DETECTION OF IRON DEFICIENCY ANAEMIA IN ANAEMIA OF CHRONIC DISEASE PATIENTS IN HUSM: A PRELIMINARY CASE CONTROL STUDY

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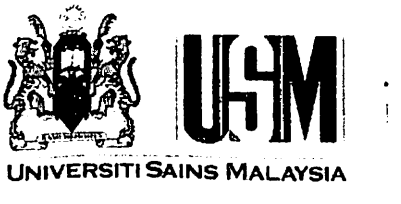

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LIST OF ABBREVIATIONS

Hb	Haemoglobin
sTfR	soluble transferrin receptor
TfR	transferrin receptor
Tf	transferrin
TIBC	total iron binding capacity
MCHC	mean cell haemoglobin concentration
MCV	mean cell corpuscular volume
RDW	red cell distribution witdh
HPX	haemopexin
ACD	anaemia of chronic disease
IDA	iron deficiency anaemia
IDE	iron deficiency erythropoiesis
TfR-F	transferrin receptor-ferritin
TNF-α	tumour necrosis factor alpha
HIV	human immunodeficiency virus
DMT	divalent metal transporter
IFN-γ	interferon gamma
IFN-β	interferon beta
IFN- α	interferon alpha
ZPP	zinc protoporphyrin
CHr	reticulocyte haemoglobin
DNA	deoxyribonucleic acid

ABSTRAK

Pengesanan kekurangan zat besi dalam pesakit yang menghidap penyakit anemia kronik adalah tidak mudah. Ini adalah kerana, ujian makmal untuk mengesan status zat besi yang selalu digunakan dipengaruhi oleh tindakbalas akut. Tujuan kajian ini adalah untuk mengetahui kekerapan kekurangan zat besi dalam pesakit yang menghidap penyakit anemia kronik menggunakan pelbagai ujian hematologikal dan membandingkan keputusan dengan kontrol.

Seramai 60 pesakit yang disahkan menghidap penyakit anemia kronik dan seramai 25 kontrol terdiri daripada sukarelawan yang sihat yang bekerja di HUSM diambil untuk menyertai kajian ini. Kedua-dua kumpulan haruslah memenuhi syarat-syarat pengambilan dan pengecualian sebelum diuji untuk mengetahui status zat besi. Ujian-ujian yang dijalankan termasuklah gambaran keseluruhan sel-sel darah, ferritin, penerima transferrin dan indeks penerima transferrin- ferritin.

Keputusan menunjukan tiada seorang daripada kumpulan kontrol yang ada kekurangan zat besi. Kekurangan zat besi dalam pesakit yang ada anemia kronik dikesan sebanyak 20% menggunakan penerima transferrin,16.7% menggunakan indeks penerima transferrin-ferritin dan tiada kes yang dikesan oleh ferritin. Kekurangan yang signifikan ditemui dari segi purata isipadu sel darah dan ferritin tetapi nilai yang lebih tinggi yang signifikan dalam ujian penerima transferrin dan keluasan pengagihan sel darah merah yang dibandingkan diantara pesakit penyakit anemia kronik dan kontrol

Didapati terdapat kekurangan yang siginifikan dari segi purata isipadu sel darah, purata kepekatan sel hemoglobin dan ferritin, nilai lebih tinggi yang juga signifikan dari segi ujian penerima transferrin dan keluasan pengagihan sel darah merah yang dibandingkan diantara pesakit yang ada kekurangan zat besi dan yang tiada dalam kumpulan penyakit anemia kronik.

Penemuan yang tersebut menunjukan profil anemia disebabkan oleh kekurangan zat besi dan menyokong kemungkinan adanya kekurangan zat besi dalam pesakit penyakit anemia kronik.

Tiada perbezaan yang signifikan didalam purata penerima transferrin di antara lelaki dan perempuan dalam kumpulan kontrol. Walaubagaimanapun, terdapatnya pertalian yang lemah apabila membandingkan umur dan sukatan penerima transferrin.

Kesimpulannya, terdapat sebahagian pesakit penyakit anemia kronik yang ada kekurangan zat besi dan pengetahuan tentang kewujudan ini adalah berguna kerana ia boleh menolong dalam pengurusan pesakit-pesakit ini.

ABSTRACT

Detecting iron deficiency anaemia (IDA) in anaemia of chronic disease (ACD) patients is not easy, as the conventional laboratory indices of iron status are often influenced by acute phase responses. The aim of the study is to determine the prevalence of iron deficiency in ACD patients using various haematological tests and to compare with the normal control.

Sixty ACD patients were purposively recruited while 25 controls were healthy volunteers among the HUSM staffs. Both groups had to fulfill their inclusion and exclusion criteria before they were tested to determine the iron status. Investigations for iron status consisted of full blood picture with other haematological indices, serum ferritin, soluble transferrin receptor (sTfR) and Transferrin receptor-ferritin index (TfR-F index).

Results showed that none of the controls were having iron deficiency anaemia. Coexisting IDA in ACD group were detected 20% by sTfR, 16.7% by TfR–F index and none using serum ferritin. There were significantly lower mean corpuscular volume (MCV) and serum ferritin but higher for red cell distribution width (RDW) and sTfR in ACD group compared to the normal controls ($P<0.001$)

Comparing IDA cases and the non-IDA cases in the ACD group, there were significantly lower mean corpuscular volume (MCV),mean cell haemoglobin concentration (MCHC) and serum ferritin but higher for red cell distribution width (RDW) and sTfR ($P<0.001$),

The findings above pointed towards "IDA-like" profile and support the possibility of co-existing iron deficiency anaemia. The mean sTfR level was not significantly different between males and females in the control group. However, a weak correlation was noted between age and sTfR levels.

In conclusion, a proportion of ACD patients had coexisting IDA and knowing this coexistence will be beneficial, as it will assist in better management of these patients.

CHAPTER 1

GENERAL INTRODUCTION

1.0 GENERAL INTRODUCTION

Iron deficiency is a common clinical problem. In many cases, iron deficiency is relatively simple to diagnose. However, in some patients with many medical problems, such as malignancy, infection, inflammation, the diagnosis can be difficult (Weiss and Goodnough, 2005).

Serum iron, total iron binding capacity (TIBC), transferrrin saturation and serum ferritin are the conventional laboratory tests to assess iron status. These tests are however influenced by inflammatory acute phase reactions, which may complicate the clinical interpretation of the tests results (Fitzsimons *et al*, 2001).

The detection of iron deficiency in the presence of chronic disease is an important diagnostic challenge because of the frequency of the problem seen in clinical practice and its direct implication on the patient management (Weiss and Goodman, 2005).

In anaemia of chronic disease (ACD) inappropriate iron therapy may aggravate the underlying disease (Vreugdenhil *et al*, 1990).Because the absence of stainable iron in the bone marrow examination is generally regarded as the definitive marker of iron deficiency, marrow examinations are generally requested to confirm iron deficiency. This method is invasive and not convenient (Punnonen *et al*, 1997)

There is an evident clinical need for non invasive and sensitive method for detection of iron deficiency, and in recent years, the serum transferrin receptor (sTfR) level has been introduced as a new tool for the diagnosis of iron depletion (Cook, 2005).

Serum transferrin receptor is largely determined by the quantum of erythropoeisis and by intracellular iron content of the cells of the erythroid lineage (Gupta *et al*, 2003). Most importantly, it is not an acute phase reactant, and remains normal in patients with inflammatory conditions (Skikne, 1998). Hence, it could serve as a better indicator for the existence of iron deficiency in ACD.

In the previous study, transferrin receptor-log ferritin ratio, the TfR-F index, was used because it was shown to improve the diagnostic efficiency for IDA compared with serum sTfR level alone or the sTfR-ferritin ratio. The TfR-F index is an accurate marker for IDA, because it represents the total-body iron stores and the availability of iron for erythropoiesis. A positive finding on the TfR-F index (>2) can accurately establish a diagnosis of IDA and may eliminate the need for bone marrow examination (Weiss and Goodnough, 2005)

I hope that this study will contribute to a better comprehension of the problem and will help to find the ideal way of detecting coexisting iron deficiency in anaemia of chronic disease patients.

CHAPTER 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 IRON METABOLISM

2.1.1 Introduction

Iron is the fourth most abundant terrestrial element, comprising approximately 4.7% of the earth's crust in the form of the minerals hematite, magnetite, and siderite. Despite its abundance in the earth's crust, iron deficiency is a serious health issue in many parts of the world. The iron nutrition status of an individual and of populations is largely a function of the amount of dietary iron, the bioavailability of that iron, and the extent of iron losses. Heme iron is an important dietary source of iron because it is more effectively absorbed than nonheme iron. From 5% to 35% of heme iron is absorbed from a single meal, whereas nonheme iron absorption from a single meal can range from 2% to 20% (Pietrangelo, 2001)

2.1.2 Iron Absorption

The majority of iron absorption takes place in the duodenum and upper jejunum.The process of iron absorption can be divided into three stages: (1) iron uptake, (2) intraenterocyte transport, and (3) storage and extraenterocyte transfer. During the intestinal phase of digestion, iron binds to specific mucosal membrane sites, is internalized, and then either is retained by the mucosal cell or is transported to the basolateral membrane where it is bound to transferrin (Tf) in the plasma pool. The process of iron absorption is controlled by intraluminal, mucosal, and somatic factors. A multitude of intraluminal factors affect the amount of iron available for absorption as either inhibitors or promoters. Mucosal factors include the amount of mucosal surface

and intestinal motility. Somatic factors that influence iron absorption include erythropoiesis and hypoxia (Roy *et al*, 2000)

Hemoglobin and serum ferritin apparently have limited roles in signaling the enterocyte about the need for iron absorption. It has been suggested that internalized plasma ferro-Tf may allow the enterocyte to monitor body iron status and regulate iron absorption. Exposure to low intracellular amounts of plasma ferro-Tf would signal the enterocyte to up-regulate iron entry into the body. Transferrin receptors (TfR) are only found at the basolateral surface of enterocytes. The amount of enterocyte TfR mRNA and protein increases during iron deficiency and decreases during secondary iron loading. Acute hemolysis, which stimulates iron absorption, does not influence enterocyte basolateral TfR number (Pietrangelo, 2001).

Active erythropoiesis, induced either by bleeding or by acute hemolysis increases the absorption of iron. It has therefore been proposed that erythropoietin is an endogenous signal for iron absorption; there is limited evidence for this hypothesis. Hypoxia increases iron absorption independently of erythropoiesis (Cavill, 2002). Increased plasma iron turnover, which occurs not only in erythropoiesis but also in disorders of ineffective erythropoiesis such as thalassemia, hemolytic anemias, and sideroblastic anemias, is associated with increased iron absorption. Other clinical disorders such as hemochromatosis, congenital ferrochelatase deficiency, and porphyria cutanea tarda result in an increased iron absorption by mechanisms that are yet unexplained. Finally, inflammatory processes may decrease iron absorption,' probably by eliciting the production of cytokines that have a direct effect on the mucosal cell (Roy *et al,* 2000).

2.1.3 Iron transport and storage

The most significant iron transport molecule is transferrin. A number of other systems may make small but important contributions in transporting iron to the tissues including heme-hemopexin (HPX), ferritin and lactoferrin (Pietrangelo, 2001).

The concentration of iron in the human body is approximately 30-40 mg/kg body weight. However, that concentration varies as a function of age and gender and the specific tissues and organs examined. About 85-90% of nonstorage iron is found in the erythroid mass. The storage iron concentration in the body varies from 0 to 15 mg/kg body weight depending on gender and iron status. The distribution of this stored iron is not uniform, as the liver contains approximately 60% of the ferritin in the body. The remaining 40% is found in muscle tissues and cells of the reticuloendothelial system. Normally, 95% of the stored iron in liver tissue is found in hepatocytes as ferritin. Hemosiderin constitutes the remaining 5% and is found predominately in Kupffer cell lysosomal remnants. However, during iron overload, the mass of hemosiderin in the liver accumulates at 10 times the rate of ferritin (Bastin *et al,* 1998).

Most cellular iron acquisition occurs via TfR uptake. The functional receptor is composed of two monomers, linked by two disulfide bridges. Virtually all cells, except mature red cells, have TfR on their surface, but the largest numbers are in the erythron, placenta and liver. In a normal adult, about 80% are in the erythroid marrow. Receptor density on proliferating cells is related to the availability of iron as deprivation of iron results in prompt induction of TfR synthesis whereas excess iron suppresses TfR

numbers. Hence, the total mass of cellular TfR depends both on the number of erythroid precursors in the bone marrow and on the number of TfR per cell, a function of iron status of the cell (Beguin, 2003).

A circulating form of TfR has been found in human as well as animal serum. Serum TfR (sTfR) is a soluble truncated monomer of tissue receptor, lacking its first 100 amino acid, which circulates in the form of transferrin and its receptor. STfR is produced by proteolysis, mediated by a membrane – associated serine protease that occurs mostly at the surface of exosomes (Shih *et al*, 1990)

2.2 ERYTHROPOIESIS AND IRON

Iron and erythropoeisis are inextricably linked. Erythropoeisis is a dynamic process that requires 30-40 mg iron each day to be supplied to the developing erythroblasts. This is a highly dynamic process based on the delicate balance between iron demand and supply. The major source of large amount of iron that the developing red cells require is delivered by transferrin which is received by the transferrin receptor on the surface of erythroblast (Cavill, 2002). The amount of iron required by the developing erythroblasts is some 10-fold greater than the total amount of iron circulating in the transferrin iron pool at any one time. Hence, this pool turnover 10 or more times each day and an iron atom entering that pool will only spend ninety minutes in the circulation before being taken up by the marrow. The transient balance between inflow and outflow is easily disturbed and this accounts for the biological lability of that pool (Pietrangelo, 2001).

In normal circumstances daily red cell production is balanced by equal red cell destruction. This releases sufficient iron to maintain haemoglobin (Hb) synthesis at its previous level.

It is established that the positive feedback control mechanism of hypoxia, erythropoietin secretion and haemoglobin concentration are the determinants of erythropoietic activity. It is evident that other mechanisms may also play a part. In normal subjects with no obvious hypoxic problems, it was shown that some marrows would produce red cells at twice the rate of others. In a fully compensated haemolysis, where red cells production are many times the normal level, the absence of hypoxic stimulus does not lead to any increase in erythropoietin secretion (Kendall, 2001)

The balance of red cell production and destruction maintains the haemoglobin concentration. When production fails to keep pace with destruction then there will be a gradual diminution in the Hb concentration until this reaches some arbitrary level that is considered to indicate anaemia (Cavill, 2002).

2.3 ANAEMIA OF CHRONIC DISEASE

2.3.1 Definitions

Anaemia of chronic disease (ACD) is the second most prevalent after anaemia caused by iron deficiency, occurs in patients with acute or chronic immune activation (Weiss, 2002).

The most frequent conditions associated with anemia of chronic disease are acute and chronic infections, inflammatory, neoplastic, chronic kidney disease and inflammatory diseases (Weiss, 2002). Syndromes similar or identical to ACD are also observed in intensive care units, in post-surgical setting and following severe trauma (Means, 2004)

The diagnosis of ACD is quite easy and based on the underlying disturbances of iron homeostasis which lead to withdrawal of the metal from the sites of erythropoeisis and the circulation to the storage compartment in the reticuloendothelial system thus causing at the same time hypoferraemia and hyperferritinaemia (Weiss, 2002).

They are usually of moderate severity with high haemoglobin (Hb) concentration ranging from 7 to 11g/dl. They are associated with a low serum iron, a low iron-binding capacity, increased tissue iron stores and a reduced rate of red cell production (Fitzsimons *et al,* 2001)

2.3.2 Pathophysiological features

i. Introduction

ACD is immune driven. Cytokines and cells of the reticuloendothelial system induce changes in iron homeostasis, the proliferation of erythroid progenitor cells, the production of erythropoietin, and the life span of red cells, all of which contribute to the pathogenesis of anaemia (Weiss *et al*, 2005).

Erythropoiesis can be affected by disease underlying anemia of chronic disease through the infiltration of tumor cells into bone marrow or of microorganisms, as seen in human immunodeficiency virus (HIV) infection, hepatitis C, and malaria (Gordeuk *et al*, 2001). Moreover, tumor cells can produce proinflammatory cytokines and free radicals that damage erythroid progenitor cells. Bleeding episodes, vitamin deficiencies (e.g., of cobalamin and folic acid), hypersplenism, autoimmune hemolysis, renal dysfunction, and radio- and chemotherapeutic interventions themselves can also aggravate anemia (Groopman, 1999).

Anaemia with chronic kidney disease shares some of the characteristics of anemia of chronic disease, although the decrease in the production of erythropoietin, mediated by renal insufficiency and the antiproliferative effects of accumulating uraemic toxins, contribute importantly. In addition, in patients with end-stage renal disease, chronic immune activation can arise from contact activation of immune cells by dialysis membranes, from frequent episodes of infection, or from both factors, and such patients

present with changes in the homeostasis of body iron that is typical of anemia of chronic disease (Eschbach *et al*, 2002)

ii. Disturbances in Iron Homeostasis

A hallmark of anaemia of chronic disease is the development of disturbances of iron homeostasis, with increased uptake and retention of iron within cells of the reticuloendothelial system. This leads to a diversion of iron from the circulation into storage sites of the reticuloendothelial system, subsequent limitation of the availability of iron for erythroid progenitor cells, and iron-restricted erythropoiesis (Weiss *et al*, 2005).

In mice that are injected with the proinflammatory cytokines interleukin-1 and tumor necrosis factor α (TNF-α), both hypoferremia and anemia develop; this combination of conditions has been linked to cytokine-inducible synthesis of ferritin, the major protein associated with iron storage, by macrophages and hepatocytes (Torti *et al*, 2002). In chronic inflammation, the acquisition of iron by macrophages most prominently takes place through erythrophagocytosis and the transmembrane import of ferrous iron by the protein divalent metal transporter 1 (DMT-1) (Andrews, 1999)

IFN-γ (Interferon-γ), lipopolysaccharide, and TNF-α upregulate the expression of DMT-1, with an increased uptake of iron into activated macrophages. These proinflammatory stimuli also induce the retention of iron in macrophages by down-regulating the expression of ferroportin, thus blocking the release of iron from these cells (Torti *et al*, 2002). Ferroportin is a transmembrane exporter of iron, a process that is believed to be

responsible for the transfer of absorbed ferrous iron from duodenal enterocytes to the circulation. Moreover, antiinflammatory cytokines such as interleukin-10 can induce anaemia through the stimulation of transferrin-mediated acquisition of iron by macrophages and by translational stimulation of ferritin expression (Tilg *et al*, 2002).

The identification of hepcidin, an iron-regulated acute-phase protein that is composed of 25 amino acids, helped to identify the relationship of the immune response to iron homeostasis and anemia of chronic disease Hepcidin expression is induced by lipopolysaccharide and interleukin-6 and is inhibited by TNF-α. Transgenic or constitutive overexpression of hepcidin results in severe iron-deficiency anemia in mice (Nicolas *et al*, 2002). Inflammation in mice that are hepcidin-deficient did not lead to hypoferremia, a finding that suggests that hepcidin may be centrally involved in the diversion of iron traffic through decreased duodenal absorption of iron and the blocking of iron release from macrophages that occurs in anemia of chronic disease The induction of hypoferremia by interleukin-6 and hepcidin occurs within a few hours and is not observed in interleukin-6-knockout mice that are treated with turpentine as a model of inflammation, a finding that suggests that hepcidin may be central to anemia of chronic disease (Nemeth *et al*, 2004). A recently identified gene, hemojuvelin, may act in concert with hepcidin in inducing these changes. Accordingly, the disturbance of iron homeostasis with subsequent limitation of the availability of iron for erythroid progenitor cells appears to impair the proliferation of these cells by negatively affecting heme biosynthesis (Andrews, 2004).

iii.Impaired proliferation of erythroid progenitor cells

The proliferation and differentiation of erythroid precursors - erythroid burst-forming units and erythroid colony-forming units are impaired and are due to the inhibitory effects of interferon-α, -β, and -γ, TNF-α, and interleukin-1.These cytokines influence the growth of erythroid burst-forming units and erythroid colony-forming units. Interferon-γ appears to be the most potent inhibitor of erythropoiesis in directly blocking colony forming unit proliferation. This notion is reflected by an inverse correlation with hemoglobin concentrations and reticulocyte counts (Weiss, 2002). The underlying mechanisms may involve cytokine-mediated induction of apoptosis, which appears, in part, related to the formation of ceramide, the down-regulation of the expression of erythropoietin receptors on progenitor cells, impaired formation and activity of erythropoietin, and a reduced expression of other prohematopoietic factors, such as stem-cell factor .Moreover, cytokines exert direct toxic effects on progenitor cells by inducing the formation of labile free radicals such as nitric oxide or superoxide anion by neighboring macrophage-like cells (Means, 2004)

iv. Blunted erythropoietin response

Erythropoietin regulates erythroid-cell proliferation centrally. Erythropoietin expression is inversely related to tissue oxygenation and haemoglobin levels, and there is a semilogarithmic relation between the erythropoietin response (log) and the degree of anaemia (linear). Erythropoietin responses in anaemia of chronic disease are inadequate for the degree of anaemia in most, but not all, conditions (Cazzola *et al*, 1996)

The cytokines interleukin-1 and TNF-α directly inhibit erythropoietin expression in vitro, a finding that is probably due, at least in part, to cytokine-mediated formation of reactive oxygen species, which in turn affects the binding affinities of erythropoietin-inducing transcription factors and also damages erythropoietin-producing cells. Although convincing data from human studies are lacking, the injection of lipopolysaccharide into mice results in reduced expression of erythropoietin mRNA in kidneys and decreased levels of circulating erythropoietin (Jelkmann, 1998).

The responsiveness of erythroid progenitor cells to erythropoietin appears to be inversely related to the severity of the underlying chronic disease and the amount of circulating cytokines, since in the presence of high concentrations of interferon-γ or TNF-α, much higher amounts of erythropoietin are required to restore the formation of erythroid colony-forming units. After binding to its receptor, erythropoietin stimulates members of the signal transduction pathways and subsequently activates mitogen and tyrosine kinase phosphorylation, processes affected by the inflammatory cytokines and the negative-feedback regulation they induce (Minoo *et al*, 2004).

The response to erythropoietin is further reduced by the inhibitory effects of proinflammatory cytokines toward the proliferation of erythroid progenitor cells, the parallel down-regulation of erythropoietin receptors, and the limited availability of iron to contribute to cell proliferation and hemoglobin synthesis. Finally, increased erythrophagocytosis during inflammation leads to a decreased erythrocyte half-life, along with anticipated damage to erythrocytes that is mediated by cytokines and free radicals (Spivak, 2002).

2.4 IMPAIRMENT OF IRON STATUS

2.4.1 Introduction.

A requirement for analysis and determination of iron status is the detection of all phases of developing iron deficiency. Real iron deficiency is to be correctly differentiated from ACD. However, in some cases the ACD can coexist with iron deficiency state which makes the laboratory evaluation of the iron status difficult. It is important to identify iron deficiency state in the patients with ACD as this determines subsequent management of the patients.

2.4.2 Definitions of impaired iron status.

Iron deficiency is defined as a reduction in total body iron to an extent that iron stores are fully exhausted and some degree of tissue iron deficiency is present.
Iron deficiency develops slowly in three stages; storage iron deficiency, iron deficiency erythropoiesis (IDE) and iron deficiency anaemia (IDA) (Cook, 2005)

Iron deficient erythropoiesis refers to impaired supply of plasma iron to the erythroid marrow for haemoglobin synthesis, either directly as a reduced iron saturation of plasma transferrin, or indirectly as signs of iron deficiency in circulating red blood cells (Worwood, 1997)

IDE is a cardinal feature of iron deficiency anaemia but it can also be seen in other clinical disorders associated with inflammation or malignancy. Iron store are totally absent in IDA, whereas IDE can occur with normal or increased amounts of storage iron resulting from impaired release of iron to the plasma from the storage iron compartment.

Functional iron deficiency is a specific form of IDE seen in patients given recombinant human erythropoietin, a treatment that exaggerates any disparity between the influxes of iron to the plasma from the storage compartment and the iron requirements erythroid marrow (Thomas *et al*, 2002).

2.5 LABORATORY EVALUATION OF ANAEMIA OF CHRONIC DISEASE

2.5.1 Screening measurements

Screening measurements identify iron deficiency erythropoiesis by demonstrating either a reduced supply of plasma iron or poor haemoglobinization of circulating red blood cells. Haemoglobin is the first screening measurement and is measured as part of the complete blood count as mean cell haemoglobin (MCH) content and as mean cell haemoglobin concentration (MCHC) (Cook, 2005).

In ACD, the haemoglobin level is normally moderately reduced (7-11g/dl). However, it has low specificity and sensitivity for identifying iron deficiency. This is because there

are numerous other causes of anaemia that can mimic iron deficiency in the laboratory (Worwood, 1997).

Screening measurements that identify IDE are helpful in reducing the diagnostic possibilities for IDA. Measurement of serum iron concentration provides little useful information because of the considerable variation from hour-to-hour and day-to-day in normal individuals. Low concentrations are found in patients with IDA and high concentrations in patients with iron overload. Low serum iron concentration can also be a response to inflammation, infection and surgery and does not necessarily indicate an absence of storage iron (Worwood, 1997).

More information can be obtained by measuring both the serum iron concentration and the total iron binding capacity (TIBC), from which the percentage of transferrin saturation with iron may be calculated. Marked diurnal variation in plasma iron values and numerous clinical disorders that affect the transferrin saturation limit its clinical utility. Normal or elevated transferrin saturation is as useful for excluding IDA as low value is for identifying it (Cook *et al*, 2003).

With the improved accuracy of measuring red-cell indices that resulted from the introduction of automated haematology analysers, the MCV became the most important red cell marker for detecting IDE It is a reliable and widely available measurement it is relatively late indicator in patients who are not actively bleeding. Thalassaemia must be considered in the differential diagnosis of a low MCV (Cook, 2005)

The percentage of circulating hypochromic erythrocytes permits earlier detection of IDE than the MCV, but a few weeks of IDE is required before the measurement becomes abnormal.

A more rapid change occurs with the reticulocyte haemoglobin content (CHr) that falls within couple of days of the onset of IDE. Falsely normal CHr can be measured with an elevated MCV or thalassaemia (Thomas *et al*, 2002).

Another simple and reliable method is the measurement of erythrocyte zinc protoporphyrin (ZPP), a product of abnormal haem synthesis (Labbe *et al*, 1999). The concentration increases in iron deficiency. The small sample size, simplicity, rapidity and reproducibility within the laboratory are advantages (Worwood, 1997). Chronic diseases that reduce serum iron concentration, but do not reduce iron stores, also increase protoporphyrin level. A significant limitation is that it increases with lead toxicity, and even the normal range varies with environmental lead (Cook *et al*, 2003)

2.5.2 Storage iron

The diagnosis of IDA requires evidence that iron stores are fully depleted. Bone marrow examination has been widely regarded the gold standard for the diagnosis. However, recent studies have shown that the marrow iron stain is often unreliable. It is also not

suitable to be done in clinical practice for the sole purpose of diagnosing IDA because of the expense, discomfort and the technical pitfalls of this approach (Cook, 2005).

The serum ferritin has been the most useful laboratory measure of iron status which offers important advantages over bone marrow examination. A valuable feature of the measurement is that the concentration is directly proportional to body iron stores in healthy individuals. It is universally available, well standardized measurement and a level of $\leq$ 12-20μg/l is a highly specific indicator of iron deficiency (Mast *et al*, 1998). Many studies have shown its superiority over other iron-related measurements for identifying IDA. Ferritin consists of H- and L-subunits. The H- subunits are regulated at the DNA level by cytokines and may act like a positive acute phase protein that increases in response to underlying infection and inflammation. In this context, serum ferritin does no longer reflect body iron stores when iron deficiency and ACD coexist, and its use may result in an underestimation of iron deficiency (Ahluwalia, 1998)

2.5.3 Tissue iron

The diagnosis of iron deficiency requires the demonstration of tissue iron deficiency. This can be demonstrated by examining the iron status of the erythroid marrow. The uptake of plasma iron by red-cell precursors is regulated by the transferrin receptor, a transmembrane glycoprotein that serves as the portal for the transferring iron to the interior of all body cells (Cook, 2005).

2.5.4 Soluble transferrin receptor and iron status

Soluble form of the transferrin receptors (sTfR) are detectable in the circulation by immunoassay and appear to reflect the number of transferrin receptors on immature red cells and thus, the level of bone marrow erythropoieisis (Cazzola *et al,* 1992)

It has been shown that, as compared to normal individuals, levels are marginally increased in nonanaemic iron-deficient subjects but more dramatically so in patients with IDA (Punnonen *et al*, 1997). In such patients, sTfR levels exhibit strong correlations with various red cell indices indicative of iron deficiency and are inversely related to serum ferritin (Olivares *et al*, 2000).

Skikne et al in 1990 have published a study on the clinical utility of sTfR as a marker of the iron status. When normal volunteers underwent graded phlebotomy, ferritin decreased progressively while sTfR did not change much during the phase of storage iron depletion.

However, sTfR increased significantly when marrow functional iron deficiency and anaemia developed. Hence, the iron status may be fully assessed by using serum ferittin as a measure of iron storage (storage iron depletion), sTfR as a measure of functional tissue iron deficiency (iron deficient erythropoiesis), and Hb as a measure of advanced iron deficiency (IDA). Because of the reciprocal relationship between sTfR and ferritin

measurements, the ratio of sTfR/ferritin describes a perfect log-linear relationship to body iron over a wide range of normal and depleted iron stores (Skikne *et al*, 1990).

This can also be obtained by the log of the ratio of sTfR to ferritin or by the sTfR-ferritin index, the ratio of sTfR to log ferritin. These ratios increase the sensitivity of sTfR in detecting latent iron deficiency (Malope *et al*, 2001).

2.5.5 Erythropoietin

Measurement of erythropoietin levels is useful only for anaemic patients with haemoglobin level of less than 10g per deciliter. This is because the erythropoietin level at higher haemoglobin concentrations remains in the normal range.

Erythropoietin levels have been analyzed for their predictive value with respect to treatment of ACD with erythropoietic agents (Ludwig *et al*, 1994)

CHAPTER 3

OBJECTIVES

3.0. OBJECTIVES OF THE STUDY

3.1. General objective

i. To detect coexisting iron deficiency anemia in patients with anaemia of chronic disease and to compare the haematological parameters between the control and the anaemia of chronic disease patients.

3.2. Specific objectives

i. To identify iron deficiency in patients with anaemia of chronic disease using serum ferritin, soluble transferrin receptor (sTfR) and transferrin receptor- ferritin index (TfR- F index)

ii. To determine the agreement between the TfR- F index and sTfR in detecting iron deficiency

iii. To correlate the sTfR value with other haematological parameters in patients with coexisting IDA.

iv. To correlate sTfR value with age and sex in the control group.

v. To compare the haematological parameters between the IDA and non-IDA group determined by sTfR and transferrin receptor-ferritin index (TfR-F index)

CHAPTER 4

METHODOLOGY

4.0 METHODOLOGY

4.1. Study design

This case control study was conducted over a one year period from November 2004 till November 2005 at Hospital Universiti Sains Malaysia. Protocol for this study was approved by the School of Medical Science Research and Ethical Committee. All subjects gave written consent.

4.2 Sampling for cases

4.2.1 Source population for cases

The source population of the subjects was those who attended Medical Clinic, Hospital Universiti Sains Malaysia November 2004 till November 2005 who was diagnosed as having anaemia of chronic diseases. The chronic diseases included in this study were chronic infections, autoimmune diseases and chronic kidney diseases.

4.2.2 Sampling frame for cases

The sampling frame was those who were diagnosed as having anaemia of chronic diseases mentioned above and fulfill the inclusion and exclusion criteria.

4.2.3 Inclusion criteria for cases

The inclusion criteria were patients who had the haemoglobin of less than 11.5g/dl for female, less than 13.5g/dl for male (according to WHO criteria for anaemia), mean corpuscular volume of less than 95fl and confirmed diagnosis of having anaemia of chronic disease. The diagnosis was confirmed by history, physical examination and laboratory tests done by the managing doctor.

4.2.4 Exclusion criteria for cases

The exclusion criteria were those who were diagnosed as having thalassaemia major, iron deficiency anaemia, megaloblastic anaemia and hematological malignancy, had previous blood transfusion in the preceding month and those on treatment with oral or parenteral iron. All the above data were obtained from the patients' clinical records.

4.2.5 Sampling method for cases

Purposive sampling was carried out for cases. All those who fulfill the criteria mentioned were included. A total of 60 patients were included in this study. The flow chart is shown in Figure 1.0

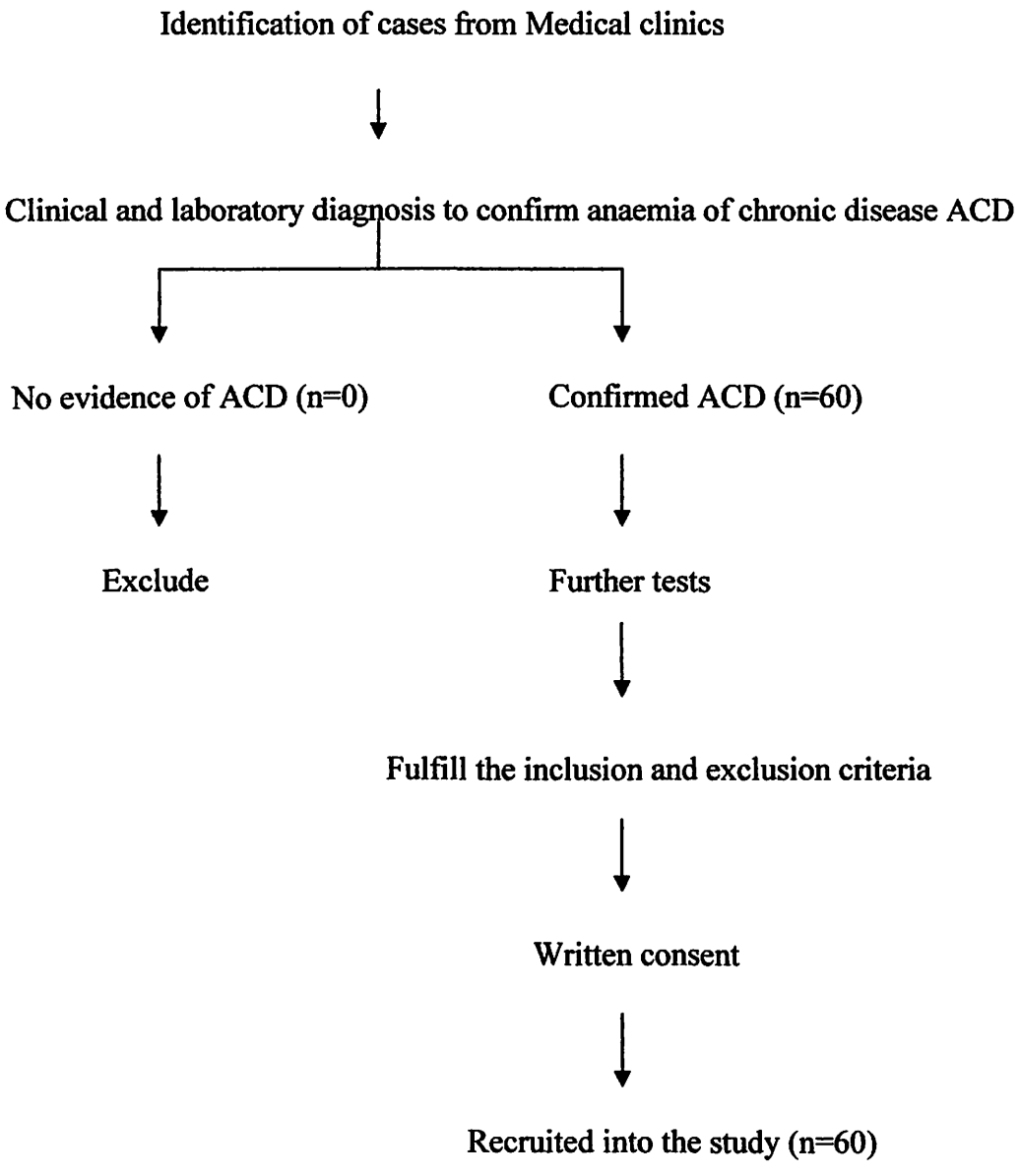

Figure 1.0 Flowchart of sampling methods for cases