EVALUATION OF IMMUNE STATUS AMONG REGULAR MALAY MALE WHOLE BLOOD DONORS

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Abs	Absolute count
APSs	Antigen presenting cells
BCR	B cell receptor
CD	Cluster of differentiation
DCs	Dendritic cells
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
FBC	Full blood count
Ig A	Immunoglobulin A
Ig G	Immunoglobulin G
Ig M	Immunoglobulin M
IFN-γ	Interferon-$^{\gamma}$
IL	Interleukin
MHC	Major histocompatibility complex
MIP1a	Macrophage Inflammatory protein 1 alpha
NF-Kb	Nuclear factor-kappaB

NK cell	Natural killer cell
NKG2D	Natural killer Group 2D
RANTES	Regulated on activation, normal T expressed and secreted
RBCs	Red blood cells
RelA	REL proto-oncogene
TCR	T cell receptor
TGF-β	Transforming growth factor beta
TH1	T helper 1
TH2	T helper 2
TH17	T helper 17
TReg cell	T regulatory cell
TNF-α	Tumour necrosis factor-α
TWBC	Total white blood cell
WBC	White blood cell

ABSTRAK

PENILAIAN STATUS IMUN DI KALANGAN PENDERMA DARAH TETAP LELAKI MELAYU

Pendermaan darah merupakan sebahagian penting dalam sistem penjagaan kesihatan. Ia adalah penting untuk memastikan bekalan darah adalah mencukupi dan selamat dari pendermaan darah tersebut. Walau bagaimanapun, adalah penting untuk memastikan bahawa kesihatan penderma tidak terjejas oleh pendermaan darah atau komponen darah. Penderma darah utuh kehilangan kira-kira $2x10^9$ sel darah putih dalam satu pendermaan darah, tanpa penurunan ketara dalam kiraan limfosit darah periferal. Kemungkinan pendermaan darah boleh mengubah status imun perlu dinilai.

Kajian ini bertujuan untuk menilai dan membandingkan tahap penanda imun antara penderma darah utuh kali pertama dan penderma darah utuh tetap , dan juga, untuk menentukan perkaitan antara tahap penanda imun dengan kekerapan pendermaan darah utuh.

Kajian perbandingan rentas telah dilakukan di Hospital USM dari Mei 2015 hingga April 2016. Sampel darah peripheral diambil daripada 40 penderma darah utuh tetap dan 40 daripada penderma darah utuh kali pertama lelaki Melayu. Parameter hematologi standard termasuk jumlah sel darah putih dengan kiraan neutrofil,

limfosit, monosit, eosinofil dan basophils dengan menggunakan XE 5000 Sysmex hematologi analisis. Flowsitometri untuk peratusan dan kiraan mutlak CD3, CD4, CD8, CD16 + 56, CD19 dan nisbah CD4:CD8 menggunakan BD FACSCANTO II. Kuantifikasi IL-2, IL-10 dan IFNγ dengan menggunakan ELISA *immunoassay*. Kuantifikasi IgG, IgA dan IgM dengan menggunakan nephlometri.

Min (SD) monosit dalam penderma darah utuh kali pertama dan penderma darah utuh tetap masing-masing adalah 0.51 (0.14) dan 0.58 (0.16) dengan nilai *p* yang signifikan dalam penderma darah tetap. Min (SD) interleukin 10 dalam penderma darah utuh kali pertama dan penderma darah utuh tetap masing-masing adalah 6.60 (3.05) dan 5.04 (2.28) dengan nilai *p* lebih rendah dalam penderma darah tetap. Min (SD) Ig A dalam penderma darah utuh kali pertama dan penderma darah utuh tetap masing-masing adalah 2.24 (0.89) dan 2.71 (1.02) dengan nilai *p* yang signifikan dalam penderma darah tetap. Parameter lain menunjukkan tiada perbezaan min yang signifikan. Analisis untuk menentukan perkaitan antara tahap penanda imun dengan kekerapan pendermaan darah utuh tidak menunjukkan hubungan yang signifikan.

Kajian ini menunjukkan bahawa penderma darah tetap tiada sebarang kesan yang serius untuk penanda imun berbanding dengan penderma darah kali pertama. Perbezaan dalam kiraan mutlak monosit, IL-10 dan IgA antara kedua-dua kumpulan mungkin berkaitan dengan perubahan aktiviti imun tanpa ada kesan klinikal. Tambahan pula, tiada hubungan yang signifikan antara tahap penanda imun dengan kekerapan pendermaan darah tetap. Dalam batasan kajian ini, tiada risiko tambahan kepada penderma darah tetap akibat daripada perubahan imun.

ABSTRACT

EVALUATION OF IMMUNE STATUS AMONG REGULAR MALAY MALE WHOLE BLOOD DONORS

Blood donations are an essential part of healthcare system. It is crucial to ensure that sufficient and safe supplies of blood from blood donation. However, it is important to ensure that the donor's health is not compromised by the donation of blood or blood component. Whole blood donors may lose approximately $2x10^9$ leukocytes in a single whole blood donation, with no significant drop in peripheral blood lymphocyte count. The possibility of blood donation may change the immune status should be assessed.

This study aimed to evaluate and compare the levels of immune markers among first time and regular whole blood donors, as well as, to determine association between the levels of immune markers with the frequency of whole blood donation.

A comparative cross sectional study was done at Hospital USM from May 2015 to April 2016. Peripheral blood was taken from 40 regular and 40 first time; Malay male whole blood donors. The immune marker parameters that were studied included TWBCs with neutrophils, lymphocytes, monocytes, eosinophils and basophils count by using XE 5000 Sysmex haematology analyzer (Koba, Japan). Flow cytometry for percentage and absolute count of CD3, CD4, CD8, CD16+56+, CD19 and CD4:CD8

ratio using BD FACSCANTO II. IL-2, IL-10 and IFNγ level quantification by using ELISA immunoassay. IgG, IgA and IgM level quantification by using nephlometry.

The mean (SD) of monocytes absolute count in first time and regular whole blood donors were 0.51 (0.14) and 0.58 (0.16), respectively with *p* value significantly higher in regular whole blood donors. The mean (SD) of interleukin 10 in first time and regular whole blood donors were 6.60 (3.05) and 5.04 (2.28), respectively with *p* value significantly lower in regular whole blood donors. The mean (SD) of Ig A in first time and regular whole blood donors were 2.24 (0.89) and 2.71(1.02), respectively with mean value significantly higher in regular whole blood donors. Other parameters showed no significant mean different. Analysis for determination of association between the levels of immune markers with frequency of whole blood donation showed no significant association.

This present study demonstrates regular blood donors showed no serious significant effects both for cellular and humoral immune markers when compared with the first time blood donors. The differences in monocytes absolute count, IL-10 and IgA between the two groups are probably related to variation of immune activity with no clinical significance. Furthermore, there was no significant association between the levels of immune markers with frequency of whole blood donation. Within limitations of this present study, there are no added risks to regular blood donors as a result of immune changes.

Chapter 1

INTRODUCTION

1.0 INTRODUCTION

Blood donations are an essential part of our healthcare system. If we did not have volunteers to donate blood, many medical procedures could not take place. Doctors and surgeons require blood and blood component products to carry out life-saving and life-enhancing treatments every day throughout the years. It is crucial to ensure that sufficient and safe supplies of blood from blood donation are available at all times, since there are no substitutes for human blood. Besides, it is important to ensure that the donor's health is not compromised by the donation of blood or blood components. Thus, it is important to practice safe blood donation to prevent complication.

Blood donation process has to follow strict guidelines to ensure that the donation does not cause any harm to the donors. Generally, the minimum interval for each whole blood donation is 8 weeks in practice and not more than 4 times a year. Healthy donors with the weight of 50 kg or more could donate blood approximately 450 to 500 ml per each donation. Each donation wills loss approximately 250 ml RBC and 250 ml plasma.

Whole blood donors also may lose approximately $2x10^9$ leukocytes in a single whole blood donation, with no significant drop in peripheral blood lymphocyte count (Strauss *et al*., 1994). No known immediate adverse effects are associated with the

slight decreases in peripheral blood lymphocyte counts following whole blood donation. The possible long term effect due to repeated loss of blood and blood component from whole blood donation was also not clear.

Few studies have been published addressing long term alteration in the immune system as an effect of chronic blood donation. Even though, there are no clinically relevant health effects attributable to multiple blood donations have been demonstrated, a few studies have revealed immunologic changes in long term whole blood donation. A decrease in total peripheral blood lymphocyte counts and significant decrease in natural killer (NK) cell activity in blood donors, with the lowest values observed in active long term blood donors was demonstrated by Lasek *et al*. (Lasek *et al.*, 1987). A decrease cytotoxic potential of NK cells in regular whole blood donors was also demonstrated by Olinescu *et al.* (Olinescu *et al.*, 1988).

Lewis *at al*. demonstrated increased expression of CR3 receptors on both monocytes and neutrophils from the whole blood donors as compared to those from non-donor controls (Lewis *et al.*, 1992). These results are consistent with findings reported by Morse et al previously, who found that complement activation occurs during blood donation. They also demonstrate that neutrophils showed an increased expression of C3b (CR1) receptor after donation as compared to before donation (Morse *et al.*, 1988).

In general, published data on the effects of long term whole blood donation on the immunologic status of individual donors remains paucity. Furthermore,

understanding of the possible clinical significance of immune activation and changes in relation to long term whole blood donation is important to avoid compromised donor wellbeing, as well as, to demonstrate immune responses which benefit the individual regular donors. Thus, can improve pre-donation counseling, and hence promote blood donation programme.

Therefore, the purpose of this study was to investigate lymphocytes subsets and mediators that are important in host defense in long term regular whole blood donors. By using flow cytometry in conjunction with various immunoassays, this study characterized peripheral blood lymphocytes and cytokines as well as immunoglobulin from regular whole blood donors and compared these findings to data obtained from first time whole blood donors (control).

Chapter 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 Introduction

In blood banking, there is accepted policy to make blood donation as convenient and safe as possible for all donors. The blood from regular blood donors frequently condemned on the claimed that the donors suffer from a poor nutritional status, hence that it is a poor source of blood component due to frequently repeated blood donations. A study by Jacobs et al revealed a high number of misconceptions about blood donation and fear that blood donation could damage the donor's health in Mwanza region, Tanzania (Jacobs and Berege, 1995).

In this recent years, it has become apparent that malnutrition can result in multiple defects in the immune system, which involving humoral responses, cell-mediated responses, phagocytosis and the complement system. Therefore, blood donors are encouraged to donate their blood only after careful assessment and clinical examination of the general condition.

Few study have been published addressing long term effect in the immune system as a regular blood donation with few studies revealed immunologic changes in regular whole blood donation. A decreased in total peripheral blood lymphocytes count and significant decreased in Natural Killer (NK) cell activity in blood donors with the

lowest level observed in active long term blood donors was demonstrated by Lasek *et al.* (Lasek et al., 1987).

Previous study by Karger et al. shown that patient undergoing pre-operative autologous blood donation prior to elective hip arthroplasty surgery experience significant enduring immunological changes following donation (Karger *et al.*, 2007). The total number of leukocytes remains constant, the number of granulocytes increases at the expense of fall in lymphocytes.

Another study by Lewis SL and Bonner PN demonstrated many plasma donors have low levels of serum protein, globulin and IgG (Lewis SL & Bonner PN, 1994). However, plasmapheresis procedure is different from whole blood donations. Plasma donors may donate larger volumes of plasma and may donate two times per weeks (American Red Cross) and depending on their body weight, which may donate up to 1800 ml of plasma per week.

Only little data is available on the long term effect of whole blood donations particularly on the immune system alteration. The existing data more focus on regular plasmapheresis in which the procedure allows the donors to donate plasma every two weeks interval.

Therefore, the aim of this study was to investigate whether long-term and regular blood donations will alter some aspect of the immune markers of the blood donors. We also studied the possibility of association of regular whole blood donors with humoral and cellular immune markers.

2.2 Blood donation

2.2.1 Introduction

Blood can be defined as the fluid that circulates in the principle vascular system of human beings and others vertebrates, consisting of plasma in which the red blood cells, white blood cells, and platelets are suspended. Blood carries oxygen and nutrient from the lungs to body cells. Blood also carries proteins and platelets that let blood to clot whenever there has been an injury. It also carries waste to be eliminated from the body. Adult may have 4-6 liters blood in their body.

Blood donation occurs whenever a person voluntarily as blood withdrawn and used for transfusion. When someone donates whole blood, only one unit (350-450 mls) will be withdrawn; healthy donors are allowed to donate 10 per cent of their blood volume. One unit can help to save or improve the lives of up to three people. In fact, some donors can help more than 500 people, over a life time. Men can only donate

whole blood four times a year, and women three times a year (due to iron level) as red blood cells takes several weeks to be restored.

One unit of RBCs transfused to an adult recipient should increase the hemoglobin concentration by 1g/dL (Klein *et al.*, 2007).

2.2.2 Types of blood donation

Blood donation can be divided into two types namely as whole blood donation and apheresis donation. Whole blood donation implies the whole blood is collected from the donors whereas in apheresis donation, only selected blood component is collected. Whole blood constitutes of red blood cells (RBCs), white blood cells (WBCs), platelet, plasma and few other components. It becomes the main source for the preparation of other blood components where the separation involves manual processing procedure under controlled temperature with specific centrifugation speed following the specific component processing guidelines.

On the other hand, the word "apheresis" is initially originated from the words "aphairesis", a Greek word, which signify "to separate", "to take away by force", or "to remove". The process started with the withdrawal of whole blood from a donor, extraction of the desired component into a collecting blood bags through a filter membrane or by centrifugation methods and returning the other unused blood

component back into the donor. Apheresis likewise can be used as a therapeutic method in some of the medical cases, act by removing specific pathologic components or substance from a patient's blood for therapeutic purpose (Winters, 2012).

2.2.3 Whole blood donation

Whole blood donation is the most common type of blood donation. Blood contains erythrocytes (RBC), leukocytes (WBC) and platelet suspended in plasma. Whole blood is the most common starting product for component preparation which targets a specific patient's need as well as enabling each component to be stored optimally.

The component therapy is more appropriate to target a patient's specific indication for transfusion; for example RBC products for symptomatic anaemia, plasma products for multiple coagulation factor deficiencies, cryoprecipitate for replacement of fibrinogen and platelet products for thrombocytopenia or platelet dysfunction.

Generally, 450 ml of whole blood is collected into a blood bag with 70 ml of anticoagulant-preservative solution, creating a product with a final haematocrit of 38%. Whole blood is stored at 1-6 °C.

2.2.4 Blood donors

Blood donation is one of the most significant contributions that a person can make towards their society. It is not harmful for an adult person to donate their blood. Hence, within few days to weeks, body of the blood donors will regenerate their blood.

The selection of appropriate blood donors is important to protect their health during and following donation, as well as to avoid any undesirable health impact. Key elements of the selection process, as part of the overall approach to blood and donor safety are the donor health history questionnaire, physical examination and infectious disease screening. The screening process includes direct questioning about specific risk behaviors, medications, travel, and other factors that potentially effect recipient or donor safety (AABB, 2014).

Blood donor should be between 18-55 years of age with a weight more than 45 kg or above with normal blood pressure, pulse rate, temperature and healthy. Both men and women can donate their blood. There are few conditions in which donors are permanently deferred for donating their blood. The donors with history of epilepsy, psychotic disorders, abnormal bleeding tendencies, malignancy should be permanently deferred for blood donation.

Potential donors will be evaluated for anything that may cause their blood unsafe to be used by others who in needs. The screening includes testing for transmitted

diseases by blood transfusion, including HIV, viral Hepatitis B, Hepatitis C and syphilis. The donor will be instructed to answer questions regarding medical history and take a short physical examination to make sure the donation is not hazardous to his or her health.

The donor's haematocrit or haemoglobin level is tested to make sure that the loss of blood will not cause them to be anaemic. Pulse, blood pressure and body temperature are evaluated. Eventually, eligible donor will be asked to give his or her consent.

2.2.5 Donor selection and donor deferral

Blood and blood component is one of the important treatment modalities and constantly in demand as they are utilized in various medical procedures. Thus, it is very crucial to ensure that the blood is safe for usage and come from the safe donors. In most of the countries in the world including Malaysia, blood supply is contributed by voluntary, non-remunerated blood donors. For that safety purpose for blood being donated as well as donor health did not compromised, blood donor criteria selection is created to screen and to ensure the individuals who fulfilled the criteria will be only accepted to donate blood (Eder et al., 2009).

Deferral of the donors who are not fulfilled the selection criteria can happen at any stage of blood donation began from registration, during pre-donation interview,

during the blood donation or even after the blood donation. This precautionary measure was taken in order to protect both of the blood donors as well as patients whom received the transfusion. Pre-donation counseling particularly is conducted to assess donor eligibility, complemented by various blood unit testing which will be done later, contribute to the safety of the unit (Sandborg and Thornton, 1994).

Pre-donation interview alone considered as one of the essential preliminary measure to capture the likelihood of the window period for certain communicable diseases, automatically reducing the risk of disease transmission, particularly in case that being undetected by standard blood serology test (Germain & Goldman, 2002).

A person who does not meet the blood donor selection criteria (e.g low haemoglobin, low blood pressure) may experience adverse donor reaction such as headache, giddiness or fainting episode. They will be temporarily deferred with appropriate advised and encourage to return for blood donation after the temporary deferral period (Halperin *et al*., 1998).

On the other hand, blood donor will be permanently excluded from blood donation due to medical condition or due to their high risk behavior and lifestyle (high risk group). Donors with medical conditions such as malignancy, bleeding abnormality as well as heart disease are prohibited from blood donation as this process will potentially affect their well-being, thus permanent deferral is indicated. High risk group (e.g. individuals with multiple sex partners, sex workers, and intravenous drug

users) will be permanently deferred in perspective of risk of transmitting transfusion transmitted disease (Lim *et al*, 1993).

Individual who do not eligible for blood donation will be deferred either temporarily or permanently relying upon the reason for deferral. A temporary deferral can be as short as twenty four hours to as long as two years, whereas permanent deferral refers to a blood donor who disqualified from blood donation indefinitely. The commonly reported cause for temporary deferral are low pre-donation haemoglobin level, low or high blood pressure, underweight, taking medications (e.g. antibiotics), medical conditions (e.g. upper respiratory tract infection), minor or major surgical procedure (e.g. acupuncture, tattoo, dental procedure) (Germain and Goldman, 2002).

The donor deferral rates may vary from a blood donation centre to another, estimated around 5% to 25%. In Malaysia, it is estimated around 15% to 20% of blood donors are reported to be deferred from blood donation in Pusat Darah Negara as well as throughout the countries every year. A large portion of them are temporarily deferred, and the top three listed for this deferral are contributed by low haemoglobin (Hb) level, followed by high or low blood pressure. About 10% of donor deferrals are permanent deferral in which majority of them are due to high risk behavior (Seong, 2013).

A retrospective study done among Turkish population according to the gender, age and education level, in 5 years beginning from 2001 until 2006 likewise

demonstrated quite similar findings in term of cause of donor deferral; low Hb (20.7%), medical condition such as upper respiratory tract infection (17.7%), hypertension (5.6%), polycythaemia (5.6%) with addition of high risk sex partner (16.7%). These are the top five listed in the entire group. The main deferral cause in male donor is upper respiratory tract infection, while in female donors is due to low level of haemoglobin. Interestingly, the education level of donors demonstrated no impact on the rate of deferral for both genders at different age group (Arslan, 2007).

Furthermore, studies conducted in Hospital Universiti Sains Malaysia, Kubang Kerian (HUSM) demonstrated approximately 5.6% blood donors are deferred in this blood centre in year 2006. Majority of the donors (64.1%) are regular donors. This study also reported similar findings for the cause of deferral as Pusat Darah Negara, caused by low haemoglobin level (40.7%) with female donors dominating (69%) in this category. The second common cause are high blood pressure (29.4%), dominated by male donor which constitute approximately 45%. The other causes include medical illness (15.6%), low blood pressure (3.5%), short interval between donation (1.7%), polycythaemia (1.7%) and other causes of deferral (5.2%) (Rabeya *et al*, 2008).

Permanent deferral could affect a donor psychologically, so that the purpose of donor deferral must be clearly explained considering it is the best manner for both blood donor and blood recipient. Understanding this will make it easier for a blood donor to accept the deferral, either temporary or permanent. Permanently deferred donors will

be counseled appropriately and professionally by trained staff so that the reason of being permanently deferred can be accepted by the donor (Lim, 1993).

Blood donor selection criteria are also regularly reviewed to improve the selection criteria and to prevent unnecessary donor deferral. Clearly, this selection guidelines help to avoid or reducing harm to the blood donors, recognize any probable significant pathology in the donors, maximizing the therapeutic quality of the blood and blood product as well as to prevent any harm to the recipient (Arslan, 2007).

2.2.6 Benefit of giving blood

Blood donors have a satisfaction and joy of saving human lives. Meanwhile, few studies on regular blood donation may reduce the risk of cardiovascular disease (Haidari *et al*., 2001; Kiechl *et al*., 1997; De Valk *et al.*, 1999).

One study conducted by Meyers et al., demonstrates blood donation in nonsmoking men was associated with reduced risk of cardiovascular event (Meyers *et al.*, 1997). Another study conducted by Salonen et al., suggested that frequent blood loss through voluntary blood donations may be associated with a reduced risk of acute myocardial infarction in middle-age men (Salonen *et al.*, 1998).

Tuomainen et al., demonstrated reduced risk of coronary events in male blood donors (Tuomainen *et al.*, 1997). However, study done by Alberto et al., revealed there was no significant associations were found between blood donation and the risk of myocardial infarction in analyses restricted to men with hypercholesterolemia or those who never used antioxidant supplements or aspirin (Ascherio *et al.*, 2001).

Another few studies suggested reduced risk of cancer occurrence with regular blood donations (Edgren *et al.*, 2007: Merk *et al*, 1990; Lasek *et al* 1994). A study by Merk *et al.* reported there was significant decreased cancer incidence among blood donors (Merk *et al.*, 1990). However, in another study conducted by Edgren *et al.* demonstrated repeated blood donation was not associated with increased or decreased risk of cancer (Edgren *et al.*, 2008).

Presence of healthy donor effect while comparing donors with the general population and active versus lapsed donors was demonstrated by Atsma Et al. (Atsma *et al.*, 2011). Furthermore, all regular blood donors may enjoy free health checkup yearly.

2.3 Immune system

2.3.1 Introduction

The immune system is an important host defense system that protects body from various infection and disease. To function normally, immune system should be able

to detect variety of pathogen, such bacteria, virus, parasitic worm infection as well as able to distinguish them from own healthy tissue.

Immune system can be classified into subsystem of innate immune system versus adaptive/acquired immune system, and humoral immunity versus cell-mediated immunity. Adaptive/acquired immune system confers immunological memory after an initial response to a specific pathogen, leading to an augment response to subsequent encounters with the same pathogen.

The immune system defends body from infection with layered defenses of increasing specificity. Initially physical barrier for example skin and mucous membrane prevent invasion of pathogens such as bacteria and viruses from entering the body. However, if a pathogen breaches these barriers, the innate immune system provides an immediate, but not specific response. If pathogen successfully overcomes the innate response, the adaptive immune system will be activated by innate response.

This advance response is then retained after the pathogen has been abolished, in the form of an immunological memory, and allows the adaptive immune system to mount faster and stronger attack each time this similar pathogen is encountered.

The induction of a proper effector function in clonal cells is intervened by the innate immune system, while both the adaptive and innate immune responses control

self/non self-discrimination. The innate, nonclonal system controls the initiation of the adaptive immune response by regulating the expression of co stimulatory activity on antigen presenting cells (APCs), and instructs the adaptive immune system to build up a particular effector response by releasing effector cytokines.

The functioning of lymphocytes bearing clonally-rearranged receptors is absolutely subject to these signals that are provided by the innate recognition system. Therefore, we consider the innate and adaptive immune responses to be integrated as a single immune system, with the innate response preceding, and being necessary for, the adaptive immune response (Medzhitov and Janeway, 1997).

2.3.2 Innate immune system

The innate immune system, otherwise called the non-specific immune system (Young and Criscitiello, 2011), is an important subsystem of the overall immune system that comprises the cells and mechanisms that defend the host from infection by various organisms.

The innate immune response is the first line of defense against infectious and noxious challenges. It is activated rapidly and usually involves cells at cutaneous and mucosal surfaces. These cells use germ-line encoded pattern recognition receptors to detect the presence of microbial molecules or products of tissue damage, and mount a stereotypic response that includes the activation of anti-microbial mechanisms and

the emission of chemotactic molecules that recruit other cells of the immune system to the site of infection or damage (Kawai & Akira, 2010).

The cells of the innate system recognize and react to pathogens in a generic way, but, unlike the adaptive immune system, the system does not confer long-lasting or protective immunity to the host (Gary M Wessel, 2004).

The innate immune system is made out of a network of cells including neutrophils, NK cells, monocytes/macrophages, and dendritic cells that mediate the earliest interactions with pathogens. Innate immune systems confer immediate defense against infection.

Neutrophils constitute the primary immune defense against rapidly dividing bacteria, yeast and fungal infections, initiate microbicidal mechanisms including generation of reactive oxygen and nitrogen species, release of proteolytic enzymes, and microbicidal peptides from cytoplasmic granules.

NK cells mediate MHC-independent cytotoxicity in the innate immune defense against viral infections and a few malignancies. Absolute numbers of NK cells increase with age, reflecting an increase in CD56 dim cells, but NK cell cytotoxicity on a per cell basis is diminished and levels of cytokines and chemokines such as RANTES, MIP1a, and IL-8 produced upon NK cell activation are also reduced (Mocchegiani *et al.*, 2009)**.**

Furthermore, in response to infection, the innate immune system will strengthens iron-withholding defenses, ensuring that the host-pathogen interface remains an ever evolving battleground for precious metal (Cassat et al., 2013).

2.3.3 Adaptive immune system

The adaptive immune system, otherwise called as the acquired immune system or, more rarely, as the specific immune system, is a subsystem of the overall immune system that is consist of highly specialized, systemic cells and processes that eliminate or prevent pathogen growth.

Adaptive immunity creates immunological memory after an initial response to a specific pathogen, and prompts an enhanced response to subsequent encounters with that pathogen. Like the innate system, the adaptive system incorporates both humoral immunity components and cell-mediated immunity components.

Unlike the innate immune system, the adaptive immune system is highly specific to a particular pathogen. Adaptive immunity can likewise provide durable protection. The cells that carry out the adaptive immune response are white blood cells known as lymphocytes.

Adaptive immunity develops through the course of days to weeks taking after exposure to foreign substances and relies on the activation, proliferation and differentiation of antigen specific B and T lymphocytes, which are involved in antibody- and cell-mediated responses, respectively (Bonilla & Oettgen, 2010).

Two important broad classes - antibody responses and cell mediated immune response are also carried by two different lymphocytes (B cells and T cells). In antibody responses, B cells are stimulated to secrete antibodies, which are proteins also known as immunoglobulins.

B cells and T cells are derived from the same multipotent haematopoietic stem cells, and are morphologically indistinguishable from each other until after they are activated. B cells play a large role in the humoral immune response, while T cells are involved in cell-mediated immune responses.

All cells are capable of presenting antigen through the function of major histocompatibility complex (MHC) molecules with the exception of erythrocytes (non-nucleated cells) (Chakrapany, 2013).

Several T cell subgroups can be stimulated by professional APCs, and each type of T cell is specially equipped to deal with each unique toxin or bacterial and viral pathogen. The sort of T cell stimulated, and the type of response generated, depends to some extent, on the context in which the APC first encountered the antigen**.**

2.4. Immune subset and immune marker

2.4.1. T cells

T helper cells are a type of T cells which play an important role in the immune system, particularly in adaptive immune system. They assist the activity of the other immune cells by releasing T cell cytokines. They are important in B cell antibody class switching, in the activation and growth of cytotoxic T cells, and maximizing bactericidal activity of phagocytes, for example macrophages. Mature T helper cells express the surface protein CD4 and considered as CD4+ T cells which play an important role in immune system. Helper T cells are capable of influencing a variety of immune cells, and the T cell response generated including extracellular signal like cytokines. Proliferating helper T cells change into effector T cells differentiate into two major subtypes, which known as T helper 1 and T helper 2.

T helper 1 (Th1)/Th2 cell balance of the patients since a Th1-type cytokine reaction pattern is should be an important defence mechanism of the adaptive cellular immune response against intracellular infectious agents, whereas a Th2-type cytokine secretion pattern regulates B-cell proliferation and antibody class switching, along these lines instructing humoral immune responses (Mosmann&Sad, 1996; Dong&Flavell, 2001; Roitt & Delves, 2004).

A study by Malaguinera showed there was major changes in the immune system associated with aging in primary lymphoid organs as well as a description of molecular mechanisms, and the impact on cancer development (Malaguarnera L, 2010).

2.4.2 NK cell

Natural killer (NK) cells were found over 30 years back. NK cells are large granular lymphocytes that belong to the innate immune system because unlike T or B lymphocytes of the adaptive or antigen-specific immune system, NK cells don't rearrange T-cell receptor or immunoglobulin genes from their germ line configuration.

Thus far it has been fully appreciated that NK cells can secrete cytokines and chemokines that impact the host's immune response, and/or kill certain infected or transformed cells via perforin/granzyme (Orange, 2006).

Interferon gamma (IFN-γ) is considered the prototypic NK-cell cytokine, and its production by NK cells is known to initiate the Th1 immune response (Mocikat *et al.*, 2003), induce APCs to further up-regulate MHC class I expression (Wallach *et al.*, 1982), activate macrophage killing of obligate intracellular pathogens (Filipe-