1. Introduction and Literature review

Anemia is still a major publi c health problem in many developing countries. The World Health Organization (WHO) Global Database on Anemia for 1993-2005 showed that 25% or 1.62 billion people globally suffer from anemia.(Benoist,*et al* 1993:WHO.2008)

Iron deficiency anemia is the most common cause of nutritional anemia throughout the world .The prevalence of iron deficiency anemia in developing countries has been reported to be more than 50% in children, which is mainly due to poor nutrition.(Cardenas,*et al* 2006)

Low dietary intakeof bioavalable iron is assumed to be a major cause of anemia in the developing world. Dietary iron consumed in resource poor areas is predominantly non-heme iron of plant origin, containing high amounts of inhibitors of iron absorption such as phytate(Hallberg,*et al 1987)*. In addition to low iron bioavailability from the diet, the high prevalence of infections and other nutritional deficiencies in developing countries can also contribute to the development of anemia.(WHO .1987)

Blood loss caused by gastrointestinal parasites, such as hookworm, is considered to be an important contributing factor in the development of poor iron status leading to iron deficiency anemia. More recently, another common infection, *Helicobacter pylori*, has been discussed in the etiology of anemia and IDA(Crompton and Nesheim.2002)

H. pylori is a common gastrointestinal pathogen world-wide with a very high prevalence in many developing countries. Once established, *H. pylori* infection is thought to persist life resulting in chronic gastritis, which is a risk factor for

development of atrophic gastritis, reduced gastric acid output and gastric cancer(Kuipers,*et al* 1995). Gastric acid is considered to be one of the most important luminal factors necessary for optimal non-heme iron absorption. (Skikne ,*et al* 1981). it is possible that *H. pylori* infections can result in a reduced ability to absorb iron due to gastric atrophy and achlorhydria, or due to a more transient hypochlorhydria during active infections. Both mechanisms would result in an increased susceptibility to IDA. Pervasive occult gastrointestinal bleeding as a result of chronic active gastritis has also been considered as a cause for iron deficiency anemia in *H. pylori* infected subjects (Yip,*et al* 1997)

Several cross-sectional studies have found an association between H.pylori and low body iron store and iron deficiency anemia and reduced response to iron supplementation.

In this study we have evaluated of iron status in patients with Hpylori bacteria to detect the effect of this bacteria in the amount of iron in human body.

1.1 Iron

1.1.1 Biochemistry and physiology of iron

In contrast to zinc, iron is an abundant element on earth and is a biologically essential component of every living organism However, despite its geologic abundance, iron is often a growth limiting factor in the environment. This apparent paradox is due to the fact that in contact with oxygen iron forms oxides, which are highly insoluble, and thus is not readily available for uptake by organisms. In response, various cellular mechanisms have evolved to capture iron from the environment in biologically useful forms. Examples are siderophores secreted by microbes to capture iron in a highly specific complex. Or mechanisms to reduce iron from the insoluble ferric iron (Fe^{+3}) to the soluble ferrous form (Fe^{+2}) as in yeasts. Many of the mechanisms found in lower organisms, have analogous counterparts in higher organisms, including humans. In the human body, iron mainly exists in complex forms bound to protein (hemoprotein) as heme compounds (hemoglobin or myoglobin), heme enzymes, or nonheme compounds (flavin-iron enzymes, transferring, and ferritin). The body requires iron for the synthesis of its oxygen transport proteins, in particular hemoglobin and myoglobin, and for the formation of heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation-reductions. Almost two-thirds of the body iron is found in the hemoglobin present in circulating erythrocytes, 25% is contained in a readily mobilizable iron store, and the remaining 15% is bound to myoglobin in muscle tissue and in a variety of enzymes involved in the oxidative metabolism and many other cell functions.

(Wood and Ronnenberg .2005:McDowell.2003)

1.1.2Human requirements

During early infancy, iron requirements are met by the little iron contained in the human milk. The need for iron rises markedly 4-6 months after birth and amounts to about 0.7-0.9 mg/day during the remaining part of the first year.Between 1 and 6 years of age, the body iron content is again doubled. Iron requirements are also very high in adolescents, particularly during the period of growth spurt. Girls usually have their growth spurt before menarche, but growth is not finished at that time. In boys there is a marked increase in hemoglobin mass and concentration during puberty. In this stage, iron requirements increase to a level above the average iron requirements in menstruating women.

Iron requirements of 97.5% of individuals in terms of absorbed iron, by age group and sex .The average adult stores about 1-3 g of iron in his or her body. A fine balance between dietary uptake and loss maintains this balance. About 1 mg of iron is lost each day through sloughing of cells from skin and mucosal surfaces, including the lining of the gastrointestinal tract.Menstruation increases the average daily iron loss to about 2 mg per day in premenopausal female adults. The augmentation of body mass during neonatal and childhood growth spurts transiently boosts iron requirements.

A dietary intake of iron is needed to replace iron lost in the stools and urine as well as through the skin. These basal losses represent approximately 0.9 mg of iron for an adult male and 0.8 mg for an adult female. The iron lost in menstrual blood must be taken into consideration for women of reproductive age.(WHO/FAO.2004:Demayer,*et al.*1989)

1.1.3 Metabolism

1.1.4 Bioavailability

Dietary iron occurs in two forms: heme and nonheme. The primary sources of heme iron are hemoglobin and myoglobin from consumption of meat, poultry, and fish, whereas nonheme iron is obtained from cereals, pulses, legumes, fruits, and vegetables. Heme iron is highly bioavailable (15%-35%) and dietary factors have little effect on its absorption, whereas nonheme iron absorption is much lower (2%-20%) and strongly influenced by the presence of other food components.On the contrary, the quantity of nonheme iron in the diet is manyfold greater than that of heme-iron in most meals. Thus despite its lower bioavailability, nonheme iron generally contributes more to iron nutrition than heme-iron. Major inhibitors of iron absorption are phytic acid, polyphenols, calcium, and peptides from partially digested proteins.Enhancers are ascorbic acid and muscle tissue which may reduce ferric iron to ferrous iron and bind it in soluble complexes which are available for absorpt(Hurrell and Egli.2010:Monsen,*et al*.1978)

1.1.5 Absorption

The fraction of iron absorbed from the amount ingested is typically low, but may range from 5% to 35% depending on circumstances and type of iron.

Iron absorption occurs by the enterocytes by divalent metal transporter 1, a member of the solute carrier group of membrane transport proteins. This takes place predominantly in the duodenum and upper jejunum. It is then transferred across the duodenal mucosa into the blood, where it is transported by transferrin to the cells or the bone marrow for erythropoiesis [producing red blood cells (RBCs)].

A feedback mechanism exists that enhances iron absorption in people who are iron deficient. In contrast, people with iron overload dampen iron absorption via hepcidin. It is now generally accepted that iron absorption is controlled by ferroportin which allows or does not allow iron from the mucosal cell into the plasma.(Mc Dowell.2003:Muir and Hopfer.1985:Hurrell.1997)

The physical state of iron entering the duodenum greatly influences its absorption. At physiological pH, ferrous iron (Fe^{+2}) is rapidly oxidized to the insoluble ferric (Fe^{+3}) form. Gastric acid lowers the pH in the proximal duodenum reducing Fe^{+3} in the intestinal lumen by ferric reductases, thus allowing the subsequent transport of Fe^{+2} across the apical membrane of enterocytes. This enhances the solubility and uptake of ferric iron. When gastric acid production is impaired (for instance by acid pump inhibitors such as the drug, prilosec), iron absorption is reduced substantially.

Dietary heme can also be transported across the apical membrane by a yet unknown mechanism and subsequently metabolized in the enterocytes by hemeoxygenase 1 (HO-1) to liberate (Fe^{+2}).(Wang and Pantopoulos.2011)

This process is more efficient than the absorption of inorganic iron and is independent of duodenal pH. It is thus not influenced by inhibitors such as phytate and polyphenols. Consequently, red meats high in hemoglobin are excellent nutrient sources of iron. Directly internalized Fe^{+2} is processed by the enterocytes and eventually (or not) exported across the basolateral membrane into the bloodstream via Fe^{+2} transporter ferroportin. The ferroportin-mediated efflux of Fe^{+2} is coupled by its reoxidation to Fe^{+2}, catalyzed by the membrane-bound ferroxidasehephaestin that physically interacts with ferroportin and possibly also by its plasma homologue ceruloplasmin. Exported iron is scavenged by transferrin,

which maintains Fe^{+3} in a redox-inert state and delivers it into tissues. The total iron content of transferrin (≈3 mg) corresponds to less than 0.1% of body iron, but it is highly dynamic and undergoes more than 10 times daily turnover to sustain erythropoiesis. The transferrin iron pool is replenished mostly by iron recycled from effete RBCs and, to a lesser extent, by newly absorbed dietary iron. Senescent RBCs are cleared by reticuloendothelial macrophages, which metabolize hemoglobin and heme, and release iron into the bloodstream. By analogy to intestinal enterocytes, macrophages export Fe^{+2} from their plasma membrane via ferroportin, in a process coupled by reoxidation of Fe^{+2} to Fe^{+3} by ceruloplasmin and followed by the loading of Fe^{+3} to transferrin.(Yeh,*et al.*2009:Theil,*et al.*2012)

Recently reported that an independent mechanism also exists for the absorption of plant ferritins mostly present in legumes. However, the relevance of the ferritin transporter is unclear as most ferritin seems to be degraded during food processing and digestion, thereby releasing inorganic iron from the ferritin shell for absorption by the normal mechanism.As one ferritin molecule contains 1000 or more iron atoms, and should also be unaffected by iron absorption inhibitors, such a mechanism would provide an important source of iron in the developing world where legumes are commonly consumed.(Hoppler,*et al.*2008)

1.1.5.1 Factors enhancing iron absorption

A number of dietary factors influence iron absorption. Ascorbate and citrate increase iron uptake in part by acting as weak chelators to help to solubilize the metal in the duodenum .Iron is readily transferred from these compounds into the mucosal lining cells. The dose-dependent enhancing effect of native or added ascorbic acid on iron absorption has been shown by researchers.The enhancing effect is largely due to its ability to reduce ferric to ferrous iron but is also due to

its potential to chelate iron.Ascorbic acid will overcome the negative effect on iron absorption of all inhibitors, which include phytate, polyphenolsand the calcium and proteins in milk products,and will increase the absorption of both native and fortification iron. In fruit and vegetables, the enhancing effect of ascorbic acid is often cancelled out by the inhibiting effect of polyphenols.Ascorbic acid is the only absorption enhancer in vegetarian diets, and iron absorption from vegetarian and vegan meals can be best optimized by the inclusion of ascorbic acid-containing vegetables. Cooking, industrial processing, and storage degrade ascorbic acid and remove its enhancing effect on iron absorption.(Conrad and Umbreit 1993:Lynch,AND Cook.1980:Bach,*et al* 2005)

1.1.5.2 Factors inhibiting iron absorption

In plant-based diets, phytate (myo-inositol hexakisphosphate) is the main inhibitor of iron absorption. The negative effect of phytate on iron absorption has been shown to be dose dependent and starts at very low concentrations of 2-10 mg/meal. The molar ratio of phytate to iron can be used to estimate the effect on absorption. The ratio should be 1:1 or preferably, 0.4:1 to significantly improve iron absorption in plain cereal or legume-based meals that do not contain any enhancers of iron absorption, or, 6:1 in composite meals with certain vegetables that contain ascorbic acid and meat as enhancers.(Hurrel,*et al*.1999)

Polyphenols occur in various amounts in plant foods and beverages, such as vegetables, fruit, some cereals and legumes, tea, coffee, and wine. The inhibiting effect of polyphenols on iron absorption has been shown with black tea and to a lesser extent with herbal teas. In cereals and legumes, polyphenols add to the inhibitory effect of phytate, as was shown in a study that compared high and low polyphenol sorghum.

Calcium has been shown to have negative effects on nonheme and heme iron absorption, which makes it different from other inhibitors that affect nonheme iron absorption only.Dose-dependent inhibitory effects were shown at doses of 75-300 mg when calcium was added to bread rolls and at doses of 165 mg calcium from milk products. It is proposed that single-meal studies show negative effects of calcium on iron absorption, whereas multiple-meal studies, with a wide variety of foods and various concentrations of other inhibitors and enhancers, indicate that calcium has only a limited effect on iron absorption.

Animal proteins such as milk proteins, egg proteins, and albumin, have been shown to inhibit iron absorption. The two major bovine milk protein fractions, casein and whey, and egg white were shown to inhibit iron absorption in humans. Proteins from soybean also decrease iron absorption.(Hallberg,*et al.*1993:Lynch,*et al.*1994).

1.1.6 Storage

Ferritin concentration together with that of hemosiderin reflects the body iron stores. They store iron in an insoluble form and are present primarily in the liver, spleen, and bone marrow.The majority of iron is bound to the ubiquitous and highly conserved iron-binding protein, ferritin. Hemosiderin is an iron storage complex that less readily releases iron for body needs. Under steady state conditions, serum ferritin concentrations correlate well with total body iron stores.Thus, serum ferritin is the most convenient laboratory test to estimate iron stores.(Wood,*et al.*2005 :Nadadur,*et al*2008 :Hunt,*et al.*2009)

1.1.7 Excretion

Apart from iron losses due to menstruation, other bleeding or pregnancy, iron is highly conserved and not readily lost from the body.There are some obligatory loss of iron from the body that results from the physiologic exfoliation of cells from epithelial surfac including the skin, genitourinary tract, and gastrointestinal tract.However, these losses are estimated to be very limited (≈1 mg/day). Iron losses through bleeding can be substantial and excessive menstrual blood loss is the most common cause of iron deficiency inwomen.(McDowell.2003:Fairbanks.1999).

1.1.8 Anemia

Anemia is functionally defined as an insufficient RBC mass to adequately deliver oxygen to peripheral tissues. For practical purposes, any of the three concentration measurements performed on whole blood can be used to establish the presence of anemia. The hemoglobin (Hb) concentration typically is expressed as graasdxmsHb per deciliter (g/dl) in the United States and as grams per liter in Europe. The hematocrit (Hct; also called the packed cell volume [PCV] or volume of packed red blood cells [vPRC]) represents the proportion of blood volume represented by RBCs, and is expressed as a percent or as a decimal. The RBC concentration is expressed in cells per microliter (10^6/μl) or cells per liter (10^{12}/L). The red cell concentration is least commonly used in the definition of anemia.(John,*et al*.2009)

1.1.9 Iron Deficiency

Iron deficiency is the state in which the content of iron in the body is less than normal. It occurs in varying degrees of severity that merge imperceptibly into one another. Iron depletion is the earliest stage of iron deficiency, in which storage iron is decreased or absent but serum iron concentration, transferrin saturation, and blood hemoglobin levels are normal. Iron deficiency without anemia is a somewhat more advanced stage of iron deficiency, characterized by decreased or absent storage iron, usually low serum iron concentration and transferrin saturation, but without frank anemia. Iron-deficiency anemia is the most advanced stage of iron deficiency. It is characterized by decreased or absent iron stores, low serum iron concentration, low transferrin saturation, and low blood hemoglobin concentration.(Kenneth,*et al*.2010)

1.1.9.1 Consequences and causes of iron deficiency

1.1.9.1.1 Consequences of iron deficiency

Iron deficiency is defined as a condition in which there are no mobilizable iron stores and in which signs of a compromised supply of iron to tissues, including the erythron, are noted.Iron deficiency can exist with or without anemia. Some functional changes may occur in the absence of anemia, but the most functional deficits appear to occur with the development of anemia.Even mild and moderate forms of iron deficiency anemia can be associated with functional impairments affecting cognitive development,immunity mechanisms, and work capacity.Iron deficiency during pregnancy is associated with a variety of adverse outcomes for both mother and infant, including increased risk of sepsis, maternal mortality, perinatal mortality, and low birth weight. Iron deficiency and anemia also reduce

learning ability and are associated with increased rates of morbidity. (Beard and Connor.2003)

1.1.9.1.2Causes of iron deficiency

Iron deficiency results from depletion of iron stores and occurs when iron absorption cannot keep pace over an extended period with the metabolic demands for iron to sustain growth and to replenish iron loss, which is primarily related to blood loss. The primary causes of iron deficiency include low intake of bioavailable iron, increased iron requirements as a result of rapid growth, pregnancy, menstruation, and excess blood loss caused by pathologic infections, such as hook worm and whipworm causing gastrointestinal blood lossand impaired absorption of iron. The frequency of iron deficiency rises in female adolescents because menstrual iron losses are superimposed with needs for rapid growth. Other risk factors for iron deficiency in young women are high parity, use of an intrauterine device, and vegetarian diets.

Nutritional iron deficiency arises when physiological requirements cannot be met by iron absorption from the diet. Dietary iron bioavailability is low in populations consuming monotonous plant-based diets with little meat. In many developing countries, plant-based weaning-foods are rarely fortified with iron, and the frequency of anemia exceeds 50% in children younger than 4 years.

When iron stores are depleted and insufficient iron is available for erythropoiesis, hemoglobin synthesis in erythrocyte precursors become impaired and hematologic signs of iron deficiency anemia appear.(Larocque,*et al.*2005:Beard.2000)

1.1.10 Group at high risk

The highest probability of suffering iron deficiency is found in those parts of a population that have inadequate access to foods rich in absorbable iron during stages of high iron demand. These groups correspond to children, adolescents, and women of reproductive age, in particular during pregnancy.

In the case of infants and adolescents, the increased iron demand is the result of rapid growth. For women of reproductive age the principle reason is the excessive blood loss during menstruation. During pregnancy, there is a significant increase in iron requirement due to the rapid growth of the placenta and the fetus and the expansion of the globular mass.In contrast, adult men and postmenopausal women are at low risk of iron deficiency and the amount of iron in a normal diet is usually sufficient to cover their physiological requirements (Dallman.1990)

1.1.11 Evaluation of iron status

Iron deficiency and eventually anemia develop in stages and can be assessed by measuring various biochemical indices. Although some iron enzymes are sensitive to iron deficiency,their activity has not been used as a successful routine measure of iron status.Laboratory measurements are essential for a proper diagnosis of iron deficiency. They are most informative when multiple measures of iron status are examined and evaluated in the context of nutritional and medical history.

The plasma or serum pool of iron is the fraction of all iron in the body that circulates bound primarily to transferrin. Three ways of estimating the level of iron in the plasma or serum include 1) measuring the total iron content per unit volume in μg/dL; 2) measuring the total number of binding sites for iron atoms on

transferrin, known as total iron-binding capacity in μg/dL[2]; and 3) estimating the percentage of the two bindings sites on all transferrin molecules that are occupied called the percentage transferrin saturation. However, marked biologic variation can occur in these values as a result of diurnal variation, the presence of infection or inflammatory conditions and recent dietary iron intake.

Zinc protoporphyrin reflects the shortage of iron supply in the last stages of hemoglobin synthesis so that zinc is inserted into the protoporphyrin molecule in the place of iron. Zinc protoporphyrin can be detected in RBCs by fluorimetry and is a measure of the severity of iron deficiency.

Serum ferritin is a good indicator of body iron stores under most circumstances. When the concentration of serum ferritin is ≥15 μg/L iron stores are present; higher concentrations reflect the size of the iron store; when the concentration is low (<12 μg/L for <5 years of age and <15 μg/L for >5 years of age) iron stores are depleted. However, ferritin is an acute phase reactant protein and its serum concentrations can be elevated, irrespective of a change in iron stores, by infection or inflammation.This means that it might be difficult to interpret the concentration of ferritin where infectious diseases are common.(Dallman.1990:WHO.2004)

Another indicator of iron status is the concentration of TfR in serum. Since TfR is mostly derived from developing RBCs, it reflects the intensity of erythropoiesis and the demand for iron. As iron stores are exhausted, the concentration rises in iron deficiency anemia indicating sever iron insufficiency. This is provided that there are no other causes of abnormal erythropoiesis. Clinical studies indicate that the serum TfR is less affected by inflammation than serum ferritin. The major advantage of TfR as an indicator is the possibility of estimating the magnitude of the functional iron deficit once iron stores are depleted.

The ratio of TfR to ferritin (TfR/ferritin) was designed to evaluate changes in both stored iron and functional iron and was thought to be more useful than either TfR or ferritin alone.TfR/ferritin has been used to estimate body iron stores in both children and adultsHowever, the high cost and the lack of standardization of the TfR assay so far have limited the applicability of the method.Low hemoglobin concentration is a measure of anemia, the end stage of iron deficiency.(Yang,*et al*.2008)

1.2 Helicobacter

Remarkably. Pylori which colonizes roughly half of the worlds population .remained undiscovered until 1982 when warren and marshall in wastren Australia overturned the dogma that bacteria could not colonized the stomach .

Over 20 species of Helicobacter are now officially recognized with more awaiting formal recognition .(David ,*et al* . 2005)

1.2.1 Description

Hpylori is aGram –negative spirally shaped bacterium .it is strictly micro – aerophilic requires carbon dioxide and rich growth media but it has atuft of sheathed unipolar flagella unlike the unsheathed flagella of campylobactetr

It is biochemically inactive in most conventional tests but produces an exceptionally powerful urease almost 100 times more active than that of proteus vulgaris which is the vital to its survival in the stomach.(David ,*et al* . 2005)

1.2.2Antigens and strain typing

Although various antigens are expressed by H.pylori serotyping is of limited practical value . However ,its genetic diversity can be exploited by molecular

typing based on DNA analysis .like campylobacters ,H.pylori exhibits considerable genetic diversity arising from natural competence , ahigh mutation rate and frequent recombination events . in consequence ahost can be colonized by apopulation of closely related variants analogous to the (quasi _species) observed with certain viruses .(David ,*et al* . 2005)

1.2.3 Pathogenesis

1.2.3.1Site of infection

H.pylori is highly adapted and lives only on gastric mucosa . colonization ceases abruptly where gastric mucosa ends , for example in arease of intestinal metaplasia in the stomach Conversely , aeares of gastric metaplasia elsewhere in the gut , notably the dudnum , may become colonized with H.pylori the setting the scene for ulceration .

The gastric antrum is the most favoured site but other site of the stomach may be colonized ,especially in patients taking an acid _lowering drug such an H2 antagonist or proton pump inhibitor , or subjects with a natural lower acid out put . The bacteria are non _invasive, being present in the must over lying the mucosa . Although gastric acid is potentially destructive to H. pylori , protection is potency by its powerful urease , which acts on the urea pass through the gastric mucosa to generate ammonium and this may neutralize acid around the bacterium colonization often extends into gastric gland .(David ,*et al* . 2005)

1.2.3.2 Manifestations

Primary infection with H .pylori is either silent or causes an illness with nausea and upper abdominal pain lasting up to weeks . Years later , the finding of gastritis and peptic ulcer disease include nausea, anorexia , vomiting , epigastric pain , and even

less specific symptoms such as belching . Many patients are asymptomatic for decades, even up to perforation of an ulcer .perforation can lead to extensive bleeding and peritonitis due to the leakage of gastric contents into the peritoneal cavity . (Kenneth,*et al*.2004)

1.2.3.3 Factorstimulatesinflammation

Neutrophils, lymphocyte s, and microabscessformation . This inflammation may be due to toxic effects of the urease or the Vac A transported into the gastric epithelial cells by the type secretion system . in side the cell , vac A causes vacuolization of endosomal compartment and has other effect including altered T_cell function . The Cag protein is injected into the gastric epithelial cell by secretion system . where it triggers multiple enzymatic reactions including those that cause reorganization of the acting cytoskeleton . Cag may also gastric mucosa .added together urease , cag , and vac A provide ample explanation for the gastritis that is universal in H. pylori infection . this prolonged and aggressive inflammatory response could lead to epithelial cell death and ulcer , but progress sion from gastritis to ulcer has not been adequately explained .(Kenneth,*et al*.

that decades of inflammation and assault by the virulence factors just described could cause metaplasia , and eventually cancer seems logical, but the specific mechanism of carcinogenesis are unknown . cag is a leading candidate . A curious paradox is that although cag+ strains are associated with ulcer and adenocarcinoma of lower stomach , they are associated with a decrease incidence of adenocarcinoma of the upper stomach (cardia) and esophagus. The gastric lymphomas may represent neoplastic transformation of B_ lymphocyte clones proliferating in response to chornic antigenic stimulation.(Kenneth,*et al*

1.2.3.4 Associateddisease

The outcome of infection by H.pylori reflects an infection between strain virulence, pro _inflammation host genotypes, and environmental factor . despite the presence of chronic active gastritis , most infection are symptomless , and endoscopic appearance of the stomach are normal. However , in some infected persons the chronic active gastritis form pad for more serious clinical outcomes , such as gastric and duodenal ulcers, non _ulcer dyspepsia and gastric malignancies. .(David ,*et al* . 2005)

1.2.3.4.1Pepticulceration

H.pylori is actively involved in the pathogenesis of peptic ulceration unrelated tonon_steroidalanti_inflammatory agents or the Zollinger_Ellison syndrome .

Infection is virtually aprerequisite for ulceration , and elimination of H.pylori allows healing of ulcer without recurrence . recurrent ulceration is almost always associated with recrudescence of infection .

The topographical pattern of gastritis is a predictor of clinical out come in antral predominant gastritis, hyperacidity induced by H. pylori via increased gastrin production promotes duodenal gastric metaplasia and this leads to colonization of the duodenum , inflammation and finally ulceration . with corpus _predominant gastritis or pangastritis , host intractions lead to the suppression of acid production and destruction of parietal cells . consequently duodenal ulceration is not evident , although hypoacidity can lead to epithelial changes and gastric gland atrophy that increase the risk of gastric ulceration ..(David ,*et al* . 2005)

1.2.3.4.2Non-ulcer dyspepsia

Some cases of non _ulcer dyspepsia are associated with H,pylori as eradication has a small but significant effect on dyspepsia and prevents the development of peptic ulcer in some patients . Although there is currently no way of identifying such patients , a ,test and treat, strategy is justified on economic grounds .

1.2.3.4.3Gastric cancer

Atrophic gastritis resulting from longstanding infection with H.pylori is associated with an increase d risk of developing gastric cancer . inaddition , gastric MALT lymphoma is strongly associated with H .pylori infection ,and in most cases complete regression has been observed after eradication of the infection ..(David ,*et al* . 2005)

1.2.3.4.4 Other disease

H.pylori infection has been associated statistically with several conditions out side the digestive tract , including coronary heart disease , iron deficiency anaemia and cot death . Although these are of great potential impotance , the links remain unproven because of possible confounding factors. Epidemiological data link eradication of H.pylori infection with increase rates of oesophageal cancer, allergy and asthma..(David ,*et al* . 2005)

1.2.3.5Epidemiology

Man appears to be the sole reservoir source of H.pylori .infection is presumed to be by the oral –oral or, possibly ,faecal –oral route ,and has been suggested to be mostly intrafamilial . volunteer studies indicate that the adult infectious dose is

relatively high ,but infections resulting from lower doses may resolve quickly whereas higher doses lead to persistent infection .(David ,*et al* . 2005)

Infection rates are strongly related to poor living conditions and overcrowing during childhood. There is a steady rise in seropositivity with increase age (about 50 % infected by the age of 60 years in industrialized countries). In developed nationsprogres. Sivelyfewer children are becoming colonized , but most children in developing countries are infected by the time they reach puberty .High rate of infections correlate broadly with high rate of gastric cancer . .(David ,*et al* . 2005)

Low gastric cancer risk, may be due to intestinal helminth infection driving the local immune response towards aprotective T helper cell2 (Th2) ,rather than the deleterious Th1 response .

Inmates of psychiatric units and orphanages and professional staff carrying out endoscopy examinations show higher than average infection from inadequately disinfected endoscopes has also occurred. .(David ,*et al* . 2005)

1.2.3.6Laboratory diagnosis

1.2.3.6.1Non- invasive tests

1.2.3.6.2Serology

Serological tests , mostly based on ELSIA latex agglutination, detect antibodies to H.pylori or its products and are used to screen patients with dyspepsia . they are less useful for screening children and are unreliable for excluding infection in elderly patients , or as a test for cure in patients who have received treatment

(owing to variable persistence of antibody). The accuracy of rapid beside tests of whole blood is poor. (David, et*al.* 2005)

1.2.3.6.3Urea breath test

This test detects bacterial urease activity in the stomach by measuring theoutput of carbon dioxide resulting from the splitting of carbon -13 or carbon -14 labelled urea into carbon dioxide and ammonia. Infected patients give high readings. The test has excellent sensitivity and specificity, but carbon _14 is weakly radioactive, so it is not used in children .A mass spectrometer is needed to assay non-radioactive carbon -13 .The urea breath test cannot be used during or directly after antibiotic therapy. (David, et*al.* 2005)

1.2.3.6.4Faecal antigen test

Stool antigen test that detect H.pylori antigens in faeces are avaible .test based on the use of monoclonal antibodies are more accurate than polyclonal antibody test and have the potential to supplant serology for routine screening .

1.2.3.6.5Polymerase chain reaction (PCR)

DNA probes for the direct detection of H.pylori in gastric juice ,faeces,dental plaque and water supplies have been developed . some can also detect genes expressing antibiotic resistance and presence of the cagA pathogenicity island.Newer versions can detect H.pylori within a few hours . present method are unsuitable for general use because clinical samples may contain compounds that inhibit the reaction .(David ,*et al* . 2005)

1.2.3.6.6 Invasive tests

1.2.3.6.7 Collection of specimens

Ideally, patients for endoscopy should not have received antibiotics or proton pump inhibitors for 1month before the test . mucosalbiopy specimens are taken from the gastric antrum within 5 cm of the pylorus , and preferably also from the body of the stomach . for maximum sensitivity , duplicate specimens are taken : one for histopathology (placed in fixative): the other for culture (placed in the neck of a sterile bottle made humid by adding a tiny amount of normal sline). Specimens for culture must be processed as soon as possible, certainly on same day , or placed in transport medium. .(David ,*et al* . 2005)

1.2.3.6.8Biopsy urease test

This is a simple and cheap test that can be perfomed at the bedside .A biopsy specimen is placed into small quatity of urea solution with a dye such as phenol red, which detects alkalinity resulting from the formation of ammonia . most infected patients (70%) give a positive result within 2h: 90% after 24h . newer tests with monoclonal antibody promises higher sensitivity and specificity .(David ,*et al* . 2005)

1.2.3.6.9 Histopathology and microscopy

Histopathology provides a permanent record of the nature and gradin of patients gastritis as well as detecting H.pylori . organism can be seen in section stained with heamatoxylin and eosin , but more specific stain make the task easier . the bacteria can also be seen in smear of biopsy material stained with Gram stain . fluorescein _based molecular probes under development are potentially able to detect H.pylori and its virulence factors ..(David ,*et al* . 2005)

1.2.3.6.10Culture

Culture is no more sensitive than skilled microscopy of histological section , but has several advantages : isolates can be tested for antimicrobial resistance and typed for epidemiological studies information about the presence of virulence factors can inform clinical outcome .

Rich growth media (commonly including lysed or whole animal blood or complement _ inactivated serum), selective agars and incubation condition similar to those used for campylobacter are used for primary isolation . sensitivity is increased if a non _selective medium is used in parallel . high humidity is essential.

Plate are left undisturbed for 3 days and incubated for a week before being discarded as negative . H.pylori forms discrete domed colonies unlike the effuse colonies of c.jejuni and c.coli.(David,*et al*. 2005)

1.2.3.7Treatment

H.pylori is sensitive to penicillin ,cephalosporines, tetracycline , erythromycin , rifampicin, aminoglycosides and nitrofurans.(Rajesh,Rattan.

1.3 Previous studies

Durdi*et al* was studded to determine the relationship between H .pylori infection and the iron status of the body. This study was conducted in ShahidBeheshti Hospital of Babol University of Medical Sciences, Babol, Iran from August 2007 to July 2008. The study group consisted of 35 patients with H. pylori and 35 matched healthy subjects as the control group. The members of both groups were enrolled in the study voluntarily. Serum iron were measured by Darman-kav Standard kit. The Collected data were analyzed by SPSS version 16. p-value of <0.05 was considered significant.

Other study was done by Tayyibe,et al was to investigate the association between iron deficiency anemia and *H. pylori* in patients with normal gastrointestinal tract endoscopy results. A total of 117 male patients with normal gastrointestinal tract endoscopy results were included in this retrospective study. The study and control groups included 69 and 48 patients with and without iron deficiency anemia.the result of this study showed therewas no association between iron deficiency anemia and *H. pylori*(Tayyibe,*et al.*2014)

1.4 Rationale

Infection with Helicobacter pylori recognized as major risk factor for chronic gastritis ,peptic ulcer disease and gastric cancer .the association between h.pylori infection and iron deficiency anaemia has been established . multiple mechanisms have been advocated to explain the relationship between h.pylori and iron status in human body (ciacci ,*et al*.2004)

In Sudan there is fewer published data about the study, so the aim of study is to increase the availability of information that may help to detect relationship between H.pylori infection and the level of iron in human body.And other researcher to continue in research.

1.5Objectiives

1.5.1 General objectives:

- To evaluate serum iron in helicobacter pylori patients.

1.5.2 : Specific objectives:

- To determine the relationship between h.pylori infection and iron status in human body.
- To study the effect of h.pylori infection in serum iron .
- To compare serum iron in h.pylori patients with normal control.
- To correlate serum iron ,age gender with patients

2. Materials and methods

2.1 Study designs

This is a case control study aimed to determine the effect of H.pylori infection in amount of iron in the human body .

This study done during the period from 15_5_2015 to 15_8_2015 in Antalya medical center

2.2 Study population

Seropositive H.pylori patients.

2.3 inclusion criteria

Individual of both sexes were H.pylori infection (who were take antibiotic or well not take),were include in the study .

2.4 Exclusion criteria

Any patients with situation effect patient iron status .

2.5 Study area

Khartoum state.

2.6 Sample size

50 venous blood samples from seropositive H.pylori patients and 20 blood sample as control collected randomly from healthy person .

2.7 Collection and processing of blood samples:

Venous blood collected using sterile disposable plastic syringe after cleaning the venipuncture area with 70% ethanol, the blood collect in suitable tube or collection container and use of li_heparin as anticoagulant then separate plasma and stored until analyzed to estimate serum iron by using cobas c111

2.8 Methodology

Serum iron was measured using automated method by cobas C111.

2.8.1 Cobas C111

The cobas c 111 analyzer is a compact random access bench top analyzer for efficient automation for small workload laboratories processing up to 80 samples a day. The cobas c 111 analyzer is available with an optional ISE unit and provides a professional solution for various essential testing requirements.

2.8.2 Features

- Compact, desktop system
- Cooled, exchangeable reagent disks
- Connectivity options
- Intuitive user interface
- Software-driven operator maintenance
- Highly stable system components
- Continuous random access testing
- Broad menu of at least 37 reagents
- Onboard capacity of 17 tests

- Matches results of larger cobassystim
- Robust system design
- Integrated safety features

2.8.3 Principle

colorimetric assay.

PH<2.0 transferrin_Fe_complex Apo transferring+Fe3+

Fe3+ascorbate Fe2+

Fe2++Ferrozine colored complex Under acidic conditions, iron is liberated from transfrrin.

Lipemic samples are clarified by the detergent .Ascorbate reduces the released Fe3+ ions to Fe2+ions which then react with Ferrozine to form colored complex . the color intensity is directly proportional to the iron concentration and mustured

2.8.4 Reagents

Use iron reagent from Roche company for cobas c111

2.8.5 Normal value

5.83-34.5 m mol/l

2.9Ethical consideration

Ethical Approval from university Ethical committee was obtained.

Permission from Hospital targeted for study also was obtained.

Data were obtained with high confidentiality and sure that data were used for research purposes only.

2.10 Data analysis

The collected data coded in master sheet and proceedfor analysis formusing independent t-test SSPS version 15.0 computerized program and data presented in form of table and graphs.

3. Results

Demographic data

Gender

Table (3-1):Frequency of gender

Gender		Frequency	Percentage%
Male	In case	14	28%
	In control	9	45%
Female	In case	36	72%
	In control	11	55%
	In case	50	100%
Total	In control	20	100%
	All	70	100%

Table (4-5) showed that the genderdistribution in test is (male to female 14:36

Age

Table (3-2):Frequency of age

Age		Frequency	Percentage%
10-20 year	In case	5	10%
	In control	1	5.3%
21-30 year	In case	8	16%
	In control	2	10.5%
31-40 year	In case	12	24%
	In control	7	36.8%
41-50 year	In case	8	16%
	In control	2	10.5%
51-60 year	In case	8	16%
	In control	5	10%
61-70 year	In case	9	18%
	In control	2	10.5%
Total	All	70	100%

Table (3-3): Frequency of antibiotic.

Answer	Frequency	Percentage%
Yes	36	72%
No	14	28%
Total	50	100%

Hematological Data

The average of s.iron level for the subject group respondent was found to (15.91) which is not significantly different from the average of s. iron for the control group (17.34) p (>.05) value shown table (3-4)

Table (3-4) variation of serum iron among case and control

Variable	Study group	Mean	Std.Deviation	p.value
s.iron	Case group	15.9102	5.66738	0.310
	Control group	17.3447	0.83325	

Table (3-5)

The variation of s.ironaccording to sex among case and control there was significant difference (p=0.00) show table (3-5)

The variation of s.iron according to age group of both sexes there was significant difference (p=0.00) show table (3-5)

The present result shown no significant difference among the patients whom under antibiotic therapy and those who did not take antibiotic therapy (p=0.701) show table (3-5)

Table (3-5) variation of serum iron among age, gender antibiotic

Variable	s.iron				
		Mean	Std.Deviation	T.test	p-value
Sex	Male	20.3252	5.74375	4.917	0.00
	Female	14.5465	3.83702		
Age		12.5951	4.96932	-21.054	0.00
Antibiotic	Yes	16.1053	5.71403	0.387	0.701
	no	15.4086	5.72574		

Discussion

The mechanism by which infection with H. pylori causes iron metabolism disturbance is of considerable interest. However, despite the importance of this, relatively little research has been done yet.Clinical and epidemiologic studies suggest that infection with Helicobacterpylori is associated with iron deficiency and IDA(Barabino,2002:Dubois and Kearney.2005)

Our study revealed no significant increase in serum iron among H.pylori patients compare to normal control group(p=0.310):although serum iron mean of cases (15.91) which was be in the normal range of the s.iron. There was significant difference in s. iron in correlation to sex (p=0.00): male group showed increase level of serum iron than female .There was significant difference in serum iron between age groups of both sexes (p=0.00). The present results shown no significant difference among the patients whom under antibiotic therapy and those who did not take antibiotic therapy in both sexes

This result was in contrast to previous study obtained by(Tayyibe ,*et al*.2014) the study was done in Istanbul _Turkey in The study and control groups included 69 and 48 patients, were calculated and compared. Results. There was no statistically significant difference found between the groups according to the prevalence of H. pylori(=0. 8 9 6) Conclusion. H. pylori is not associated with iron deficiency anemia in male patients with normal gastrointestinal tract endoscopy results.other study (Hsiang-Yao et al). Studied 882 patients in Taiwan and showed no significant association between chronic H. pylori infections and anemia.

Other study disagree with this study is done by(Durdi,*et al*.2011) in ShahidBeheshti Hospital of Babol University of Medical Sciences showed significant association between H.pylori infections and anemia (p=0.047)

In the present study, serum iron is normal in H.pylori patients may be associated with sample size if we study a large group we found iron deficiency ,but here in our study relatively smaller group we did not found deficiency . or may be associated with these person life style, for example, good nutrition and they are free of infection or non-infectious disease .

Conclusion

The study showed there was no significant relationship between the H.pylori infection and the amount of iron in the serum of those patients, also there was significant difference in serum iron in correlation to gender and age group and they no effect of antibiotic in the serum iron

The results of the present study show H.pyloricannotimpair iron metabolism and the H.pylori infection has no effect in serum iron.

Recommendations

After completion this work recommended by the following:

More investigation should be done .and the diagnostic method of detection of H.pylori should be more sensitive,Different diagnostic methods for *H. pylori* contribute to the variation of the pooled estimates because of their different sensitivities

Increase sample size and further studies are needed to elucidate the pathological basis of this observation.

Iron profile should be done in further studies.

Reference

Beard JL, Connor JR.(2003). Iron status and neural functioning. Annu Rev Nutr.; 23:41–58.

BeardJL.(2000) Iron requirement in adolescent females. Symposium: Improving adolescent iron status before childbearing. J Nutr. 2000; 130:S440–2.

Benoist Bd, McLean E, Egll I, Cogswell M.(1993) worldwide prevalence of anaemia: WHO. (2008).global database on anaemia:p.40.

BarabinoA. (2002)Helicobacter pylori-related iron deficiency anemia: a review. Helicobacter ;7:71–5

Cardenas VM, Mulla ZD, Ortiz M, Graham DY. (2006).Iron deficiency and Helicobacter pylori infection in the United States. Am J Epidemiol ; 163: 127-34

CiacciC,SabbatiniF,CavallaroR,CastiglioneF,DiBellaS,IovinoP,PalumboA,TortoaR, AmorusoD,Mazzacca G.(2004). Helicobacter pylori impairs iron absorption in infected individuals .Dig Liver Dis.:36(7):455-60

Conrad ME, Umbreit JN. (1993). a concise review: Iron absorption – the mucin-mobilferrin-integrin pathway. A competitive pathway for metal absorption. Am J Hematol; 42:67:-73

Crompton DW,Nesheim MC.(2002). Nutritional impact of intestinal helminthiasis during the human life cycle .AnnuNutr.:22:35-99

Dallman P.(1990).Iron. In: Brown ML, editor. Present Knowledge in Nutrition. 6th ed. Washington DC: Nutrition Foundation; pp. 241–50

David G,MikeB,RichardS,Will L.(2005) medical microbiology.18th ed Churchill livingstone:309-310

DeMaeyer EM, Dallman P, Gurney JM, Hallberg L, Sood SK, Srikantia SG.(1989). Geneva: World Health Organization; WHO. Preventing and controlling iron deficiency anaemia through primary health care: A guide for health administrators and programme managers; p. 58.

DurdiQ,MaryamS,Shahreyar S.(2011) Association between helicobacter pylori infection and serum iron profile caspiain journal of internal medicine :2(3): 266-269.

Fairbanks VF.Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ, (1999). Modern Nutrition in Health and Disease. 10th ed. Baltimore: Lippincott Williams & Wilkins;.pp. 193–221

FAO/WHO.(2004).Expert Consultation on Human Vitamin and Mineral Requirements, Vitamin and mineral requirements in human nutrition: Report of joint FAO/WHO expert consolation; p. 341.

Hallberg L, Rossander L, Skanberg A.(1987). Phytates and the inhibitory effect of bran on iron absorption in man. Am J Clin Nutr; 45: 988-996

Hallberg L, Rossander-Hulthen L, Brune M, Gleerup A.(1993).Inhibition of haem-iron absorption in man by calcium. Br J Nutr; 69:533–40

Hoppler M, Schoenbaechler A, Meile L, Hurrell RF, Walczyk T. (2008). Ferritin-iron is released during boiling and invitro gastric digestion. J Nutr.; 138:878–84.

Hsiang-Yao,K.Fu-Chen,S.Sophie(2013)..Helicobacter pylori infection and anemia in taiwanese adults .Gastroenterlogy Research and Practice, vol.2013.Article ID 390967,4 pages

Hunt JR, Zito CA, Johnson LA.(2009).Body iron excretion by healthy men and women. Am J ClinNutr; 89:1–7.

Hurrell R, Egli I. (2010). Iron bioavailability and dietary reference values. Am J ClinNutr.; 91:1461–7S

Hurrell RF, Reddy M, Cook JD. (1999).Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages.Br J Nutr.;81:289

John p, John Foerster,George M,*(*2009*)*wintrobs clinical Hematology 12thEidition ,Lippincott willims and wilkins :p780

Kenneth k,Marshall A,Ernest B(2010)Williams Hematology8thEidition:McGraw-Hill:p 854

KennethJ.R,C.GeorgeR,NafeesA,W.LawrenceD,JamesJp.(2004)SHerris medical microbiology 5thed. mcgraw –hill:p365-368

Kuipers EJ, Uyterlinde AM, Peña AS, Roosendaal R, Pals G, Nelis GF, Festen HP.(1995).Meuwissen SG. Long-term sequelae of Helicobacter pylori gastritis. The Lancet; 345: 1525-1528

Larocque R, Casapia M, Gotuzzo E, Gyorkos TW.(2005) Relationship between intensity of soil-transmitted helminth infections and anemia during pregnancy. Am J Trop Med Hyg.;73:783–9.

Lynch SR, Cook JD. (1980).Interaction of vitamin C and iron. Ann N Y Acad Sci.; 355:32–44

Lynch SR, Dassenko SA, Cook JD, Juillerat MA, Hurrell RF.(1994).Inhibitory effect of a soybean-protein-related moiety on iron absorption in humans. Am J ClinNutr; 60:567–72.

McDowell LR. (2003). 2nd ed. Amsterdam: Elsevier Science; Minerals in Animal and Human Nutrition; p. 660.

Monsen ER, Hallberg L, Layrisse M, Hegsted DM, Cook JD, MertzW. (1978).Estimation of available dietary iron.Am J ClinNutr. 1; 31:134–41

Muir A, Hopfer U. (1985).Regional specificity of iron uptake by small intestinal brush-boarder membranes from normal and iron deficient mice. Am J Physiol.; 248:G376-9

Nadadur SS, Srirama K, Mudipalli A.(2008).Irontransport and homeostasis mechanisms: Their role in health and disease. Indian J Med Res; 128:533–44.

Rajesh B,RattanLallchhpujani(2011)Essentials of Medical Microbiology 3th edjaypeebrothers:p 313

Skikne B, Lynch S, Cook J.(1981).Role of gastric acid in food iron absorption.Gastroenterology; 81: 1068-1071

TayyibeS,SakirOK,SibelK,SuleymanA,GulayO.(2014).Hpylorimaynot be associated with iron deficiency anemia in patients with normal gastrointestinal tract endoscopy results .Advances inHematology.Volume 2014. Article ID 375915,4 pages

Theil EC, Chen H, Miranda C, Janser H, Elsenhans B, Núñez MT.(2012). Absorption of iron from ferritin is independent of heme iron and ferrous salts in women and rat intestinal segments. J Nutr.; 142:478–83

Wang J, Pantopoulos K. (2011).Regulation of cellular iron metabolism.Biochem J; 434:365-81

WHO.(1987). Prevention and control of intestinal parasitic infections.Report of a WHO Expert Committee Ginebra Technical Report Series. ; 749: 1-86

Wood RJ, Ronnenberg A. Iron. In: Shils ME, Shike M, Ross AC, Caballero B, Cousins RJ. (2005). Modern Nutrition in Health and Disease. 10th ed. Baltimore: Lippincott Williams & Wilkins; pp. 248–70.

Yang Z, Dewey KG, Lonnerdal B, Hernell O, Chaparro C, Adu-Afarwuah S.(2008). Comparison of plasma ferritinconcentration with the ratio of plasma transferrin receptor to ferritin inestimating body iron stores: Results of 4 intervention trials. Am J ClinNutr; 87:1892–8

Yeh KY, Yeh M, Mims L, Glass J. (2009). Iron feeding induces ferroportin 1 and hephaestin migration and interaction in rat duodenal epithelium. Am J PhysiolGastrointest Liver Physiol; 296:55–65.

Yip R, Limburg PJ, Ahlquist DA, Carpenter HA, O'Neill A, Kruse D, Stitham S,Gold BD, Gunter EW, Looker AC, Parkinson AJ, NobmannED,PetersenKM,EllefsonM,SchwartzS.(1997).Pervasiveoccultgastrointestinal bleeding in an Alaska native population with prevalent iron deficiency. JAMA; 277: 1135-1139

Appendix No (1)

Master sheet of case group

No	Name	Sex	Age	disease	Antibiotic	s.iron
1	Ameramoh	F	33	+ve	Yes	15.69
2	Emansoliman	F	27	+ve	Yes	17.64
3	Fatmaabdalrhim	F	50	+ve	YES	15.74
4	Mona mochtar	F	37	+ve	No	12.18
5	Marwaabdalwhab	F	28	+ve	Yes	12.55
6	Alsoramosa	F	62	+ve	No	18.83
7	Huda ahmed	F	43	+ve	No	10.27
8	Khaldaslah	F	18	+ve	No	4.67
9	Ehsanyons	F	35	+ve	Yes	17.85
10	Mysramosa	F	22	+ve	No	14.58
11	Ayshamohmmed	F	66	+ve	Yes	20.37
12	Moshyraadallh	F	30	+ve	Yes	11.72
13	Ozazabdalrhman	F	47	+ve	Yes	9.26
14	Aloyaahmed	F	62	+ve	Yes	13.98
15	Mhasenkhald	F	32	+ve	Yes	21.96
16	Mohmmedsalih	M	58	+ve	Yes	14.10
17	Ahmed mohmmed	M	70	+ve	No	10.96
18	Khalid ali	M	54	+ve	Yes	38.7
19	EhsanOsman	F	44	+ve	No	14.56
20	Rhabmohammed	F	43	+ve	No	15.66
21	Mona alfadl	F	20	+ve	Yes	12.87
22	Ebraheem	M	38	+ve	No	27.89
23	Mostfafarog	M	40	+ve	Yes	17.97
24	Manahilslah	F	37	+ve	Yes	10.67
25	Madinaalmaky	F	63	+ve	Yes	21.88
26	Ameramohammed	F	54	+ve	Yes	9.56
27	Sdeegebrahim	M	68	+ve	Yes	20.89
28	Om alhasssanba	F	55	+ve	Yes	18.77
29	Khansaalagb	F	23	+ve	No	12.41
30	Zobeedaabdalmotalb	F	62	+ve	No	17.43
31	Hanadyaltyb	F	22	+ve	Yes	11.55
32	Fatmaahmedalzain	F	30	+ve	Yes	4.89
33	Altybgafr	M	50	+ve	Yes	23.15
34	Alsheekhmubark	M	41	+ve	No	18.44
35	Habsaomer	F	57	+ve	Yes	15.88
36	Huda alnoor	F	43	+ve	No	14.54
37	Eslamaltreefy	F	19	+ve	Yes	7.86
38	Mostafamohammed	M	66	+ve	Yes	15.77
39	Omer alhady	M	28	+ve	Yes	20.43
40	Akrammohsen	M	31	+ve	Yes	19.87

41	Hager hamid	F	33	+ve	Yes	14.78
42	Howa abdalhy	F	57	+ve	No	23.30
43	Wegdanabdalmonem	F	34	+ve	Yes	12.29
44	Sara abdalla	F	36	+ve	Yes	15.75
45	Snaamohmed	F	20	+ve	Yes	16.32
46	Zakreeyamohmmed	M	53	+ve	Yes	18.90
47	Emanalsmany	F	38	+ve	Yes	12.89
48	Atifabdalrman	M	61	+ve	Yes	17.54
49	Lylaomer	F	58	+ve	Yes	15.21

Appendix No (2)

Master sheet of control group

No	Name	Sex	Age	disease	antibiotic	S.iron
1	Fatmataha	F	33	-ve		14..56
2	Aloyamosa	F	54	-ve		12.29
3	Hassan fdl	M	65	-ve		23.54
4	Zkeeyaahmed	F	58	-ve		16.70
5	Yosraalmahy	F	40	-ve		15.64
6	Elhammohammed	F	47	-ve		14.43
7	Awatifyaseen	F	34	-ve		17.76
8	Mlwaldyng	M	27	-ve		24.17
9	Nasr eldeenahmed	M	55	-ve		19.66
10	Adilabdalrhman	M	64	-ve		23.67
11	Awadabdalmonim	M	57	-ve		14.65
12	Hassan taha	M	35	-ve		16.66
13	Samwalabdallah	M	28	-ve		21.89
14	Nansyawad	F	33	-ve		12.87
15	Slmaaltyb	F	18	-ve		16.89
16	Nglaalhag	F	40	-ve		15.80
17	Ebraheemmohammed	M	44	-ve		17.98
18	Fatmaalemam	F	53	-ve		15.96
19	Nuhamohammed	F	34	-ve		14.43

Appendix No (3)

Questionnaire

Sudan University of Science and technology

College of Graduate studies

Questionnaire No ( )

No…….

Age……

Gender: Male ( ) Female ( )

Residence……

Telephone Number……

Receiving of antibiotic :

Yes ( ) No ( )

Other disease ……

Investigation:

Serum iron……

Date: / / sig…….

Appendix No (4)

Cobas C111