $p < 0.0093$**
- **Vitamin A - significant decrease between delivery and 3 months $p < 0.0000$**
- **Vitamin A - significant decrease between 1 week and 3 months $p < 0.0186$**
- **Placebo - significant decrease between baseline and 3 months $p < 0.0000$**
- **Placebo - significant decrease between delivery and 1 week p , 0.0149**
- **Placebo - significant decrease between delivery and 3 months $p < 0.0000$**
- **Placebo - significant decrease between 1 week and 3 months $p < 0.0044$**

Figure 9

LYMPHOCYTES - TRENDS

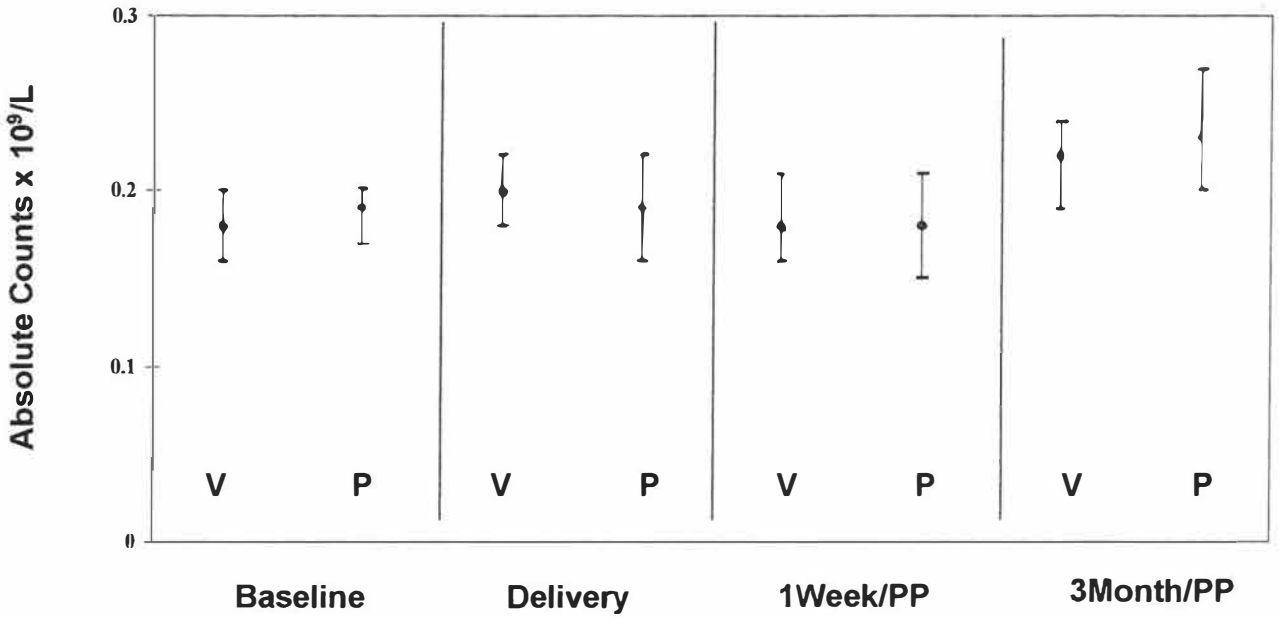
- **Vitamin A - significant increase between baseline and 3 months $p < 0.0285$**
- **Vitamin A - significant increase between baseline and 1 week $p < 0.0006$**
- **Vitamin A - significant increase between delivery and 1 week $p < 0.0034$**
- **Vitamin A - significant increase between delivery and 3 month $p < 0.0077$**
- **Placebo - significant increase between baseline and 3 month $p < 0.0429$**
- **Placebo - significant increase between baseline and 1 week $p < 0.0001$**
- **Placebo - significant increase between delivery and 1 week $p < 0.0223$**

Figure 10
B CELLS - CD19 - TRENDS

- **Vitamin A - significant decrease between baseline and 1 week p < 0.0024**
- **Vitamin A - significant decrease between delivery and 1 week p < 0.0157**
- **Vitamin A - significant increase between 1 week and 3 months p < 0.0016**
- **Placebo - significant decrease between baseline and 1 week p < 0.0005**
- **Placebo - significant decrease between delivery and 1 week p <0.0036**
- **Placebo - significant increase between 1 week and 3 months p <0.0025**

Figure 11

B CELLS - CD19 - TRENDS



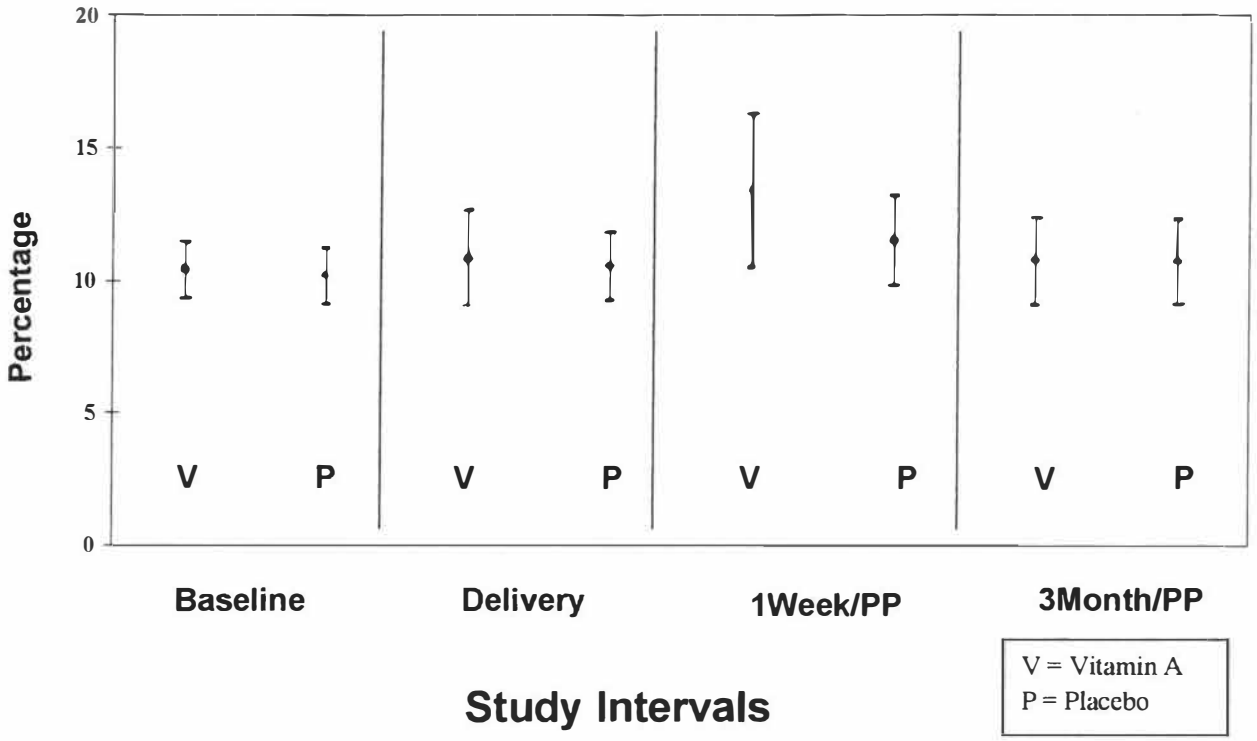
Study Intervals

V = Vitamin A
P = Placebo

- **Vitamin A - significant increase between baseline and 3 months $p < 0.0459$**
- **Vitamin A - significant increase between 1 week and 3 months $p < 0.0464$**
- **Placebo - significant increase between baseline an 3 months $p < 0.0111$**
- **Placebo - significant increase between 1 week and 3 months $p < 0.0035$**

Figure 12

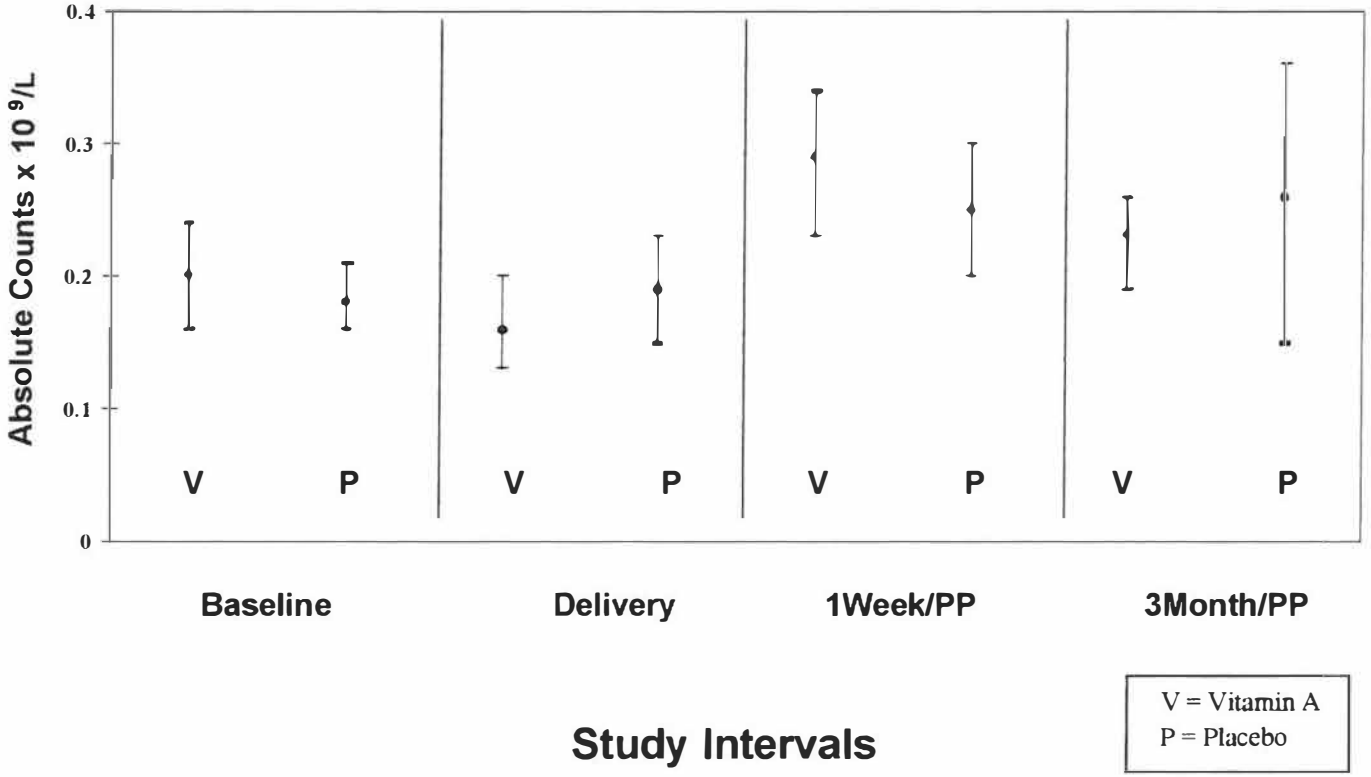
NATURAL KILLER CELLS - CD56 -TRENDS



- **Vitamin A - significant increase between baseline and 1 week $p < 0.0215$**
- **Vitamin A - significant increase between delivery and 1 week $p < 0.0325$**
- **Vitamin A - significant decrease between 1 week and 3 months $p < 0.0062$**
- **Placebo - significant increase between delivery and 1 week $p < 0.0478$**

Figure 13

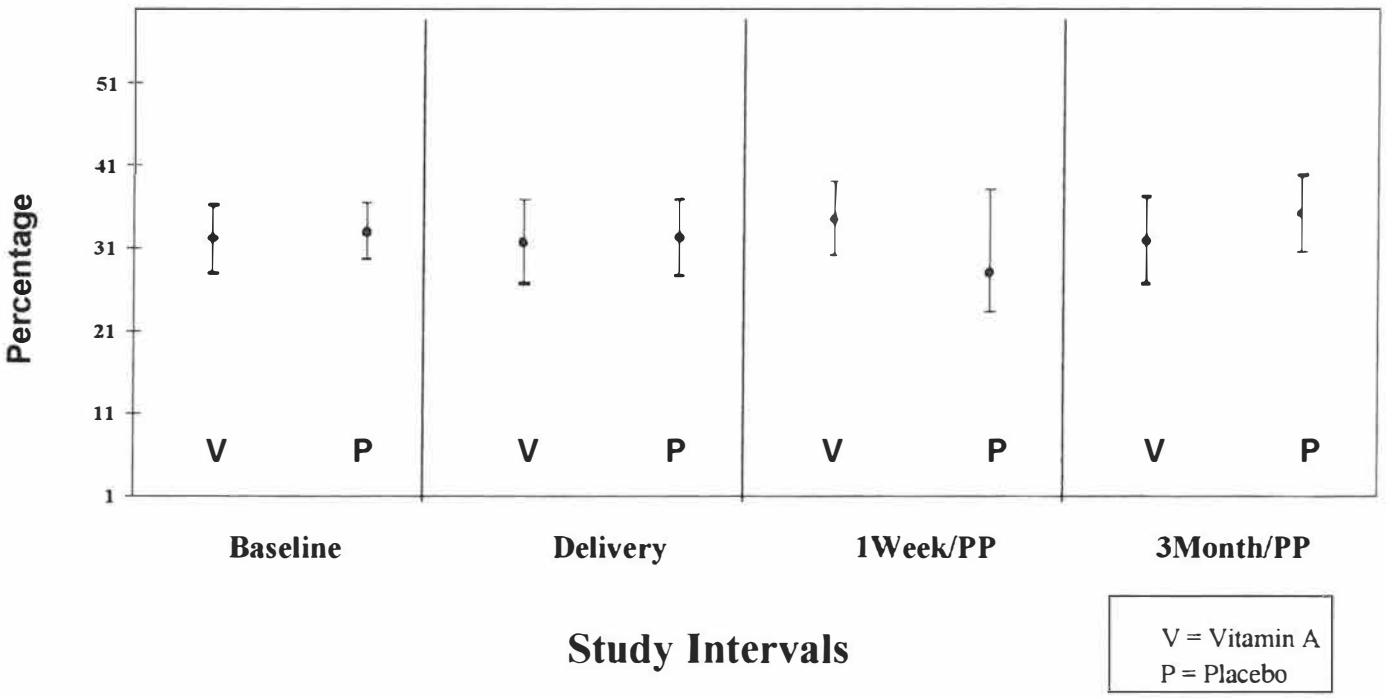
NATURAL KILLER CELLS - CD56 - TRENDS



- **Vitamin A - significant increase between baseline and 1 week $p < 0.0170$**
- **Vitamin A - significant increase between delivery and 1 week $p < 0.0004$**
- **Vitamin A - significant increase between delivery and 3 month $p < 0.0133$**
- **Vitamin A - significant decrease between and 1 week and 3 month $p < 0.0054$**
- **Placebo - significant increase between baseline and 1 week $p < 0.0088$**
- **Placebo - significant increase between delivery and 1 week $p < 0.0072$**

Figure 14

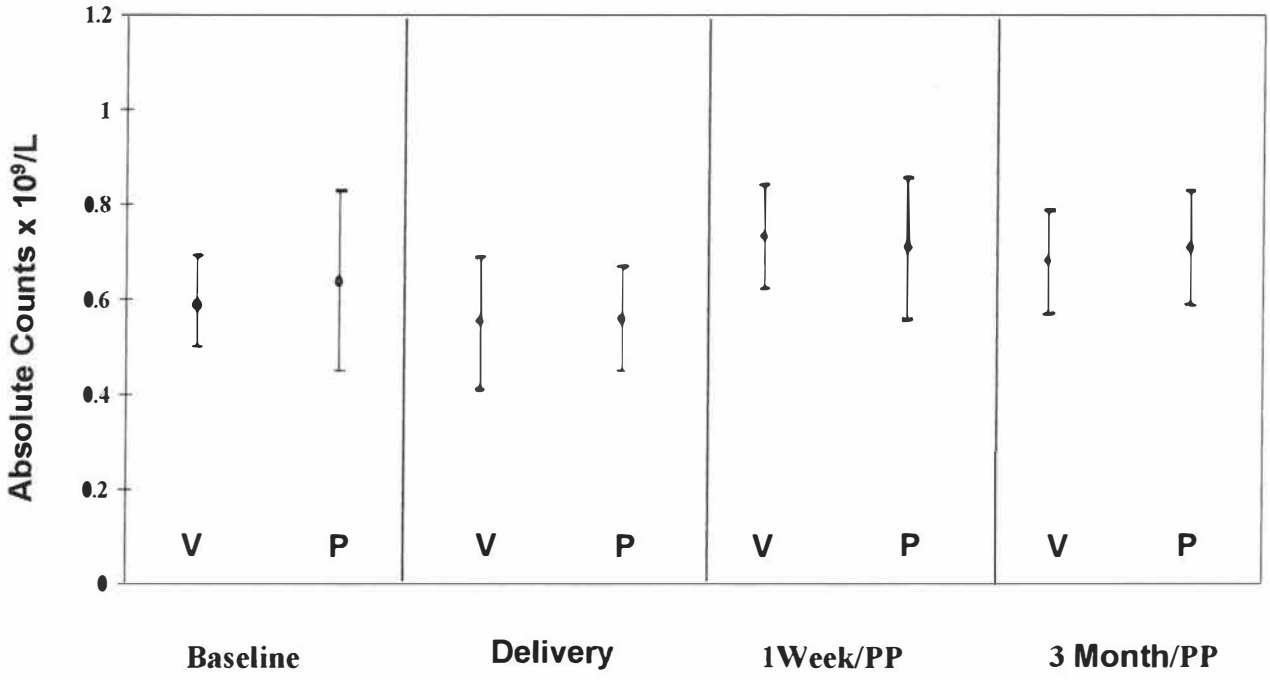
ACTIVATION MARKER - I2 - TRENDS



- **Vitamin A - no significant changes noted**
- **Placebo - no significant changes noted**

Figure 15

ACTIVATION MARKER - I2 - TRENDS



- **Vitamin A - significant increase between delivery and 1 week $p < 0.0452$**
- **Placebo - significant increase between delivery and 1 week $p < 0.0141$**

CHAPTER 5

DISCUSSION

Baseline Parameters

These were assessed when study subjects were enrolled. The following parameters were tested – haemoglobin, haematocrit, platelets, white cells, absolute lymphocyte counts, B lymphocyte percentages and absolute counts, Natural killer cell percentages and absolute counts and the activation marker percentages and absolute counts. No differences were present at baseline between the placebo and vitamin A treated group (Table 1).

Table 1

Baseline parameters in Vitamin A and Placebo HIV(+) Black Mothers.

	VITAMIN A	PLACEBO
n	103	105
Hb G/dl	10.51 (10.26-10.76)	10.63 (10.41-10.85)
HCT %	29.96 (26.89-31.03)	29.02 (27.51-30.54)
PLT $X10^9/l$	258.40 (243.39-273.37)	253.30 (238.29-268.31)
WCC $X10^9/l$	7.80 (7.41-8.19)	7.92 (7.44-8.39)
LYMPHS $X10^9/l$	1.77 (1.64-1.89)	1.71 (1.60-1.81)
CD56 %	10.42 (9.29-11.55)	10.15 (9.11-11.19)
CD56 ABS $X10^9/l$	0.20 (0.16-0.24)	0.18 (0.16-0.21)
CD19 %	10.26 (9.55-10.97)	10.87 (10.17-11.56)
CD19 ABS $X10^9/l$	0.18 (0.16-0.20)	0.19 (0.17-0.20)
I2 %	32.13 (27.93-36.32)	33.03 (29.67-36.39)
I2 ABS $X10^9/l$	0.59 (0.50-0.69)	0.64 (0.45-0.83)

Results are presented as a Mean with (95% Confidence Intervals)

Baseline Parameters in HIV(+) Black Mothers

Both groups were considered together for the purposes of describing our HIV(+) black African cohort. These were compared to reported parameters in HIV(-) normal pregnant females.

Haemoglobin and Haematocrit

In normal pregnancy, the maternal blood volume increases markedly (80,81,82,83). The blood volume at or very near term increases by approximately 45%. The degree of expansion varies considerably. In some women there is only a modest increase and in others the blood volume nearly doubles. The maternal blood volume starts to increase during the first trimester, and eventually plateaus out during the last several weeks of pregnancy. The increase in blood volume results from an increase in both plasma and erythrocytes. Although more plasma than erythrocytes is usually added to the maternal circulation, the increase in the volume of circulating erythrocytes is considerable. In spite of the augmented erythropoiesis, the concentration of haemoglobin, erythrocytes and the haematocrit commonly decrease during pregnancy. The normal range for haemoglobin in pregnancy is 11.0 - 14.0g/dl (84). In this study (see figure 1) the mean haemoglobin at baseline was 10.51 g/dl (CI=10.26-10.76) in the vitamin A group and 10.63g/dl (CI=10.41 - 10.85) in the placebo group. The haemoglobin range was 7.6 - 13.5g/dl. Of the patients 65.4% (136 out of 208) had haemoglobin levels of $\leq$ 11.0g/dl. Five patients had haemoglobin levels of $\leq$ 8.0g/dl.

Alger *et al*, reported that HIV+ patients had a significantly lower haemoglobin (10.6 ± 1.2g/dl) at recruitment than the HIV- patients (11.3 ± 1.3g/dl)(42).

Various factors including poor dietary intake, lack of supplements during pregnancy, blood loss and infections may contribute to the anaemia.
In this study the haematocrit values were proportional to their haemoglobin levels. The mean haematocrit levels were 29.96% (CI=26,89 - 31.03) in the vitamin A group and 29.02% (CI=27.51 - 30.54) in the placebo group.

Platelets

Conflicting reports have been made about platelet counts during pregnancy. Some early reports show increase in platelet counts and others show no significant change. Some more recent reports using modern automated technology show a progressive fall in pregnancy(85,86,87,88). Average platelet counts reported for normal pregnancy include 219 X 10^9/l during 27 - 30 weeks(89), 183.9 X 10^9/l in the third trimester(85), and 284.18 X 10^9/l during 26 – 30 weeks of pregnancy(86). In this study (see figure 2), the mean platelet count at baseline in the vitamin A group was 258.40 X 10^9/l (CI=243.39-273.37) and in the placebo 253.30 X 10^9/l (CI=238.29-268.31). Six patients had thrombocytopenia with counts <150 X10^9/l, of whom 2 had counts of <100 X 10^9/l. The reasons for the thrombocytopenia were not noted. Two patients had counts of >450 X 10^9/l.

Total White Cell Count

Total leucocyte count rises early in gestation and remains elevated during pregnancy, with neutrophils accounting mainly for the increase(52,53,54,90,91). Moore *et al* established a mean leucocyte count of 8.55 ± 1.13 x10^9/l in normal pregnant women between 29-39 weeks(65). Pitkin *et al*, reported leucocyte counts of 5.60-12.21 x 10^9/l in the third trimester of pregnancy. Maclean *et al* established a mean white cell count of 10.7 X10^9/l in the 28th week of pregnancy. In this study (see figure 3) the mean leucocyte counts at baseline was 7.86 x 10^9/l with a range of 4.0-16.7 X 10^9/l. Eleven out of 208 patients (5.2%) of our cohort had white cell counts <5.0 X 10^9/l. Two patients in the study had white cell counts of >16 X 10^9/l.

Lymphocytes

The reports in the literature are conflicting. In normal pregnancy there is no change in the lymphocyte count (54,55,57,91,). Pitken *et al* and Johnstone *et al* found that absolute lymphocyte counts decline in pregnancy (90,92). Pitken et al, reported a lymphocyte range of 1.13-2.58 X 10^9/l in the third trimester of pregnancy(90).

Maclean *et al*, reported a lymphocyte mean value of 1.8 X 10^9/l at 28 weeks of gestation(54). Moore *et al* found a mean value of 1.57 X 10^9/l at 29 - 39 weeks of pregnancy(65). In this study (see figure 4) the mean lymphocyte count (X 10^9/l) at baseline was 1.77(CI=1.64-1.89) and 1.71(CI=1.60-1.81) in the vitamin A

and placebo groups respectively.

Lymphopenia was noted in 9.1% (19 out of 208) with lymphocyte counts of >0.3≤1.0 $X10^9$/l. Lymphocytosis of >3.5 X 10^9/l was noted in 2 patients while the remaining 187 patients (89.9%) had normal lymphocyte counts.

B Cells

Dodson *et al* reported that B cell counts in gravid women is normal throughout gestation(55). They reported a B cell count of 10% between 25-30 weeks of pregnancy. Strelkauskas *et al* found that B cell counts (as measured by the presence of surface immunoglobulin or the presence of B cell surface antigens) were increased(56). Tallon *et al* established a B cell count of 9.4% and a decrease in the absolute count in the third trimester of normal pregnancy(52). In this study (see Table 1), the B cell (CD19) percentage was 10.26% (CI=9.55 - 10.97) and 10.87% (CI=10.17 - 11.56) in the vitamin A and placebo groups respectively. The B cell mean absolute count ($X10^9$/l) was 0.18 (CI=0.16 - 0.20) and 0.19 (CI=0.17 - 0.20) in the vitamin A and placebo groups respectively.

Natural Killer Cells

Very limited research has been performed on Natural Killer cells using CD56 as a marker in this setting.

In this study (see Table 1) the Natural Killer cell (CD56) mean percentage was 10.42 (9.29 - 11.55) and 10.15% (CI=9.11 - 11.19) in the vitamin A and placebo groups respectively. The Natural Killer cell mean absolute count was 0.20

(CI=0.16 - 0.24) and 0.18 (CI=0.16 - 0.21) X 10^9/l in the vitamin A and placebo groups respectively.

Kuhnert *et al* reported a mean Natural Killer cell percentage of 14.0 ± 6.3% in non-pregnant females and 12.2 ± 5.4% in pregnant females in the third trimester of pregnancy(93). They also reported an absolute count 0,253 x 10^9/L in non-pregnancy and a count of 0.192 x 10^9/L in the third trimester of pregnancy.

Activation Marker

Moore *et al* concluded that the mean Ia (activation marker) percentage in the 29-39 weeks of normal pregnancy was 20.9% (65). Maclean *et al* found that lymphocytes from pregnant women had increased potential rather than increased activity. This group of workers established that the value for Ia (activation marker) in the 28th week of pregnancy was 0.11 X 10^9/l (54). In this study (see Table 1) the activation marker (I2) mean percentages were 32.13 (CI=27.93 – 36.32) and 33.03%(CI=29.67 - 36.39) in the vitamin A and placebo groups respectively. The activation marker mean absolute values were 0.59 (CI=0.50 – 0.69) and 0.64 (CI=0.45 - 0.83) X 10^9/l in the vitamin A and placebo groups respectively.

VITAMIN A

Table 2

Retinol levels at Baseline in the Vitamin A group and Placebo Group

	VITAMIN A	PLACEBO		
Retinol Levels <20ug/dl	37.5% (n=15)	38.5% (n=20)		
Retinol Levels >20<40 ug/dl	50.0% (n=20)	55.7% (n=29)		
Retinol Levels >40 ug/dl	12.5% (n=5)	5.7% (n=3)		
Mean Retinol Levels ug/dl	25.65 (21.71-29.60)	24.38 (21.80-26.97)	P-VALUE 0.5758	RANGE 7.8 – 64.5

The normal retinol level in non-pregnant and pregnant females is 20--60ug/dl(94). Retinol levels for the two groups were comparable and there was no significant differences noted (p = 0.5758). The mean retinol levels were 25,65ug/dl (CI=21.71 - 29.60) and 24.38ug/dl (CI= 21.80 - 26.97) in the vitamin A and placebo groups respectively. The retinol range was 7.8 - 64.5ug/dl.

In this cohort 92 patients had retinol levels measured (see Table 2). There were 40 patients in the vitamin A group and and 52 patients in the placebo group. It was established that 15 patients (37.5%) in the vitamin A group and 20 patients (38.5%) in the placebo group were deficient. In the vitamin A group

62,5% (25 out of 40) and 61,5% (32 out of 52) in the placebo group had normal retinol levelsIn the patients with normal vitamin A levels >80% of the values were low normals (20 - 40ug/dl).

Coutsoudis *et al* found that women attending the King Edward VIII hospital maternity service were not, a vitamin A deficient population(12).
Another local study done at this hospital showed multiple nutritional deficiencies associated with decreased or inadequate immunity in HIV positive patients(95).
In a study done in the United States mean vitamin A levels were lower in blacks than in Hispanics and whites(96). Pregnancy, HIV infection, low socioeconomic status and race may all be associated with vitamin A deficiency.

Pregnant women may be at higher risk for vitamin A deficiency because of increased demands for vitamin A by the developing fetus. In HIV positive women vitamin A deficiency may occur as a result of many possible factors including low intake and malabsorption of vitamin A-rich foods, liver disease and abnormal urinary losses of vitamin A during infection(97). Stephensen *et al* also reported that patients with pneumonia and sepsis excrete significant amounts of retinol and retinol binding protein (RBP) in their urine, unlike healthy individuals who excrete only trace amounts(97). Therefore, the requirement for vitamin A is greatly increased during acute infections which might be related to the loss of retinol in the urine. A high prevalence of vitamin A deficiency has been demonstrated among HIV-infected adults(98). During infectious illnesses vitamins are utilised in greater amounts than in the normal state and therefore

body vitamin stores are depleted(98). The reasons proposed for the low levels in pregnancy were an increase in circulating blood volume, an inadequate dietary vitamin intake, an increase in renal vitamin clearance, fetal sequestration and an increase in vitamin catabolism and tissue retention. The rise was attributed to the end of the pregnancy as well as factors like oral contraceptives.

Baker et al found that there was progressive decrease in retinol during pregnancy up to 28 weeks which starts early in pregnancy(99). In contrast, others found an increase in retinol levels during pregnancy(100,101). Bruinse *et al* observed low retinol levels in pregnancy and a rapid significant increase in retinol levels within days after delivery(102). The reasons for the low levels in pregnancy were Baranowitz *et al* studied 84 HIV+ patients and found that the majority were deficient or had low normal serum carotene levels(100).

TRENDS NOTED AT VARIOUS INTERVALS OF STUDY

Haemoglobin

Haemoglobin levels at 3 months post partum are significantly increased for both groups (see figure 5). These probably reflect basal non-pregnancy values for the study individuals, and were similar in both groups. The placebo group showed an earlier increase in the haemoglobin level between baseline and delivery compared to the vitamin A group which increased between delivery and 1 week post partum. Similar significant differences were noted within subjects between delivery and 1 week in the vitamin A group and between baseline and delivery in the placebo group.

Alger *et al*, reported a modest increase in the haemoglobin values at delivery in the HIV+ patients(10.6-10.9g/dl). At delivery there was no difference between the infected and uninfected patients(42). Our study showed a similar modest increase between baseline and delivery in the vitamin A (10.51 $\rightarrow$10.83g/dl) and (10.63 $\rightarrow$ 11.12g/dl) in the placebo group.

Haematocrit

In parallel with haemoglobin levels seen the haematocrit trends in our study were also elevated at 3 months compared to baseline in the two groups (see figure 6). The groups were comparable at 3 months and were reverting to normal levels post partum.

Pritchard *et al* reported that in normal pregnancy the haematocrit

returns to normal 6-7 days post partum(82). It is possible that vitamin A therapy may have contributed to earlier normalisation. The significance of this difference is uncertain.

Platelets

The groups were comparable at 3 months with no significant difference in their means (see figure 7). The higher platelet counts at 3 months probably reflect normal non pregnant values. The placebo group and the vitamin A group showed no significant change in pregnancy. The groups behaved similarly after delivery with a rise at 1 week and a subsequent fall at 3 months.

Fenton *et al* found that platelet counts do not change during normal pregnancy(89). Cairns *et al* reported that there is a small decline in platelet counts throughout pregnancy when measured using automated platelet counters(86).

Fay *et al* reported a significant decrease in platelet counts during normal pregnancy which he attributed to increased consumption of platelets(87).

White Cell Counts

The vitamin A and placebo group showed a non significant increase in white cell counts during pregnancy. In both groups there were significant decreases in white cell counts between delivery and 1 week and between 1 week and 3 months (see figure 8). The 3 month values (vitamin A 5.62 and placebo 5.56 X

10^9/l) in both groups were significantly lower than the baseline values (vitamin A 7.80 and placebo 7.92 X 10^9/l). The groups were comparable at 3 months with no significant difference in their mean values.

Alger *et al* found increased white cell counts from enrollment to delivery in both HIV+ and HIV- mothers(42). Pitken et al reported decrease in the total white cell count in the 3rd trimester which continued after delivery. Their 6 week post partum values (non pregnant basal values) ranged between 4.32 - 7.64 X 10^9/l(90). Both groups of our cohort at 3 months were within a similar range.

Lymphocytes

Lymphocyte counts showed significant increases between baseline and 3 months in the vitamin A and placebo groups (see figure 9). At 3 months the groups were comparable with no significant difference in their mean values. In both groups there was no change during pregnancy, a significant increase by 1 week and no change between 1 week and 3 months.

Various authors (52,53, 90,91,92) have reported lower absolute lymphocyte numbers during pregnancy (not always significant) which increase after delivery. In this study the lymphocyte counts between 1 week and 3 months remained the same in the vitamin A group (mean 2.17 – 2.17 x 10^9/l) while there was a non-significant fall in the placebo group (2.15 – 2.04 x 10^9/l). Vitamin A may have a protective effect in maintaining the lymphocyte numbers but further studies are required to assess this.

Alger *et al* reported lower absolute lymphocyte counts at enrollment in a cohort of HIV infected women, which increased at the time of delivery (1.714 $\rightarrow$ 2.214 x 10^9/l) compared to values of the HIV negative cohort (2.365 $\rightarrow$ 2.294 x 10^9/l) (42). These patients did not have vitamin A supplements. This observation was not made in either of the study groups but the trends were similar to those reported in the literature.

B Cell (CD19) Percentage

The two groups showed a significant decrease between delivery-1 week in their B cell percentage counts and a significant increase between 1 week and 3 months (see figure 10). At 3 months both groups were comparable.

Dodson *et al* reported normal T and B cell populations in pregnancy(55). The mean percentages of B cells varied between 10-20% . This cohort showed similar results in the vitamin A and placebo groups.

B Cell Absolute Counts

In both groups significant increases were noted between 1 week and 3 months (see figure 11). Although the actual lymphocyte counts increased between delivery and 1 week the absolute B cell counts remained unchanged during this period. This suggests that the initial lymphocyte increase was most likely due to T cell and Natural Killer cell recovery.

Reports on absolute B cell counts in pregnancy are conflicting.

Tallon *et al* reported that the absolute number of B lymphocytes decreased significantly in the third trimester of pregnancy and post partum(52).

Baines *et al* found that pregnancy has little or no effect on the proportions of B cells in maternal peripheral blood(57).

Strelkauskas *et al* reported an increase in the absolute B cell counts in pregnancy(56).

In this study the 3 months absolute B cell counts were significantly higher than baseline levels with no difference between the groups. These values probably reflect the "non-pregnant" levels. By inference the baseline values and the values at delivery and 1 week were lower than these "non-pregnant" values.

Natural Kiler Cell (CD56) Percentage

The placebo group showed a non significant increase between baseline, delivery, and 1 week and a non-significant decrease at 3 months post partum (see figure 12). There was a significant increase for this parameter between baseline and 1 week in the vitamin A group. The vitamin A group showed a decrease between 1 week and 3 months. The percentages of CD56 positive Natural Killer cells at 3 months were virtually identical in the 2 groups.

Kuhnert *et al* reported a Natural Killer cell percentage of 15.1 $\pm$ 7.1% in women 1 week post partum(93).

Natural Killer Cell Absolute Count

In the vitamin A and placebo group there was no significant changes between baseline and delivery. In both groups there was a significant increase in the absolute count between delivery and 1 week (see figure 13). The placebo group did not show any decrease at all study intervals. However the vitamin A group showed a decline twice. At 1 week post delivery there is a significant rise in the vitamin A group and a gradual fall is noted to 3 months. Theoretically, if blood samples had been collected 1 week after the baseline supplementation a rise in the counts of Natural Killer cells in the vitamin A group could have been recorded prior to the fall noted at delivery. However to verify this theory further studies are required to validate the transient booster effect of vitamin A on Natural Killer cells.

Kuhnert *et al* reported a Natural Killer absolute count of 0.266 x 10^9/L in women 1 week post partum(93).

Very limited research has been performed using as the CD56 Natural Killer cell marker in this setting.

Activation Marker (I2) Percentage

No significant changes were noted for this parameter throughout the study. The two groups were comparable at 3 months with no statistically significant difference in their means (see figure 14).

Activation Marker (I2) Absolute Count

There was no significant change between baseline and delivery although there was a downward trend. The vitamin A and placebo groups showed a similar and significant increase between delivery and 1 week post partum (see figure 15). At 3 months the groups were comparable and the means showed no significant difference. The counts did not alter much between 1 week and 3 months. The two groups had no differences at any of the intervals. The increase between delivery and 1 week coincides with the changes noted in the total absolute lymphocyte counts (probably the recovery of the T and Natural Killer cells). The increase in the activation marker at this point probably indicates an activation of T cells. Monocytes expressing the activation marker I2 were excluded by the gating of lymphocytes only.

Moore *et al* reported that the activation marker (Ia) percentage and absolute numbers showed no significant change in non pregnant as well as pregnant subjects(65).

NNIP-S STUDY:

This was a large controlled study of the effects of vitamin A and beta-carotene supplementation during pregnancy. The results of this study are relevant to our study. Preliminary reports from the NNIP-S (Nepal Nutritive Intervention Project Sarlahi-2) study were presented at the XVIII IVACG Meeting in Cairo (1998). Some of the findings reported at this congress follow. Malnutrition and micronutritional deficiencies (chronic vitamin A deficiency) were significant problems in mothers and infants. Vitamin A treatment reduced maternal mortality by 38 % and morbidity by 31% while Beta-carotene treatment reduced these by 50% and 47% respectively. The incidence of night blindness in Nepal was 11%. Treatment with vitamin A or Beta-carotene reduced night blindness significantly – by 40% and 65% respectively. Vitamin A reduced anaemia during and after pregnancy and in infants. There was a reduction in the incidence of malaria in the vitamin A group. There was no increase in birth defects; a slight protective effect with decrease in severe cranial and ocular abnormalities was noted with vitamin A treatment.

Importance of this Study

At the time of conceptualisation of the hypothesis vitamin A supplementation had been shown to result in decreased morbidity and mortality in infants in Nepal.

SUMMARY

In this study biochemical vitamin A deficiency was noted in approximately 38% of the patients at baseline. Anemia at baseline was present in 65.4% of the patients. Haemoglobin levels showed a significant increase in both groups at 3 months. The placebo group recovered earlier than the vitamin A group. Their haematocrits values were proportional to the haemoglobin values at baseline. The haematocrit values increased earlier in the vitamin A group (delivery to 1 week) than the placebo group (1 week to 3 months).

Six patients had thrombocytopenia at baseline. In both groups counts increased after delivery with a subsequent fall at 3 months. The platelet counts during pregnancy were lower than in the non – pregnant state (baseline compared to 3 months).

Of the patients studied 5.2% had white cell counts $<5.0 \times 10^9/l$ at baseline. Both groups showed a significant decrease between delivery and 1 week and 1 week and 3 months. Pregnancy is associated with increased white cell counts which corrects after delivery.

Lymphopenia was noted in 9.1% of the patients at baseline. Total absolute lymphocyte counts showed a significant increase between delivery and 1 week post delivery in both groups. There was no fall in the vitamin A group and a non significant fall in the placebo group.

B lymphocyte counts at baseline were similar to counts reported in normal

pregnancy. The white cell count decreased, and the total lymphocyte count increased early (1 week after delivery). B lymphocyte recovery occurred between 1 week and 3 months. T cells and Natural Killer cells are presumed to have recovered earlier. Values for B lymphocytes were lower during pregnancy.

The Natural Killer cell counts were comparable in the two groups at baseline. Placebo group did not show decrease at any of the intervals, while the Natural Killer cells showed decreases at delivery (non significant) and at 3 months in the vitamin A group.

Activation marker (I2) showed an increase at baseline (32 – 33%) compared to 20% reported in the literature for normal pregnancy. There was an increase between delivery and 1 week probably reflecting the activation of T cells.

This study found no significant effect of vitamin A on the B cells at any point. Although Natural Killer cells showed a non-significant increase in the vitamin A group the counts in the vitamin A group fell below the placebo group at delivery and at 3 months. If there was a "booster" effect it was very transient. However the point of concern is that the Natural Killer cells in the vitamin A group fell below the placebo group at 3 months. No differences were noted in the activation marker in the 2 groups at any point. No beneficial or detrimental effect was noted for vitamin A for the above parameters.

In the Nepal study patients having night blindness were symptomatic with

vitamin A deficiency suggesting that the deficiency was more severe. Vitamin A deficiency is a chronic problem which is compounded by malnutrition, infection, including malaria and parasitism in Nepal. The big reduction in maternal mortality was not seen in our study probably because the cause of death was multifactorial in Nepal and due to the absence of maternal mortality in both our groups.

CONCLUSION:

- No beneficial effects were noted on natural killer cells, B lymphocytes and the (I2) activation marker.

Recommendations Arising from this Study:

- To study normal non-HIV subjects in pregnancy to document various haematological and immunological parameters with emphasis on the CD56 marker for natural killer cells. Very few reports are published and local data is lacking
- To study the protective effect of vitamin A on the absolute lymphocyte numbers and to assess the short term "booster" effect on killer cells.

APPENDIX

Table 3

Differences in the Haematological Parameters between Baseline and Delivery in Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	BASELINE	DELIVERY	BASELINE	DELIVERY
n	103	45	105	45
Hb G/dl	10.51 (10.26 – 10.76)	10.83 (10.42 – 11.24)	10.63 (10.41 - 10.85)	11.12* (10.73 – 11.51)
HCT %	29.96 (26.89 – 31.03)	26.60 (23.00 – 30.25)	29.02 (27.51- 30.54)	26.97 (23.30 – 30.64)
PLT $X10^{9}/l$	258.40 (243.39 – 273.37)	264.47 (236.38 – 292.57)	253.30 (238.29 - 268.31)	230.08 (208.34 – 251.81)
WCC $X10^{9}/l$	7.80 (7.41 - 8.19	8.63 (7.37 – 9.90)	7.92 (7.44 - 8.39)	8.38 (7.34 - 9.41)
LYMPHS $X10^{9}/l$	1.77 (1.64 - 1.89)	1.72 (1.50 – 2.00)	1.71 (1.60 - 1.81)	1.80 (1.60 - 2.00)

Results are presented as a mean with (95% confidence intervals).

*Significance = $p<0.05$

Table 4

Differences in the Immunophenotype Markers between Baseline and Delivery in the Vitamin A and Placebo Groups

	VITAMIN A		PLACEBO	
	BASELINE	DELIVERY	BASELINE	DELIVERY
n	103	45	105	45
CD56 %	10.42 (9.29 - 11.55)	10.80 (9.03 – 12.57)	10.15 (9.11 - 11.19)	10.51 (9.24 – 11.79)
CD56 ABS $X10^{9/l}$	0.20 (0.16 - 0.24)	0.16 (0.13 – 0.20)	0.18 (0.16 - 0.21)	0.19 (0.15 - 0.23)
CD19 %	10.26 (9.55 - 10.97)	9.99 (9.00 – 10.98)	10.87 (10.17 - 11.56)	10.87 (9.82 – 11.92)
CD19 ABS $X10^{9/l}$	0.18 (0.16 - 0.20)	0.20 (0.18 – 0.22)	0.19 (0.17 - 0.20)	0.19 (0.16 - 0.22)
I2 %	32.13 (27.93 – 36.32)	31.74 (26.68 – 36.81)	33.03 (29.67 - 36.39)	32.35 (27.79 - 36.90)
I2 ABS $X10^{9}/l$	0.59 (0.50 - 0.69)	0.55 (0.41 - 0.69)	0.64 (0.45 - 0.83)	0.56 (0.45 - 0.67)

Results are presented as a mean with (95% confidence intervals)

Table 5

Differences in the Haematological Parameters between Baseline and 1 Week post partum in the Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	BASELINE	1 WEEK	BASELINE	1 WEEK
n	103	48	105	45
Hb G/dl	10.51 (10.26 – 10.76)	11.81* (11.37 – 12.35)	10.63 (10.41 – 10.85)	11.26* (10.80 – 11.72)
HCT %	29.96 (26.89 – 31.03)	31.54 (28.47 – 34.60)	29.02 (27.51 – 30.54)	30.20 (27.04 – 33.36)
PLT X 10^9/l	258.38 (243.39 - 273.37)	364.84* (317.42 – 412.26)	253.30 (238.29 – 268.31)	367.29* (334.36 – 400.21)
WCC X 10^9/l	7.80 (7.41 – 8.19)	6.84* (6.23 – 7.44)	7.92 (7.44 – 8.39)	7.86 (6.18 – 9.54)
LYMPHS X 10^9/l	1.77 (1.64 – 1.90)	2.17* (2.00 – 2.37)	1.71 (1.60 – 1.81)	2.15* (1.91 – 2.40)

Results are presented as a mean with (95% confidence intervals)

*Significance = $p<0.05$

Table 6

Differences in the Immunophenotype Markers between Baseline and 1 Week post partum in the Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	BASELINE	1 WEEK	BASELINE	1 WEEK
n	103	48	105	45
CD56 %	10.42 (9.29 – 11.55)	13.36* (10.54 – 16.19)	10.15 (9.11 – 11.11)	11.51 (9.84 – 13.18)
CD56 ABS X 10^9/l	0.20 (0.16 – 0.24)	0.29* (0.23 – 0.34)	0.18 (0.16 – 0.21)	0.25* (0.20 – 0.30)
CD19 %	10.26 (9.55 – 10.97)	8.42* (7.58 – 9.25)	10.87 (10.17 – 11.56)	8.53* (7.35 – 9.71)
CD19 ABS X10^9/l	0.18 (0.16 – 0.20)	0.18 (0.16 – 0.21)	0.19 (0.17 – 0.20)	0.18 (0.15 – 0.21)
I2 %	32.13 (27.93 – 36.32)	34.56 (30.09 – 39.03)	33.03 (29.67 – 36.39)	33.25 (28.03 – 38.47)
I2 ABS X 10^9/l	0.59 (0.50 – 0.69)	0.73 (0.62 – 0.84)	0.64 (0.45 – 0.83)	0.71 (0.56 – 0.86)

Results are presented as a mean with (95% confidence intervals)

*Significance = $p<0.05$

Table 7

Differences in the Haematological Parameters between Baseline and 3 Months post partum in the Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	BASELINE	3 MONTH	BASELINE	3 MONTH
n	103	46	105	39
Hb G/dl	10.51 (10.26 – 10.76)	12.15* (11.18 – 12.45)	10.63 (10.41 – 10.85)	11.79* (11.11 – 12.48)
HCT %	28.96 (26.89 – 31.03)	33.58* (30.78 – 36.38)	29.02 (27.51 – 30.54)	35.49* (34.30 – 36.68)
PLT X 10^9/l	258.38 (243.39 – 273.37)	282.16 (261.47 – 302.85)	253.39 (238.29 – 268.31)	286.84* (261.33 – 312.36)
WCC X 10^9/l	7.80 (7.41 – 8.19)	5.62* (5.15 – 6.08)	7.92 (7.44 – 8.39)	5.56* (5.07 – 6.06)
LYMPHS X 10^9/l	1.77 (1.64 – 1.90)	2.17* (1.93 – 2.42)	1.71 (1.60 – 1.81)	2.04* (1.85 – 2.23)

Results are presented as a mean with (95% confidence intervals)

*Significance = $p<0.05$

Table 8

Differences in the Immunophenotype Markers between Baseline and 3 Months post partum in the Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	BASELINE	3 MONTH	BASELINE	3 MONTH
n	103	46	105	39
CD56 %	10.42 (9.29 – 11.55)	10.73 (9.09 – 12.38)	10.15 (9.11 – 11.19)	10.72 (9.12- 12.31)
CD56 ABS X 10^9/l	0.20 (0.16 – 0.24)	0.23 (0.19 – 0.26)	0.18 (0.16 – 0.21)	0.26 (0.15 – 0.36)
CD19 %	10.26 (9.55 – 10.97)	10.38 (9.09 – 11.67)	10.87 (10.17 – 11.56)	10.35 (9.95 – 12.74)
CD19 ABS X 10^9/l	0.18 (0.16 – 0.20)	0.22* (0.19 – 0.24)	0.19 (0.17 – 0.20)	0.23* (0.20 – 0.27)
I2 %	32.13 (27.93 – 36.32)	31.99 (26.77 – 37.22)	30.03 (29.67 – 36.39)	35.18 (30.49 – 39.87)
I2 ABS X 10^9/l	0.59 (0.50 – 0.69)	0.68 (0.57 – 0.79)	0.64 (0.45 – 0.83)	0.71 (0.59 – 0.83)

Results are presented as a mean with (95% confidence intervals)

*Significance = $p<0.05$

Table 9

Differences in the Haematological Parameters between Delivery and 1 Week post partum in the Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	DELIVERY	1 WEEK	DELIVERY	1 WEEK
n	45	48	45	45
Hb G/dl	10.83 (10.42 – 11.25)	11.81* (11.37 – 12.25)	11.12 (10.73 - 11.51)	11.26 (10.80 – 11.72)
HCT %	26.60 (22.96 – 30.25)	31.54* (28.47 – 34.60)	26.97 (23.30 - 30.64)	30.20 (27.04 – 33.36)
PLT $X10^9/l$	264.47 (236.38 – 292.57)	364.84* (317.42 – 412.26)	230.08 (208.34 - 251.81)	367.29* 334.36→400.21
WCC $X10^9/l$	8.63 (7.37 - 9.90)	6.84* (6.23 – 7.45)	8.38 (7.34 - 9.41)	7.86 (6.19 – 9.54)
LYMPHS $X10^9/l$	1.72 (1.50 - 2.00)	2.17* (1.97 – 2.37)	1.80 (1.60 - 2.00)	2.15* (1.91 - 2.40)

Results are presented as a mean with (95% confidence intervals)

*Significance = $p<0.05$

Table 10

Differences in the Immunophenotype Markers between Delivery and 1 Week post partum in the Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	DELIVERY	1 WEEK	DELIVERY	1 WEEK
n	45	48	45	45
CD56 %	10.82 (9.03 - 12.57)	13.36 (10.54 – 16.19)	10.52 (9.24 - 11.80)	11.51 (9.84 – 13.18)
CD56 ABS $X10^9/l$	0.16 (0.13 - 0.20)	0.29* (0.23 – 0.34)	0.19 (0.15 - 0.23)	0.25 (0.20 - 0.30)
CD19 %	9.99 (9.00 - 10.98)	8.42* (7.58 – 9.25)	10.87 (9.82 - 11.92)	8.53* (7.35 - 9.71)
CD19 ABS $X10^9/l$	0.29 (0.06 - 0.51)	0.18 (0.16 – 0.21)	0.19 (0.16 - 0.22)	0.18 (0.15 - 0.21)
I2 %	31.74 (26.68 – 36.81)	34.06 (29.03 – 39.09)	32.35 (27.79 - 36.90)	33.25 (28.03 – 38.07)
I2 ABS $X10^9/l$	0.55 (0.41 - 0.69)	0.73* (0.62 – 0.84)	0.56 (0.45 - 0.67)	0.71* (0.56 - 0.86)

Results are presented as a mean with (95% confidence intervals)

*Significance = $p<0.05$

Table 11

Differences in the Haematological Parameters between Delivery and 3 Months post partum in the Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	DELIVERY	3 MONTH	DELIVERY	3 MONTH
n	45	46	45	39
Hb G/dl	10.83 (10.42 – 11.25)	12.15* (11.85 – 12.45)	11.12 (10.73 - 11.51)	11.79 (11.11 – 12.48)
HCT %	26.60 (22.96 – 30.25)	33.58* (30.78 – 36.38)	26.97 (23.30 - 30.64)	35.49* (34.30 – 36.68)
PLT $X10^9/l$	264.47 (236.38 – 292.57)	282.16 (261.47 – 302.85)	230.08 (208.34 – 251.81)	286.84* (261.33 – 312.36)
WCC $X10^9/l$	8.63 (7.37 – 9.90)	5.62* (5.15 – 6.08)	8.38 (7.34 - 9.41)	5.56* (5.07 - 6.06)
LYMPHS $X10^9/l$	1.72 (1.50 – 2.00)	2.17* (1.93 – 2.42)	1.80 (1.60 - 2.00)	2.04 (1.85 - 2.23)

Results are presented as a mean with (95% confidence intervals)

*Significance = $p<0.05$

Table 12

Differences in the Immunophenotype Markers between Delivery and 3 Months post partum in the Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	DELIVERY	3 MONTH	DELIVERY	3 MONTH
n	45	46	45	39
CD56 %	10.80 (9.03 - 12.57)	10.73 (9.08 – 12.38)	10.51 (9.24 - 11.80)	10.72 (9.12 - 12.31)
CD56 ABS $X10^9/l$	0.16 (0.13 – 0.20)	0.23* (0.19 – 0.26)	0.19 (0.15 - 0.23)	0.26 (0.15 - 0.36)
CD19 %	9.99 (9.00 - 10.98)	10.38 (9.09 – 11.67)	10.87 (9.82 - 11.92)	11.35 (9.95 - 12.74)
CD19 ABS $X10^9/l$	0.19 (0.16 - 0.21)	0.22 (0.19 - 0.24)	0.19 (0.16 - 0.22)	0.23 (0.20 - 0.27)
I2 %	31.74 (26.68 - 36.81)	31.99 (26.77 – 37.22)	32.35 (27.79 – 36.90)	35.16 (30.49 - 39.87)
I2 ABS $X10^9/l$	0.55 (0.41 - 0.69)	0.68 (0.57 - 0.79)	0.56 (0.45 - 0.67)	0.71 (0.59 - 0.83)

Results are presented as a mean with (95% confidence intervals)

*Significance = $p<0.05$

Table 13

Differences in the Haematological Parameters between 1 Week and 3 Months post partum in the Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	1 WEEK	3 MONTH	1 WEEK	3 MONTH
n	48	46	45	39
Hb G/dl	11.81 (11.37 - 12.25)	12.15 (11.85 – 12.45)	11.26 (10.80 - 11.72)	11.79 (11.11 - 12.48)
HCT %	31.54 (28.47 - 34.60)	33.58 (30.78 – 36.38)	30.20 (27.04 - 33.36)	35.49* (34.30 - 36.68)
PLT $X10^9/l$	364.84 (317.42 – 412.26)	282.16* (261.47 – 302.85)	367.29 (334.36 - 400.21)	286.84* (261.33 – 312.36)
WCC $X10^9/l$	6.84 (6.23 - 7.45)	5.62* (5.15 - 6.08)	7.86 (6.18 - 9.54)	5.56* (5.07 - 6.06)
LYMPHS $X10^9/l$	2.17 (2.00 - 2.37)	2.17 (1.93 - 2.42)	2.15 (1.91 - 2.40)	2.04 (1.85 - 2.23)

Results are presented as a mean with (95% confidence intervals)

*Significance = $p<0.05$

Table 14

Differences in the Immunophenotype Markers between 1 Week and 3 Months post partum in the Vitamin A and Placebo groups

	VITAMIN A		PLACEBO	
	1 WEEK	3 MONTH	1 WEEK	3 MONTH
n	48	46	45	39
CD56 %	13.36 (10.54 - 16.19)	10.73 (9.09 - 12.38)	11.51 (9.84 - 13.18)	10.72 (9.12 – 12.31)
CD56 ABS $X10^9/l$	0.29 (0.23 - 0.34)	0.23 (0.19 - 0.26)	0.25 (0.20 - 0.30)	0.26 (0.15 - 0.36)
CD19 %	8.42 (7.58 - 9.25)	10.38* (9.09 - 11.67)	8.53 (7.35 - 9.71)	11.35* (9.95 – 12.74)
CD19 ABS $X10^9/l$	0.18 (0.16 - 0.21)	0.22 (0.19 - 0.24)	0.18 (0.15 - 0.21)	0.23* (0.20 - 0.27)
I2 %	34.56 (30.09 - 39.03)	31.99 (26.77 – 37.22)	33.25 (28.03 - 38.47)	34.18 (30.49 – 37.87)
I2 ABS $X10^9/l$	0.73 (0.62 - 0.84)	0.68 (0.57 - 0.79)	0.71 (0.56 - 0.86)	0.71 (0.59 - 0.83)

Results are presented as a mean with (95% confidence intervals)

*Significance = $p<0.05$

ABBREVIATIONS

MTI - mother-to-infant

ANC - Ante natal clinic

NK - Natural killer

IU - international units

RBP - Retinol binding protein

CRBP - Cytoplasmic retinol binding protein

RAR - Retinoic acid receptor

RXR - Retinoic X receptor

ADCC - Antibody dependent cell cytotoxicity

SCID - Severe combined immunodeficiency

HLA - Human leucocyte antigen

MHC - Major histocompatibility complex

Ig - Immunoglobulin

umol - micromols.

ug - micrograms

IFN - Interferon

IL - Interleukin

PCP - Pneumocystis carinii pneumonia

SIV - Simian immunodeficiency virus

HTLV - Human T-Lymphocyte virus

RT - Reverse transcriptase

RNA - Ribonucleic acid

Tat - Trans-activator

Rev - Regulator of HIV structural proteins

mRNA - messenger ribonucleic acid

Nef - Negative factor

Vif - Viral infectivity factor

IP/ml - Infectious particles per milliliters.

HSV - Herpes simplex virus

CMV - Cytomegalovirus

EBV - Epstein Barr virus

TNF - Tumor necrosis factor

GM-CSF - Granulocyte/monocyte colony stimulating factor

CTL - Cytotoxic T-lymphocyte

TGF - Transforming growth factor

MTB - Mycobacterium tuberculosis

MAC - Mycobacterium avium complex

HPV - Human papillomavirus

PID - Pelvic inflammatory disease

PTK - Protein tyrosine kinase

BCRs - B cell receptors

SCF - Stem cell factor

IgM - Immunoglobulin M

IgD - Immunoglobulin D

PBL - Peripheral blood lymphocyte

gp - Glycoprotein

PWM - Pokeweed mitogen

SIgM - surface Immunoglobulin M

LGL - Large granular lymphocyte

LAK - Lymphokine activated killer

APA - Antiphospholipid antibody

TcR - T cell receptor

LCMV - Lymphocytic choriomeningitis virus

HSV-FS - Herpes simplex virus type I

Ir - Immune response

FITC - Fluorescein isothiocyanate

FALS - Forward angle light scatter

RALS - Right angle light scatter

PE - Phycoerythrin

PMT - Photomultiplier tube

IFM - Immunofluorescent microscopy

PBMC - Peripheral blood mononuclear cells

FS - Forward scatter

LSS - Log side scatter

LFL - Log fluorescence

EDTA - Ethylenediamine tetra-acetic acid

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