**ADDIS ABABA UNIVERSITY**

**COLLEGE OF HEALTH SCIENCES**

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# Assessment of Effect of Helicobacter pylori and Helminths infection on Anemia with emphasis on ferritin level of school children in Batu town, Oromia Region, Ethiopia, 2019


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This is to certify that the thesis prepared by Raji Tesfaye entitled "**Assessment of Effect of Helicobacter pylori and Helminths infection on anemia with emphasis on ferritin level of school children in Batu town, Oromia Region, Ethiopia, 2019**" submitted in partial fulfilment of the requirements for the Degree of Masters of Sciences in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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## Abbreviations

| | |
|---|---|
| BMI | Body Mass Index |
| CagA | Cytotoxin-associated gene A |
| DHS | Demography Health Survey |
| DMT | Divalent Metal Transporter |
| DRERC | Departmental Research and Ethics Review Committee |
| Hb | Hemoglobin |
| H.pylori | Helicobacter pylori |
| IDA | Iron Deficiency Anemia |
| kDa | Kilodalton |
| MDG | Millennium Development Goal |
| NSAID | Non Steroid Anti-Inflammatory Drug |
| PAI | Pathogenicity Island |
| RBC | Red Blood Cell |
| SC | School Children |
| SPSS | Statistical Package for Social Sciences |
| SST | Serum Separator Tube |
| STH | Soil-Transmitted Helminthiasis |
| VacA | Vacuolating cytotoxin A |
| WHO | World Health Organization |

## Abstract

**Background:** In infants and young children, severe chronic anemia may lead to delayed growth and long term effects on neurodevelopment and behaviour. The main causes of anemia are: dietary iron deficiency; infectious diseases, deficiencies of other key micronutrients or inherited conditions that affect red blood cells (RBCs). Iron deficiency accounts for about 50% of all cases of anemia which results in iron deficiency anemia (IDA). According to the Demography Health Survey, the prevalence of anemia among Ethiopian children under the age of 15 is estimated to be about 24%, classifying it as a moderate public health problem as identified by WHO.

**Objective**: To investigate the effect of Helicobacter pylori and Helminths infection on anemia with emphasis on ferritin level of school children in Batu town, Oromia Region, Ethiopia.

**Methods**: A comparative study was conducted on 161 primary school children aged 4-14 years. Serum samples were collected for serum ferritin level measurement using Beckman Coulter chemistry analyzer. Secondary data on haemoglobin and RBC indices were extracted using format. SPSS version 21 was used to enter and analyze data. Statistical significance was determined between the groups using an independent t-test and a non-parametric Mann-Whitney-U test with a P value less than 0.05.

**Results:** A total of 161 samples were analyzed in this study, with 77 (47.8%) of them being males. Of them, 109 (67.7%) were in the cases group, while 52 (32.3%) were negative for both *H. pylori* and helminths. There was no statistically significant difference in serum ferritin levels between *H. pylori* stool antigen positive or *H. pylori* antibody positive children compared with the control groups, (P=0.787, P= 0.350) respectively. There was no significant effect on serum ferritin as well as hemoglobin, RBC indices between helminths infection when compared with a control group. The serum ferritin level (P=0.286), hemoglobin concentration (P=0.563), MCV (P=0.646), MCH (P=0.485), and MCHC (P=0.975) all increased as a result.

**Conclusion and Recommendation:** The presence of Helicobacter pylori or helminths has no effect on serum ferritin levels or the development of iron deficiency. Cohort studies are recommended for establishing a cause and effect relationship between *H.pylori* infection or helminths infection and serum ferritin levels.

***Keywords:*** *H.pylori, Helminths, serum ferritin, Anemia, Iron deficiency*

# 1. Introduction

## 1. 1 Background

Anemia is a condition in which the body's physiologic needs are not met because the number of red blood cells (and therefore their oxygen-carrying capacity) is inadequate. At sea level, the World Health Organization developed a cut-off value for hemoglobin concentration to diagnose anemia: 11 g/dl in children aged 6 to 59 months, 11.5 g/dl in children aged 5 to 11 years, and 12g/dl in older children (aged 12 to 15years). Mild anemia is defined as hemoglobin levels between 11.0 g/dL and the cut-off points, moderate anemia is defined as Hb 8.0–10.9 g/dL, and severe anemia is defined as Hb8.0 g/dL [1].

Anemia is a global public health problem that affects both developing and developed countries, with serious implications for human health and social and economic growth. This has an influence on countries with medium, middle, and high incomes [2, 3]. The World Health Organization (WHO) reports that two billion people are anemic, characterized as having hemoglobin levels below recommended levels [4]. It affects people at all stages of their lives, but it is more common in pregnant women and young children [2]. Dietary iron deficiency; infectious diseases like malaria, hookworm infections, and schistosomiasis; deficiencies of other primary micronutrients like folate, vitamin B12, and vitamin A; or hereditary disorders that damage red blood cells (RBCs) are the most common causes of anemia[4].

*Helicobacter pylori* (*H. pylori*) are a spiral-shaped pathogenic bacterium that lives on the mucosa of the human stomach [5]. *H. Pylori* infection is the most common infection in the world, with a global prevalence of more than 50%. Geographical, racial, age, and socioeconomic factors can all have an impact on the prevalence. Infection with *H. Pylori* is very common in developing countries, and it is particularly prevalent among children and people of lower socioeconomic status. *H. Pylori* colonization is normally asymptomatic, although about 20% of the infected population develops chronic gastritis and peptic ulcers [6].

It has a variety of gastric, intestinal, and extra gastric symptoms. Chronic gastritis, peptic ulcer, gastric mucosa associated lymphoid tissue lymphoma, and gastric adenocarcinoma are all caused by *H.pylori*. It also induces more than 50 extragastric manifestations, including cardiac, dermal, endocrine, gynaecological, obstetrical, neurological, ophthalmological, and iron deficiency (ID) or iron deficiency anemia (IDA) [7].

STH stands for soil-transmitted helminthiasis, a group of parasitic diseases caused by nematode worms and transmitted to humans by faeces-contaminated soil [8]. Infection is linked to stunting of linear development, physical weakness, low educational achievement, intestinal obstruction, hepatic and biliary diseases, and iron deficiency anemia in an estimated 400 million school-aged children among the world's 3.5 billion infected individuals [9]. *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, and *Ancylostoma duodenale* are soil-transmitted helminths of significant concern to humans [8]. Intestinal parasitic infections may have a detrimental effect on the health of a significant number of school-aged children, causing iron deficiency anemia as well as a decline in work ability and fitness [10].

The molecule responsible for oxygen transport is hemoglobin. Hemoglobin is made up of four polypeptide chains, each carrying a heme group, and has a molecular weight of 64,500. The amino acid glycine is the first step in the heme synthesis process. As intermediate steps, porphobilinogen, uroporphyrinogen, coproporphyrinogen, and protoporphyrin are produced. Serum transferrin supplies iron (Fe2+), which combines with protoporphyrin to form heme. One heme molecule binds to one globin chain to form the hemoglobin molecule, which binds oxygen avidly [11].

Iron serves a number of important roles in the body. It functions as a carrier of oxygen from the lungs to the tissues through red blood cell hemoglobin, as a medium for electron transport within cells, and as a component of important enzyme systems in various tissues. Iron is reversibly processed in the liver as ferritin and hemosiderin, and it is transported throughout the body by the protein transferring mechanism [12].Ferritin is an iron storage protein present in both prokaryotes and eukaryotes. It is made up of 24 subunits and a protein shell with a molecular mass of around 500 kDa. The protein shell surrounds a ferric-hydroxy-phosphate center that can accommodate up to 4000 iron atoms [13].

Childhood anemia and iron deficiency have a major effect on long-term health, so it is important to recognize risk factors early in a child's life. If a growing child's iron needs are not met, his or her learning capacity, work performance, and immune function will suffer before other

Symptoms of anemia appear. If they're on deficiency is not treated, it may lead to problems with height and weight, learning and actions, and physical and mental development [14].

## 1.2 Statement of Problem

Anemia is a common public health concern among school children, with significant implications for their health, as well as their social and economic growth. Anemia affects about 33% of school-aged children worldwide. Anemia affects1.62 billion people globally, accounting for 24.8 percent of the population [2].According to the Demography Health Survey (DHS), the prevalence of anemia among Ethiopian children under the age of 15 is estimated to be about 24%,classifying it as a moderate public health problem as identified by WHO. IDA affects over 8 million children under15 years of age [15].

Anemia has a number of negative effects, including reduced cognitive output, poor growth of babies, preschool, and school-aged children; impairment of physical capacity; decrease of immune competence; and increased morbidity from infections in all age groups, according to some literature on anemia in children prevalence ranging from 15.5 to 37.6% in different regions of Ethiopia [16].While the etiology of anemia is complex and multifactorial, iron deficiency accounts for about half of all cases of anemia, resulting in iron deficiency anemia (IDA) [2].

Anemia is a significant public health problem in developing countries, with ID accounting for about half of all cases. In developing countries, factors such as low iron consumption, low dietary iron bioavailability, and gastrointestinal parasite infections lead to the high prevalence of ID/IDA. *Helicobacter pylori* infect more than half of the world's population, and it is most commonly acquired in early childhood, especially in preschool age. *H. pylori* infection in young children may have potentially more complex clinical effects than in developed countries, including not only iron deficiency or iron deficiency anemia but also growth retardation, diarrheal disease, malabsorption, and impaired cognitive functioning low-resource settings where the infection co exists with other morbidities such as malnutrition and parasitic and enteropathogen infections. [17].

Intestinal parasitic infections are the 17th most neglected tropical disease, according to the World Health Organization, and the 4th most common cause of communicable disease with a high rate of disability. The helminthic infection induces anemia by reducing iron absorption from the intestine, sucking blood directly, and interfering with iron metabolism both directly and indirectly [18]. As a result, the aim of this study is to see how *Helicobacter pylori* and Helminths infection affect anemia and ferritin levels in primary school children.

### 1.3 Significance of the study

From a health policy standpoint, maternal and child health is a top priority in Ethiopia. The findings of the study will be used to advise intervention strategies aimed at reducing anemia and iron deficiency in school-aged children. Despite the fact that children's mortality has decreased in order to reach Development goals, such information is useful to guide policy makers. As a result, this research may help to reduce morbidity and mortality in this age group. The impact of *Helicobacter pylori* and Helminths infection on anemia and ferritin levels in school children is crucial in determining the prevalence and severity of anemia in children. There has been no research on serum ferritin levels in school children to assess iron deficiency anemia. Furthermore, the results of the study can be used as a starting point for further studies and to encourage concerned bodies to introduce various measures at various levels of society in order to protect children from anemia complications and promote child health.

# 2. Literature Review

## 2.1 Iron deficiency anemia

Anemia is estimated to impact 1.62 billion people worldwide, according to the World Health Organization (WHO). Anemia is a major global issue that affects 305 million (25.4 percent) school-aged children. Anemia is estimated to affect 13.2% of African school children, according to the WHO. Severe 40 or higher, Moderate 20.0–39.9, Mild 5.0–19.9, and normal 4.9 or lower are the WHO classifications of public health importance of anemia in populations based on prevalence estimates [2].The developing world has the highest incidence of anemia, which has a number of causes [19]. Anemia is three to four times more common in developing countries than in developed countries. Pregnant women (56 percent), school-aged children (53 percent), non pregnant women (44percent), and preschool children (42percent) are the most impacted demographic groups in developing countries [20].

### 2.1.1 Pathophysiology

Every cell in the body requires iron. It is involved in oxidative metabolism, cellular growth and proliferation, and oxygen transport and storage, among other things. To perform these functions, iron must be bound to protein compounds [21]. The total amount of iron in the body is around 3-4 g, with two-thirds of it coming from red blood cells (RBCs) and recycled iron from RBC destruction. Just 1–2 mg of iron is absorbed in the intestinal tract and distributed in the blood, while the rest is contained in ferritin/hemosiderin [22].

Iron is needed for a variety of metabolic processes. The average amount of iron in an adult human body is 50mg/kg for males and 40mg/kg for females. The largest component is circulating hemoglobin, with 450 mL (1 unit) of whole blood containing about 200 mg of iron [23]. Iron is consumed in two ways from the diet: inorganic iron (nonheme-iron), which is primarily contained in vegetables and plant-based foods, and heme-iron, which is derived from the breakdown of hemoglobin (Hb) or myoglobin in animals [24].

The heme is released from its apoprotein when it is exposed to acid and proteases found in gastric juices. Hemin is formed when heme iron is oxidized to its ferric state. Hemin appears to reach the mucosal cell intact, most likely through binding to an unspecified receptor. Nonheme iron, on the other hand, is usually delivered in its ferric ($Fe^{3+}$) form, which must be converted to ferrous ion before it can be absorbed [25]. The majority of iron moves from the stomach to the duodenum and upper jejunum [26], where it is absorbed by enterocytes in the proximal duodenum. A ferri reductase located on the surface of enterocytes reduces incoming iron in the Fe3+ state to Fe2+. A proton-coupled divalent metal transports iron from the apical surfaces of enterocytes to the interiors of the cells [27].

Absorption normally admits approximately 5% to 10% of a total dietary intake of 10 to 20 mg/day. The majority of iron absorbed is bound to the liver-produced plasma protein transferrin. The glycoprotein transferrin is a beta globulin. Within the intestinal lumen, transferrin chelates iron and transports it to the small intestine's mucosal cells [4].Iron is transported via the blood stream by transferrin. Two Fe3+ ions are joined by each transferrin molecule. Specific receptors, which are abundant in the tissues that need iron most urgently (bone marrow, liver, and placenta), are needed for the body to use iron properly. In most cases, the bone marrow absorbs 70 to 90 percent of the iron for hemoglobin production. In the liver, spleen and bone marrow, iron may be deposited, bound to ferritin and hemosiderine up to twenty times beyond its normal amount [28].

When there is a negative iron balance, iron resorption rises from its normal level of 5–10 percent to a maximum of 20%. The erythropoiesis becomes dysfunctional when the iron reserves are fully depleted, as shown by a decrease in serum iron and ferritin and an increase in transferrin. Red blood cells that are abnormally small and poorly hemoglobinized (hypochromic) are formed. The overall hemoglobin concentration declines after a few weeks, suggesting iron deficiency anemia. The synthesis of other iron-dependent proteins (e.g. cytochromes, myoglobin, and flavoproteins) is also impaired if the iron deficiency is serious [11].

In a 2010 study on the prevalence and correlates of anemia among children aged 9 to 11 years in70 elementary schools in rural Shaanxi, China, the overall anemia incidence was 21.5 percent when they used a blood haemoglobin cut-off value of 115 g/L. on the opposite, When they used a cut-off value of 120 g/L as a cut-off, they found that 39% of students had haemoglobin levels that categorized them as anemic. Anemia has a clear association with the students' heights and weights, according to this report. Anemias cause children to have lower height for age (HAZ) scores and are more likely to be stunted [29].

Around 14 000 children in three age groups (7-11 years, 12-14 years, and >15 years) were enrolled in basic education in rural schools in Ghana, Indonesia, Kenya, Malawi, Mali, Mozambique, Tanzania, and Vietnam, according to various studies conducted in eight African and Asian countries. Anemia is prevalent in many African and Asian countries, especially in Africa. Anemia was discovered to be a serious public health issue in five African countries, with a prevalence of >40 percent anemic in these five countries, according to this report. In the two Asian countries surveyed, anemia was not a public health issue among the children. Anemic children were more likely to be boys than girls, and children who started school late were more likely to be anemic than children who started school on time [30].

In 2016, data on the prevalence of anemia in Egypt's informal primary school children was obtained from 2826 children aged 6 to 19. According to the findings, the overall prevalence of anemia among the children surveyed was 59.3 percent. Mild anemia was diagnosed in the majority of them (82.5%). Just 0.1 percent of the children had severe anemia. For boys and children, the mean hemoglobin levels were 10.9±1.2 and 10.7±1.1 mg, respectively. BMI was used as a criterion to classify underweight, overweight and obesity. The result findings showedthat, 75.8 percent of underweight children were anemic, 63.1 percent of overweight children were anemic and 60.2 percent of obese were anemic. The magnitude of anemia in this study (59.3 percent) is considered a severe public health problem according to the World Health Organization standards [31].

A random sample of 143 pupils aged 6 to 12 years in Ghana's Volta Region participated in across-sectional study conducted in 2014.When using serum ferritin concentration cut off values: ferritin 30 ng/ml, the prevalence of low iron stores (serum ferritin concentration 30ng/ml) among study participants was 71.3 percent, with females accounting for 68 percent and males accounting for 74 percent, and the overall prevalence of anemia among the studied children being 30.8 percent. In comparison to males, females had a higher prevalence of anemia [32].

In January 2011, a cross-sectional study on the prevalence and seriousness of anemia among school children was conducted in Jimma Town, Southwest Ethiopia, on 423 children aged 6–14 years. The study discovered that 152(37.3%) of the children had anemia, with 73(18.1%) having mild anemia and severe anemia was noted in 79 (19.6%) of them. Anemia was found to be prevalent in children aged 6–11years, with a prevalence of 118(40.5%), and 34(30.1%) in children aged 12–14 years. According to this report, anemia is related to a number of factors such as a family's educational history, monthly income, and marital status [33].

Another cross-sectional research was performed in Kersa, Eastern Ethiopia, from January to February 2012. The research included 1755 school children aged 5 to 14 years old from twelve primary schools, with 750 girls and 1005 boys. The children's average age was 10.7 years. Anemia was found to be present in 27.1 percent of primary school students, with 13.8 percent being mildly anemic, 10.8 percent being moderately anemic, and 2.3 percent being seriously anemic. There was no statistically significant difference in anemia prevalence between male and female children, who were 27.3 percent and 26.8 percent; respectively. The prevalence was related to the age of the infant, paternal education, and irregular legume consumption. Anemia among primary school children is a moderate public health concern, according to the prevalence found in this report [34].

In 2017, researchers conducted a study in seven primary schools in Gondar, Ethiopia, and discovered that 81 out of 523 school children were anemic. Anemia was found to be prevalent in 15.5 percent of SC. The participants' Hb levels ranged from 9.7g/dl to 16.3g/dl, with a mean of 12.72 ± 1.008 g/dl. 56(69.1%) and 25(30.9%) of the anemic children were mildly and moderately anemic, respectively [35].

## 2.2 Impact of Parasitic infection on serum ferritin level

Helminths infestations have long been recognized as major causes of anemia in endemic countries, and they also play a significant role in the burden of anemia in Sub-Saharan Africa. Soil-transmitted helminths (STHs), such as *Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms *Necator americanus* and *Ancylostoma duodenale*, are the most common causes of human intestinal helminthiasis [36]. Anemia is often linked to parasitic infections including malaria and hookworm infections. Hookworms cause anemia by causing chronic intestinal blood loss, which causes iron deficiency. *Ancylostoma duodenale* and *Necator americanus*, two hookworm species, cause around 0.2 mL and 0.15 mL blood loss per day, respectively. Anti-clotting agents (such as coagulase, a blood thinner) are also released by hookworms, ensuring constant blood flow. *Trichuris* and *Ascaris* infections with high severity have been known to affect nutritional status [37].

In a 2003 study conducted in two primary schools in a rural area close to the south east of Lake Langano, Ethiopia, they discovered that the prevalence of infection with various intestinal helminths and protozoan parasites for Kime and Langano SIM school children was the following. Out of 170 stool samples obtained from Kime school children, 150 (88.2%) were positive for at least one parasite. Similarly, 67 (75.3%) of the 89 stool samples obtained from Langano SIM students tested positive for at least one parasite. The study participants combined from both schools had an average prevalence of at least one intestinal parasitic infection of 83.8 percent. Hookworm was the most common intestinal helminths, accounting for 64.7 percent of Kime school children and 51.7 percent of Langano SIM. Children from Kime School had higher rates of *Schistosoma mansoni*, *Trichuris trichuria*, *Ascaris lumbricoides*, and Taenia spp. than those from Langano SIM [38].

Between May and June of 2001, an Ethiopian cross-sectional analysis was conducted in Babile, in eastern Ethiopia. The overall prevalence of 27.2 percent was observed in 415 school children (113 out of 415 children). The most common parasite found was *H. nana*, which was found in 42 students (10.1%), followed by hookworm in 28 students (6.7%), and *S. mansoni* in 18 students (4.3percent).The prevalence of Soil Transmitted Helminths (STH) was 14.2% (59 out of 415 students) [39].

### 2.3 Impact of Helicobacter pylori infection on serum ferritin level

Colonization of the gastric mucosa by *Helicobacter pylori* (*H. pylori*) can reduce iron absorption and increase iron loss, potentially contributing to IDA [40]. Due to gastric atrophy and achlorhydria, or due to a more temporary hypochlorhydria during active infections, H. Pylori infections can cause a reduced ability to absorb iron. Both mechanisms will result in IDA sensitivity being increased [41]. Another cause of iron deficiency in *H. pylori* patients is chronic blood loss caused by chronic gastritis, which can be serious enough to cause erosive gastritis, particularly in patients with active bleeding peptic ulcers and those who take NSAIDs such as aspirin on a regular basis [7].

Furthermore, as compared to patients infected with less virulent strains, extremely virulent strains such as *H. pylori* with the cytotoxin-associated gene A (CagA) linked to gastric tumor and the vacuolating cytotoxin A (VacA) linked to gastric ulceration, act by molecular mimicry mechanisms to generate or magnify iron deficiency in patients. Another theory may be the association of VacA and the Cag-pathogenicity island (PAI), since there are two forms of H.pylori strains, type I and type II, that are based on the presence or absence of both the Cag-PAI and the Vac A. PAI-positive H.pylori strains are found to be more virulent than those that do not .H.pylori uses CagA and VacA to take iron from mucosal epithelial cells, according to a new report. Cag A prevents VacA-induced apoptosis by preventing pinocytotic up take of VacA, resulting in a decrease in vacuole formation within the host cell. Furthermore, CagA increases transferrin up take from the basolateral surface and transfers it to the apical surface for release into the lumen, while VacA increases transferrin-receptor uptake from the surface and transfers it to bacterial attachment sites, resulting in a reduction in serum iron levels [7].

Intestinal parasites, *H. Pylori* infection, and anemia or iron status among school-aged children in rural Bangladesh have all been studied. The overall prevalence of parasitic infection among children was 84 percent, with Ascaris (71 percent) having the highest prevalence, followed by Trichuris (67 percent), and Hookworm (64 percent) (31 percent).*Helicobacter pylori* infection was found in 39.7% of people. A total of 26.8% of the children were anemic, with low iron status characterized as a low serum ferritin (less than 20 g/L) or an elevated transferrin receptor (more than 5.0 mg/L) in 18% and 11% of the children, respectively. There was no statistically significant connection between anemia, hemoglobin concentration, or low iron status and infection with either of the intestinal parasites or H. pylori infection [41].

Seventy one school-aged Iranian children with dyspepsia, epigastric, and vague abdominal pain were studied in 2019 to see if there was a connection between Helicobacter pylori infection and iron deficiency anemia. Hemoglobin, mean corpuscular volume (MCV), serum ferritin, total iron binding capacity (TIBC), and serum iron levels were contrasted between children with and without *H. Pylori* infection using gastrointestinal endoscopy. Between *H. pylori* positive and negative patients, the proportion of children with IDA was not statistically different (26.2 percent vs. 14.3 percent; P=0.48).In this study, statistical significance was found between *H. Pylori* positive and negative patients' regarding hemoglobin concentrations (P=0.01), but no statistical significance was found between *H. pylori* positive and negative patients' MCV, TIBC, serum ferritin, and serum iron levels [42].

Serum ferritin levels and *H. pylori* antibody titers (IgG) among 6-12 year old healthy primary school children in Tehran were assessed in a cross-sectional study involving 1665 students. Anti-*H.pylori* antibody titers were observed in 429(26%) of the participants, with 220 (23.4%) being girls with an ISR (Immune status Ratio) of 0.75 ±0.39 and 209 (29.1%) being boys with an ISR of 0.79 ±0.42.*H. pylori* infection was found to be significantly more prevalent in boys than in girls in the study (P=0.029). There is no significant relationship between low serum ferritin levels and positive *H.pylori* IgG titers, and no significant relationship between mean serum ferritin levels in IgG seropositive and *H. Pylori* seronegative individuals, according to this report [43].

Another 132 participants participated in a cross-sectional analysis in Medan, Indonesia on the association between iron deficiency anemia and intestinal parasitic infection in school-age children. There were 54.5 percent boys and 45.5 percent girls among them. In total, 34 students (25.8%) were parasite-infected, with 18.9% protozoa infections and 6.9% STH infections among the participants. Furthermore, 10 students (7.5%) were anemic (Hb11,5 g/dl), with 11.3 percent having low serum iron levels, 41.4 percent having high TIBC, 45.2 percent having microcytic erythrocytes, and 12.1percent having hypochromic erythrocytes. The findings of this study revealed a significant correlation between MCV and intestinal parasitic infection (p-value 0.05), but no significant correlation between anemia and other IDA variables including serum iron, TIBC, or MCH(p-value>0.05) [18].

The relationship between *H. pylori* infection and Iron deficiency anemia (IDA) in preschool children was investigated in a case-control study involving 134 Iranian children under the age of six. There were 64 children with iron deficiency anemia and 70 who were control. Both patients with IDA and non-anemic controls had a positive antibody level for *H. pylori* specific IgG after a serological examination for the existence of *H. pylori* infection. The difference between the two classes was statistically significant (P<0.0001), according to the results [44].

In Nigeria, researchers looked into the connection between intestinal helminthiasis and serum ferritin levels in school children. There were a total of 246 children aged 5 to 12 years old. The prevalence of intestinal helminthic infections was found to be 29.7%. Parasitic infections were observed in 26.5 percent of males and 31.9 percent of females, respectively. Anemia was observed in 36.2 percent of the children examined. Intestinal parasite infections were found to have a statistically significant connection. In children with intestinal helminthiasis, serum ferritin levels were significantly lower than in controls [45].

Effect of early and current *Helicobacter pylori* infection on the risk of anemia in 6.5-year-old children was studied in a birth cohort study in Butajira, Ethiopia, with a total of 856 children enrolled. They discovered that 34.8 percent of children enrolled at the 6.5-year follow-up visit had anemia. *H. pylori* infection was observed in 41% of three-year-olds and 44% of five-year-olds, respectively. The prevalence of was higher in children infected with *H. pylori* (37.0%) than in children who were not infected (34.5%) at the 6.5-year follow-up visit, but the difference was not significant (p = 0.38). Most red cell indices assessed at age 6.5 years showed a non-significant decrease in *H. pylori* infected children relative to non-infected children. While red cell indices were regressed against any *H. pylori* infection up to age 6.5 years, infection was associated with lower red cell indices ; MCV (p =0.01), MCH (p=0.01)and MCHC p =(0.01) [46].

All in all as reviewed above some studies found no association between *H pylori* of helminths infection and serum ferritin levels while most also found the association to be significant. The study from Ethiopia, which do not measure Iron status indicator parameters like serum ferritin, also found no association with RBC indices at the age of 6.5 years though the difference was significant with *H pylori* infection anytime up to 6.5 years and red cell indices. The current study, therefore aimed to assess the association of *H pylori* and helminths infection with serum ferritin levels in addition to anemia in school aged children from Batu town.

# 3. Objective

## 3.1 General Objective

To investigate the effect of *Helicobacter pylori* and Helminths infection on anemia or ferritin level of school children in Batu town, Oromia Region, Ethiopia.

## 3.2 Specific Objectives

- To determine the effect of *Helicobacter pylori* infection on the serum ferritin levels of school children in Batu.
- To compare the serum ferritin level between helminths infected and non–infected school children in Batu
- To determine the effect of helminths and *Helicobacter pylori* infection on RBC indices of school children in Batu.

# 4. Hypothesis

- There is no significant difference on the serum ferritin level or red cell indices values *Helicobacter pylori* and Helminths infected students and non-infected controls among schoolchildren in Batu.

# 5. Materials and methods

## 5.1 Study area

Batu is one of the woredas in the Oromia Region of Ethiopia. Part of the Arsi Zone located in the Great Rift Valley; the town is situated at 162 km south of Addis Ababa. It is located on the road connecting Addis Ababa to Nairobi in the East Shewa Zone of the Oromia Region of Ethiopia. Batu has a latitude and longitude of 7°56′N 38°43′E with an elevation of 1643 meters above sea level. Based on 2007 national census reported a total population for this woreda was120, 862, of whom 60,379 were men and 60,483 were women. The city also has both government and private schools of which 14 are primary schools from them Sher and Batu primary schools were included. The present study was conducted using serum samples which were collected from school children in Batu between May-June 2016.

## 5.2 Study design and period

A comparative study was done on samples obtained from school students in two schools in Batu East Shewa District between May and June 2016, and the sample for this study was recruited from a stored sample in April 2020.

## 5.3 Population

### 5.3.1 Source Population

- The source population for the original study were school children aged 4 to 14 years old from all primary schools in Batu city. For the current study, all the stored serum samples collected from the selected children were the source samples.

### 5.3.2 Study population

- Primary school children between the ages of 4 and 14 who were chosen from Batu primary schools and who met the inclusion criteria were the study population. The study samples were also selected using additional criteria as shown below.

## 5.4 Inclusion and Exclusion criteria

### 5.4.1 Inclusion criteria

Serum samples which were stored at-80$^{O}$C were selected when they show no hemolysis, and complete data is available for hemoglobin, *Helicobacter pylori* antibody and antigen tests, and

Stool samples were tested for Helminths presence or absence. The participants ranged in age from 4 to 14 years.

### 5.4.2 Exclusion criteria

The original study has excluded school aged children with the following conditions:

- Any child who has taken any drug (haematinics or iron supplements included) in the previous month
- Any child who has taken anti-helminths and H.pylori medications in the previous six months

For the current study the additional exclusion criteria was inadequacy of sample volume for ferritin analysis.

## 5.5 Study variables

### 5.5.1 Dependent variables

- Ferritin level
- Hgb
- RBC indices (MCV,MCH,MCHC)

### 5.5.2 Independent variables

- Age
- Sex
- Helminths infection
- *Helicobacter pylori* infection

## 5.6 Measurement and data collection

### 5.6.1 Sample size determination

The convenient sampling method was used to select 161 samples from all stored sample that fulfil the selection criteria, all of which were obtained from school children living in Batu. From the total sample, children aged 4 to 14 years old were recruited for this study. The selected161 samples were categorized as follows:

- A total of 52 helminths-free and helicobacter pylori antibody-negative control samples were included in Group A.

- Group B contained 42 children who had been infected with helminths (all included).
- There were 49 *Helicobacter pylori* antibody positive samples in Group C.
- All the18 *Helicobacter pylori* antigen positive samples were included in Group D.

#### 5.6.2 Sampling method

Convenient sampling technique was used to select samples. All *H.pylori* antigen positives and all helminths positives were included. Secondary data on haemoglobin and RBC indices were extracted from previously stored data. Age and sex matching was considered to include samples from helminths and *H pylori* non-infected children and from those positive for *H pylori* antibodies.

#### 5.6.3 Laboratory analysis

##### 5.6.3.1 Specimen preparation

The original samples were collected in plane tubes following standard operating procedures. The serum was transported to the Ethiopian Public Health Institute (EPHI) using cold box for storage at $80^{O}C$ until analysis. For the current study, the samples were selected based on the aforementioned criteria and immediately transferred the serum into clean, labelled microcentrifuge tube using a permanent marker and transported the sample in triple package to testing laboratory (Santé Diagnostic Laboratory) for serum ferritin level determination. After the preparation of material and equipment the serum sample were measured the serum ferritin level of each students sample using clinical chemistry analyzer.

##### 5.6.3.2 Serum Ferritin test

**Principle**

Serum ferritin level was determined using Beckman Coulter chemistry analyzer. Latex agglutination reactions occur as a result of antibody-coated latex beads aggregating if antigen is present in sufficient quantity. Immune complexes formed in solution scatter light in proportion to their size, shape and concentration. Under conditions of antibody excess, increasing amounts of antigen result in higher scatter. Turbidimeters measure the reduction of incident light due to reflection, absorption, or scatter.

In the Beckman Coulter procedure, the measurement of the decrease in light intensity transmitted (increase in absorbance) through particles suspended in solution as a result of complexes formed during the antigen-antibody reaction, is the basis of this assay. The Ferritin reagent is a suspension of polystyrene latex particles, of uniform size, coated with polyclonal rabbit anti-ferritin antibody. When a sample containing ferritin is mixed with the Ferritin reagent, an agglutination mixture occurs. This is measured spectrophotometrically on Beckman Coulter Chemistry Analyzers.

**Interpretation**

Ferritin levels less than 15ng/ml is considered low, and while that greater than15ng/ml is considered normal. Each sample's result in ng/ml was automatically printed out.

## 5.7 Data Quality Assurance

### 5.7.1 Pre analytical

The specimen collected was labelled by using unique identification code for every participant sample in this research. Using the right test tube, appropriate container, adequate volume of sample and kept in appropriate conditions until it was processed. Checking all materials and reagent before preceding the procedure like expiry date, lot numbers, etc was done.

### 5.7.2 Analytical

To assure the procedure is correct first quality controls was done using a quality control material and followed the standard operation procedure or the kit insert.

### 5.7.3 Post analytical

Recording the result appropriately in prepared log sheet and writing legibly, cross checking of results and inserting data in computer.

- The analysis was carried out in chemistry laboratory using Beckman Coulter AU480 analyzer chemistry machine present in Santé Diagnostic centre which is accredited by Ethiopian National Accreditation Office (ENAO).

## 5.8 Data analysis and interpretation

Obtained data was entered, and analyzed by using Statistical package for the Social Science SPSS (Version 21) software the descriptive data were analyzed as mean with 95% Confidence Interval (CI), median with Inter Quartile Range (IQR) and percent where appropriate. Normally distributed continues variables like age, ferritin level and hemoglobin were compared using independent t-test. The non-parametric Mann-Whitney-U test between Helicobacter pylori and

Helminths infected with control sample were used to assess effect of Helicobacter pylori and Helminths infection on anemia or ferritin level of school children significance level. P values less than 0.05 was taken as statistically significant. The analyzed data was present using frequency tables, diagrams and figures to illustrate our finding.

## 5.9 Ethical considerations

Ethical approval has been obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences; Department of Medical Laboratory Sciences. Official permissions from the study sites were obtained. Informed consent for the children to participate in the study was obtained from the parents or guardians to be eligible for enrolment into the study. In order to participate in this study, children over the age of 12 must have written assent. Other than the investigations needed for the research goals out lined in the consent form, no other tests were performed on the sample. All participant information was kept private, and children who required medical attention were referred to a health centre or hospital for proper assessment and care. To use the stored serum samples for the current study, ethical clearance was obtained from DRERC.

## 5.10 Dissemination of the result

Addis Ababa University's College of Health Sciences, Department of Laboratory Sciences will receive copies of the paper. As a result, it can be used as a library reference. After completion, this study will be used as a reference material by health practitioners, academics, experts, and policy makers for intervention; presentations will be made at scientific conferences, and the paper will be published in authoritative journals.

## 5.11 Operational definitions

- Anemia was defined as Hb level less than 11.5g/dl for aged 5 to 11 years and Lessthan12.0g/dl for age12 years to 14years
- School age children: students whose ages are between 4 and 14 years old
- Control was defined as Helicobacter pylori antibody and antigen negative and Helminths free.
- Case group was defined as sample which is positive for Helicobacter pylori antibody and antigen and sample contain Helminths.

### 5.12 Workflow

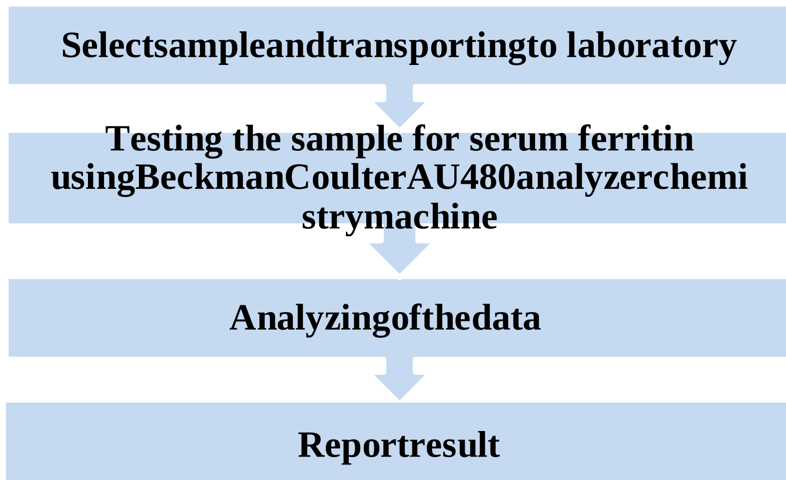


Figure1. Flowchart

# 6. Result

## 6.1 Characteristics of the study participants

A total of 161 samples were analyzed in this study, with 77 (47.8%) of them being males and 84(52.2%) being females. The total participants' mean age (95 percent confidence interval) was 8.58 (8.2-9.0), with a range of 4-14 years. Of the total children, 109 of the research participants were positive for. *H pylori* and Helminths labelled as cases group, while 52 non-infected children were in the control group. The proportion of types of cases were 42 stool helminths positive, 18 stool *H-pylori* antigen positive and 49 serum *H-pylori* antibody tests positive out of all study participants (n=161), The cases and control groups have no significant difference in age and sex, see table one(Tables 1 a and b).

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Table1a.Characteristics of school aged children from Batu, central Ethiopia (n=161)

| Characteristics | Frequency | Percent | |
|---|---|---|---|
| **Sex** | | | |
| **Female** | 84 | 52.2 | |
| **Male** | 77 | 47.8 | |
| **Age(Years)** | | | |
| **4-9** | 102 | 63.4 | |
| **10-14** | 59 | 36.6 | |
| **Variables** | **Total** | **Case(n=109)** | **Control(52)** |
| **All negative** | 52 | 0 | 52 |
| **Helminths positive** | 42 | 42 | 0 |
| **H. pylori Antigen positive** | 18 | 18 | 0 |
| **H. pylori Antibody positive** | 49 | 49 | 0 |
| **Total** | 161 | 109 | 52 |

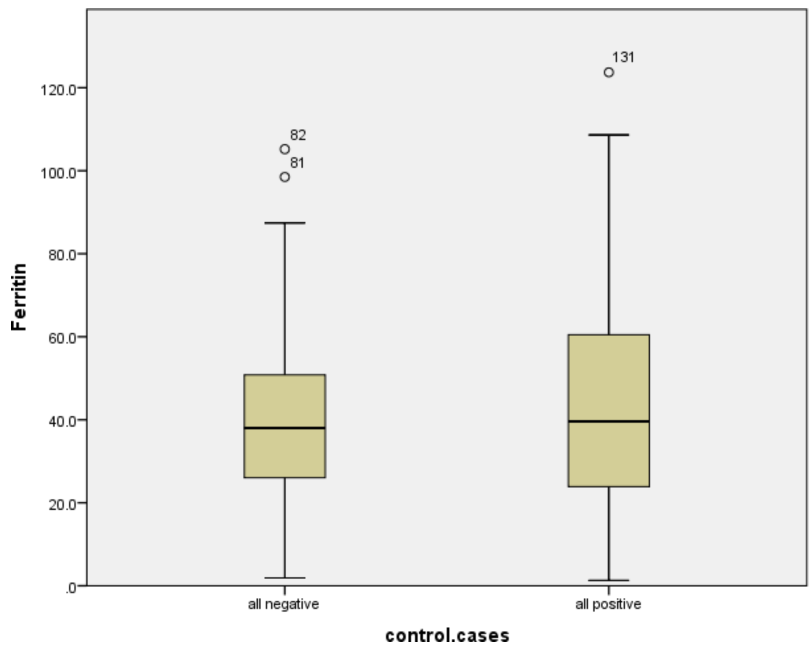
Table1b.Comparison of demographic data between the cases and control groups among school children in Batu, Central Ethiopia (n=161)

| Demographic data | | | | |
|---|---|---|---|---|
| Age(normally distributed between cases and control group**)** | | | | |
| **Age** | Total | Cases | Control | P value |
| **Mean with 95% CI** | 8.58(8.16-9.0) | 8.77(8.2-9.3) | 8.17(7.5-8.9) | 0.192 |
| Chie$^2$ test used to cross tab gender $v_{s.}$ cases /control | | | | |
| **Male** | 77 | 53 | 24 | 0.769 |
| **Female** | 84 | 56 | 28 | |

## 6.2 Serum ferritin levels

The median (IQR) and mean (95% CI) value of serum ferritin level in all study subjects were 38.9(24-58) and 42.5(36-47) respectively. Figure2 displays distribution of ferritin level between control groups and all case groups (Figure 2A) and a control groups with each cases groups (Figure 2B).Although the distribution of serum ferritin level is wider in the helminths and *H.pylori* positive groups, as shown Table 2, there was no statistically significant difference between children negative for both infections and the positive groups (Table2).

A

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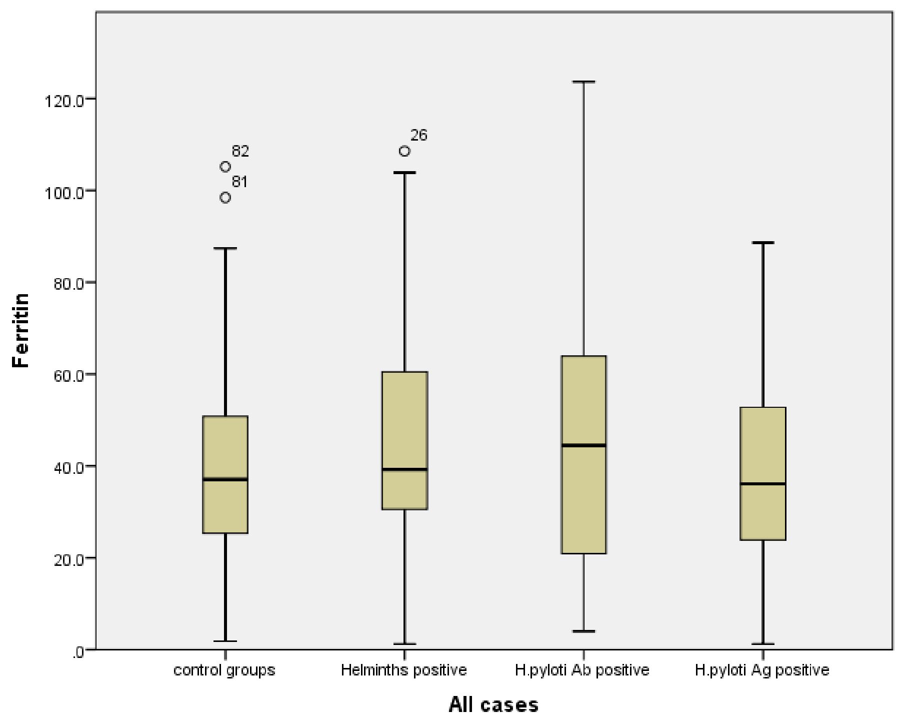


Figure 2: Distribution of Ferritin level between control groups and all case groups (A) and a control groups with each cases groups (B).

### 6.3 Comparison of serum ferritin level between all cases and controls

The serum ferritin values were nearly normally distributed between the case and control group as shown in the figures (Figure 2A). As shown in Figure 3 below, the abnormal ferritin level below15ng/ml was detected in 26 (23.9%) of the 109 positive cases and 18 of the 52 (34.6%) children who were negative for both *H. pylori* and helminths infections, though the difference was not statistically not significant (p=0.856).

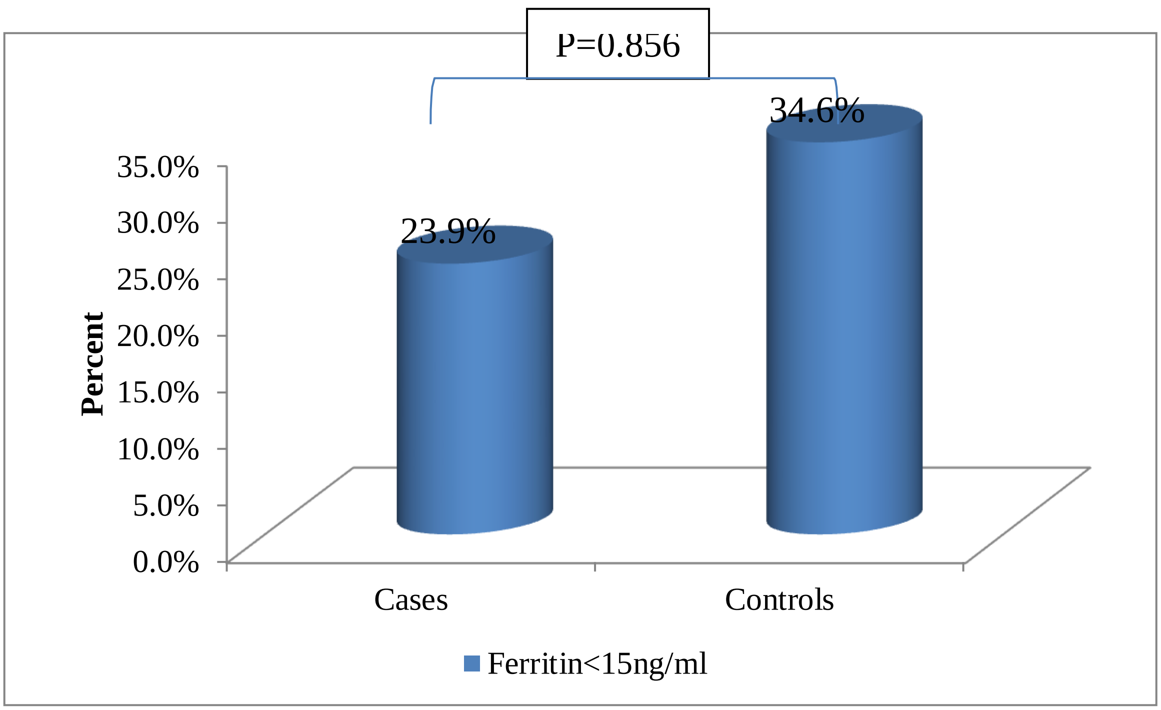


Figure3. Magnitude of abnormal ferritin level between cases and controls among school age children from Batu, Ethiopia

Moreover, Table 2 displays that both parametric (students t-test) and nonparametric test (Mann-Whitney U test) were used to see differences in any mean and median values, respectively. The result showed that there is no statistically significant difference in ferritin mean values between cases and controls (43.8, 95% CI 38-49 for cases and 40.9, 95%CI 34-47 for controls, mean difference=2.9(-11-6, p=0.518).The same is true in a comparison of the median serum ferritin

Levels between the controls (Median=38, IQR=26-51) and the Cases group (Median =39.6, IQR=24.4-60.5) with a p-value of 0.641 (see Table 2). Similarly, As shown in Table 2 there was no statistically significant association between gender and serum ferritin levels between males and females, with p-values for mean (p=0.520) and for median (p=0.840).

Correlation analysis between ferritin levels and age revealed no statistically significant association between the two(r=0.020, p=0,996).

Table 2. Univariate analysis showing factors associated with serum ferritin level among the study participants, Batu, Ethiopia

**Mann-Whitney U and students T-test for median and mean values of serum ferritin Comparison between Cases and control group**

| **Ferritin level ng/ml** | Cases | Control | Median/Mean Difference | P-value |
|---|---|---|---|---|
| **median with IQR** | 39.6(24-60.5) | 38(26-51) | 1.6 | 0.641 |
| **Mean with 95%CI** | 43.8(38-49) | 40.9(34-47) | -2.9 | 0.518 |

**Mann-Whitney U and students T-test for median and mean values of serum ferritin comparison between males and females**

| **Ferritin Level ng/ml** | Male | Female | Median/Mean difference | P-value |
|---|---|---|---|---|
| **Median with IQR** | 38.9(26.3-57.6) | 39.7(24.2-58.6) | 0.8 | 0.840 |
| **Mean with 95%CI** | 41.5(36-47) | 44.20(38-50.5) | -2.73 | 0.520 |

## 6.4 Effect of *H. Pylori* on serum ferritin level, hemoglobin and Red blood cell indices

In this study the result shows that there were no statistically significant differences regarding serum ferritin, Hgb, MCV, MCH, MCHC levels between *H.pylori* stool antigen positive or *H.*pylori antibody positive and control groups: Serum ferritin (P=0.787, P= 0.350), Hgb (P=0.587,P=0.153), or MCV (P=0.211, P= 0.992), MCH (0.163, P=0.963) and MCHC (P = 0.541, P=0.952) for *H. Pylori* antibody or antigen, respectively (Table3).

Figure 3 also displays the distribution of serum ferritin, Hgb and red cell indices levels between *H pylori* (Antigen and Antibody, separately) positive and control groups. The non-parametric Man Whitney U test revealed there was no statistical difference in these parameters as compared to the values in H pylori negative children (Figure 4).

Table3.Comparison of Serum ferritin level and hematological indices in *H.pylori* Ag positive and *H.pylori* Ab positives with control groups, Batu, Ethiopia

| **Parameter** | **Mean(SD)** | | **P-value(95 %CI)** | **Mean(SD)** | | **P-value(95%CI)** |
|---|---|---|---|---|---|---|
| | **H.pylori stool Antigen positive** | **Control groups** | | **H.pylori antibody positive** | **Control groups** | |
| **Ferritin** | 38.2(23.25) | 40.02(25.14) | 0.787 (-11.63-15.28) | 45.11(29.3) | 40.02(25.14) | 0.350 (-15.85–5.66 ) |
| **HGB** | 13.6(1.6) | 13.8(1.2) | 0.587 (-0.50-0.88) | 13.5(1.09) | 13.8(1.2) | 0.153 (-0.12–0.76) |
| **MCV(fl)** | 88.23(6.237) | 86.65(3.624) | 0.211 (-4.083-0.920) | 86.66(4.764) | 86.65(3.624) | 0.992 (-1.738–1.721) |
| **MCH** | 29.48(2.502) | 28.78(1.396) | 0.163 (-1.679-0.289) | 28.80(2.008) | 28.78(1.396) | 0.963 (-0.723–0.689) |
| **MCHC (ρg)** | 33.35(0.914) | 32.62(4.888) | 0.541 (-3.131-1.657) | 32.56(4.952) | 32.62(4.888) | 0.952 (-1.933–2.055) |

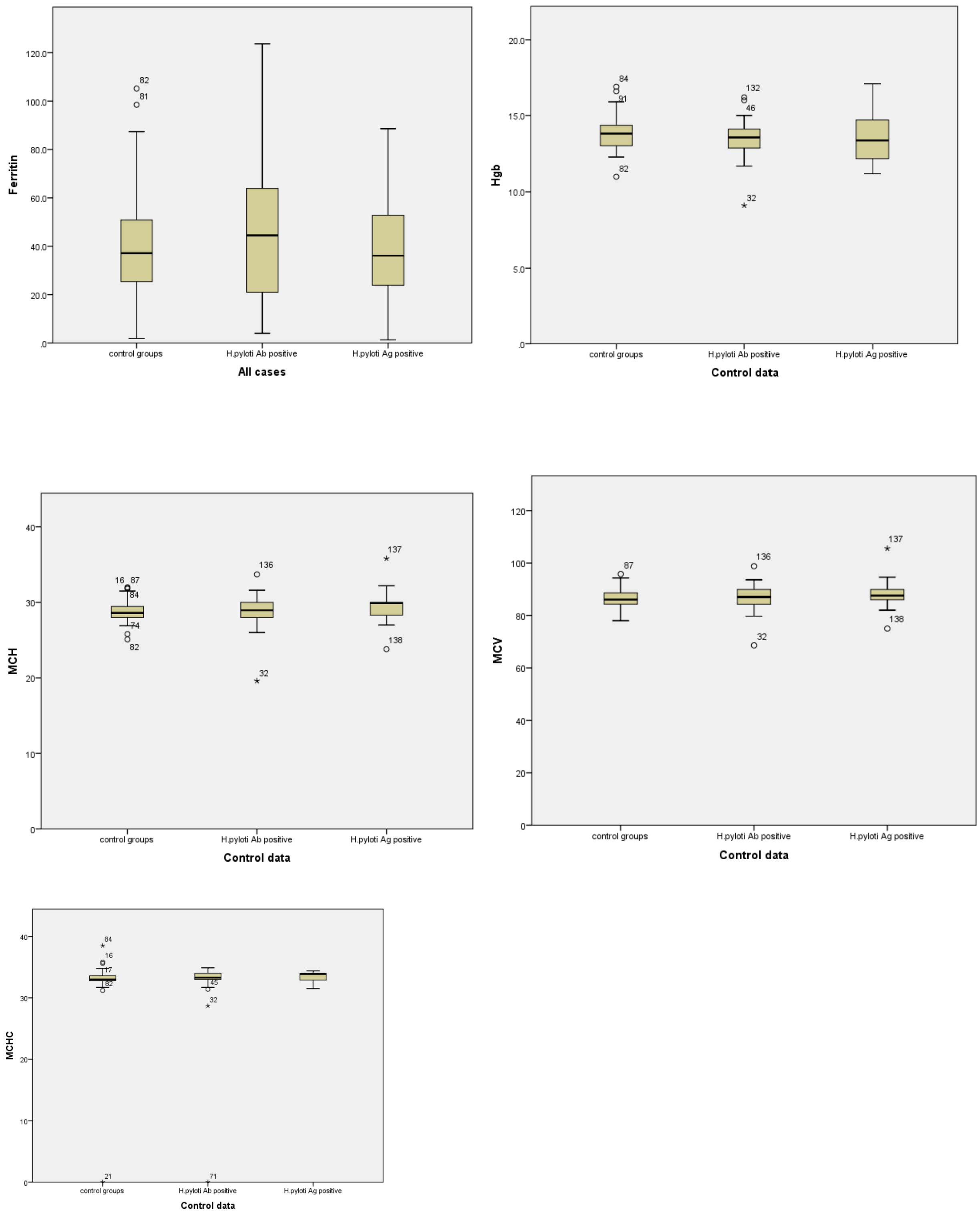


Figure4:-Distribution of Ferritin level, Hgb concentration, MCV, MCH and MCHC values in control groups and H.pylori antibody and antigen positive Groups, Batu, Ethiopia

## 6.5. Effect of Helminths on serum ferritin level, haemoglobin and Red blood cell indices

In this study, 42 (26.1%) of the students were infected with parasites, while 52 (32.3%) of the students were used as control groups, free of protozoa and helminths. There was no significant effect of helminths infection on serum ferritin as well as hemoglobin, MCV, MCH, MCHC. The mean serum ferritin level, hemoglobin concentration, and RBC indices of the helminths positive sample and control groups were compared using an independent samples t test. None were statistically significant; the serum ferritin level (P=0.286), hemoglobin concentration (P=0.563), MCV (P=0.646), MCH (P=0.485), and MCHC (P=0.975); none were statistically significant. (Table4).The distribution of these parameters between helminths positive and negative children is shown in Figure 5.

Table4.Comparison of Serum ferritin level and hematological indices between Helminths positive and negative children in Batu, Ethiopia

| Parameter | Mean (SD) | | P-value(95%CI) |
|---|---|---|---|
| | **Helminths positive** | **Control groups** | |
| **Ferritin** | 45.86(27.5) | 40.02(25.14) | 0.286(-16.636 –4.964) |
| **HGB** | 13.6(1.44) | 13.8(1.2) | 0.563(-0.3765 --0.687) |
| **MCV(fl)** | 87.10(5.607) | 86.65(3.624) | 0.646(-2.417--1.507) |
| **MCH** | 29.03(1.990) | 28.78(1.396) | 0.485(-0.969--0.464) |
| **MCHC(ρg)** | 32.58(5.203) | 32.62(4.888) | 0.975(-2.071--2.137) |

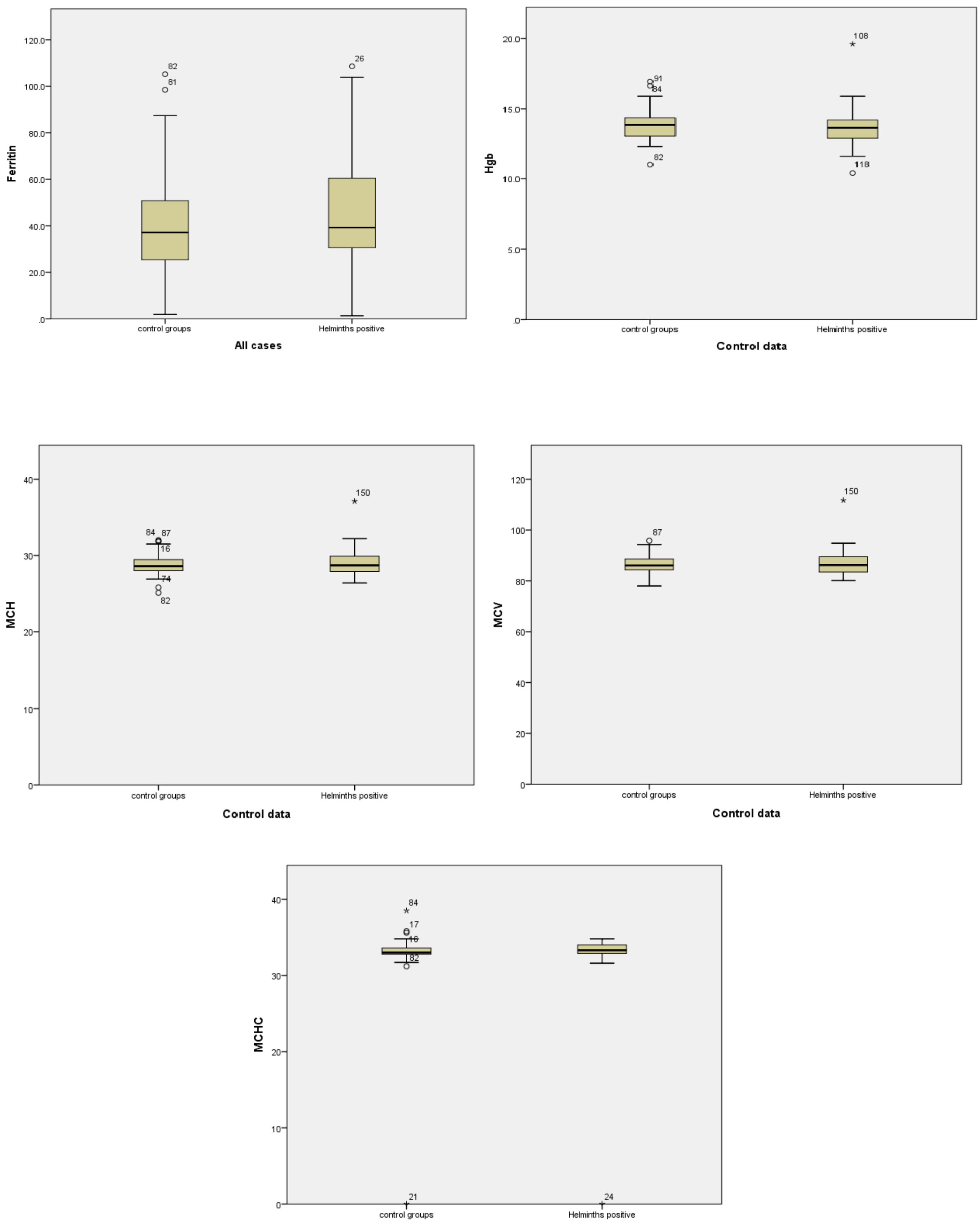


Figure 5: -Distribution of serum Ferritin level, Hgb concentration, MCV, MCH and MCHC, in both control groups and helminths positive Groups, Batu, Ethiopia.

# 7. Discussions

This study aimed to determine the effect of *H pylori* and Helminths infection on the serum ferritin and other red cell indices of children aged 4-14 years in Batu, Oromia region, Ethiopia. The findings suggest that in the group of school-aged children in Batu city, there was no significant effect of *H. pylori* infection or the presence of helminths on serum ferritin levels. The mean serum ferritin level was normal in all the groups: *H.pylori* stool antigen positive cases, *H. Pylori* antibody positive cases, helminths, and control groups. Similarly, other hematological parameters such as hemoglobin, MCV, MCH, and MCHC were not statistically significantly different between children who are positive for the two infections and those negative counterparts.

## 7.1 Effect of H. Pylori infection on ferritin level

There was no evidence of a statistically significant effect of *H.pylori* infection on serum levels or RBC parameters between the *H.pylori* positive sample and the control groups. The mean serum ferritin level, hemoglobin concentration level, and RBC indices in both H.pylori positive and control groups suggest that the presence of *H.pylori* does not influence or contribute to a deficiency of serum ferritin level in the blood.

Previous studies in various environments, such as Iranian school aged children, Tehran, primary school children [43], and Butajira under 6.5-year-old children [46], have not shown significant associations between *H.pylori* infection and serum ferritin or other hematological parameters.

In the Iranian study which recruited 71 children aged 18 years and conducted between November 2016 and May 2017, the proportion of children with IDA differed between *H. pylori* positive and negative patients, but the difference was not statistically significant (26.2%vs.14.3%;P=0.48). Hence, they found no association between *H. pylori* infection and low serum ferritin levels or IDA [42].This finding is comparable to another study in Iranian children aged 6-12 years with a case cross-sectional study design including 1665 students from those participants Anti *H. pylori* antibody titers were positive in 429 (26%).This result also showed that there was no association between *H. pylori* infection and IDA [43], Another Ethiopian birth cohort study found that 34.8 percent of children enrolled at the 6.5-year follow-up visit had anemia due to early and existing *Helicobacter pylori* infection. Children infected with *H.pylori* had a higher incidence of (37.0%) than children who were not infected (34.5%), but the difference was not statistically significant. Most red cell indices assessed at age 6.5 years showed a non-significant reduction in *H. pylori* infected children relative to non-infected children [46], a fining which is consistent with the current study,

In Iran, however, case control study was conducted on 134 children under the age of six. There were 64 cases of iron deficiency anemia and 70 controls from that participant. They discovered that *H. pylori* infection was present in both IDA 52(81.3%) patients and non-anemic controls, with 10 (14.3%) having a positive *H. pylori* antibody level. In contrast to the current study, there was an association between *H. Pylori* infection and IDA [44].

The absence of association between *H pylori* and serum ferritin level or hemoglobin levels was also supported with other studies. For example, a study from Bangladesh found no statistically significant connection between anemia, hemoglobin concentration, or low iron status and infection with either of H.pylori infection or intestinal parasites [41].

## 7.2 Effect of Helminths on ferritin level

In Ethiopia, parasitic diseases that cause anemia are common, but the current study when helminths positive samples with control groups were compared based on the mean serum ferritin value and other hematological parameters including hemoglobin and RBC indices, the current findings indicated that the effect of helminths have no statistical significance.

In a study conducted in Nigeria on the relationship between intestinal helminthiasis and serum ferritin levels among school children aged 5 to 12 years, the prevalence of intestinal helminthic infections was found to be 29.7%.Anemia was found in 36.2 percent of the people surveyed. In contrast to the current findings, serum ferritin levels in children with intestinal helminthiasis were significantly lower than in controls [45]. This is expected since helminthic infection results in chronic blood loss which result iron deficiency and ultimately full blown anemia.

Another cross-sectional study conducted in Medan looked into the relationship between iron deficiency anemia and intestinal parasitic infection in school-aged children. Despite the fact that a p-value of 0.05 indicated a strong connection between MCV and intestinal parasitic infection, 34 students (25.8%) were parasite-infected, with 18.9% protozoa infections and 6.9% STH infections. Although the fact that there was no statistical significant correlation between anemia and other IDA variables such as serum iron, TIBC, and MCH (p-value>0.05) [18].

All in all, since this study was conducted with a limited sample size, the findings should be viewed with caution. If the sample size were increased, the outcome could change. On the other hand as has been seen in one Iranian study [43], the absence of significant association between *H pylori* or helminths and serum ferritin level in the current study may not be attributed to sample size only as has been evidenced by the Iranian study which recruited large number of children (n=1665) aged 6-12years and still cannot demonstrate an association.

The body iron store indicator, Serum ferritin, is a common acute phase reactant and inflammatory marker that is elevated non-specifically in a number of inflammatory conditions such as chronic kidney disease, rheumatoid arthritis, and other autoimmune disorders, acute infection, and malignancy. It's unclear if *H. pylori* infection has any effect on the acute phase reactants. If this were the case, the inverse association between serum ferritin levels and H.pylori seropositivity or helminths infection would be weakened [47, 48].

It is also possible that in the case of helminths, light infection might have contributed to the absence of effect on serum ferritin or the other RBC parameters levels. In this regard, it is worth mentioning that the deworming program might have contributed to the observed result as light infections are reported by other studies in Ethiopia [49].

# 8. Strength and Limitation of the study

## 8.1 Strength of the study

- This study has tried to show the effect of *H, pylori* or helminths infection on serum ferritin level. Information regarding Serum ferritin, which is an indicator of individual's iron store, is limiting in *H. pylori* positive individuals. Both stool antigen and antibody were measured.
- One of the strength of this research is that using case control research design method

## 8.2 Limitation

Our study has some limitations, such as the fact that it does not differentiate between different types of *H. pylori* strains, which is significant because some strains are more pathogenic than others and have an effect on serum levels. Another disadvantage is the presence of a small number of *H.pylori* stool antigens positive sample.

# 9. Conclusion and Recommendation

## 9.1 Conclusion

The presence of *Helicobacter pylori* or helminths has no effect on serum ferritin levels or the development of iron deficiency in school-aged children. Because of the limited sample size and observational evidence, these findings should be viewed with caution. More large-scale research is needed to determine the possible risk factors. Furthermore, cohort studies are recommended for establishing a cause and effect relationship between *H.pylori* infection or helminths infection and serum ferritin levels. Adding other iron panel tests would also provide a good picture of the impact of *H. Pylori* or helminths infection on serum ferritin levels.

## 9.2 Recommendation

Since no studies on the effects of *H.pylori* and Helminths on serum ferritin levels in school-aged children have been conducted in Ethiopia, further research is needed to complement the results of this study, which will be used to develop potential recommendations for *H.pylori* treatment and strengthening of mass deworming of school-aged children. Although there was no association between *H. pylori*, Helminths, and serum ferritin levels in our study, increasing the number of participants could yield different results. Furthermore, knowing the *H. pylori* species type may have an effect on the study's outcome.

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# 11.Annexes

## Annex I: SOP for serum iron determination

### Intended Use

Immuno-turbidimetric test for the quantitative determination of ferritin in human serum and plasma on Beckman Coulter AU analysers

Measurement of ferritin in serum or plasma is a useful indicator of body iron stores in normal persons, and in individuals with iron deficiency. A ferritin level of less than 10 µg/l usually indicates iron deficiency anemia. An increased ferritin concentration is observed in a large number of chronic diseases. These include chronic infections; chronic inflammatory disorders; heart disease; and malignancies, especially lymphomas, leukemias, breast cancer and neuroblastoma. In patients who have any of these disorders together with iron deficiency, ferritin concentration is often normal. Ferritin concentrations may be extremely high in patient suffering from hemochromatosis and certain liver diseases.

### Methodology

Latex agglutination reactions occur as a result of antibody-coated latex beads aggregating if antigen is present in sufficient quantity. Immune complexes formed in solution scatter light in proportion to their size, shape and concentration. Under conditions of antibody excess, increasing amounts of antigen result in higher scatter. Turbidimeters measure the reduction of incident light due to refection, absorption, or scatter.

In the Beckman Coulter procedure, the measurement of the decrease in light intensity transmitted (increase in absorbance) through particles suspended in solution as a result of complexes formed during the antigen-antibody reaction, is the basis of this assay. The Ferritin reagent is a suspension of polystyrene latex particles, of uniform size, coated with polyclonal rabbit anti-ferritin antibody. When a sample containing ferritin is mixed with the Ferritin reagent, an agglutination mixture occurs. This is measured spectrophotometrically on Beckman Coulter Chemistry Analysers.

**Patient Preparation:**

Not required.

TYPE OF SPECIMEN

Serum, Li-heparin plasma and EDTA plasma samples are the recommended specimens.

**Handling of sample:**

Serum ferritin sample is Stable in serum and plasma for 7days when stored at 2…8°C and for 12 months when stored at-20°C.

Strongly lipemic samples should be avoided.

**Equipment:**

Beckman Coulter AU480 analyzer

**Materials:**

Beckman Coulter AU System serum ferritin Reagent

Final concentration of reactive ingredients:

Glycine buffer (R1: pH8.3, R2: pH 7.3) 170 mmol/L

Latex particles coated with rabbit anti-human ferritin

Test tubes 12-16 mm indiameter or sample cups

REAGENT PREPARATION

R1 is ready for use and can be placed directly on board the instrument. R2 should be mixed by inversion 5 – 10 times before placing on board the instrument.

**Precautions:**

For invitro diagnostic use.

Causes mild skin irritation.

If skin irritation occurs: Get medical advice/attention.

**Indications of Deterioration:**

The reagents are stable unopened, up to the stated expiry date when stored at 2…8°C. Once open, reagents stored on board the instrument are stable for 60 days. Once on board the R2 must be mixed at weekly intervals

**Procedure**

- **Torun patient sample**

1. On the white rack, put the sample cup.
2. Label the patient ID number.
3. Request the sample from the sample management section.
4. Dispense 200-500µl of serum to the labelled sample cup.
5. Load the white rack on AU-480, and then run machine.
6. Review the print out result for any discordant.
7. Enter the result to SLIS [Santé laboratory information system].
8. Make are view for proper transcription of the result and Print the result.

- **Torun Quality Control**

    1. From refrigerator get the Beckman Coulter control levels.
    2. Just put the control at room temperature for 20 minute.
    3. Dispense the control on the sample cup.
    4. Load the green rack and run the machine.
    5. Review the QC result for the breaches.
    6. Document the result.

- **Torun Calibration**

    1. Dispense the calibrators on the sample cup. At yellow rack and the blank [DI water] at blue.
    2. Load the racks and run the machine.
    3. Review the calibrations for any failed calibration.

**Performance Characteristics:**

The following data was obtained using this serum ferritin Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained at individual facilities may differ.

PRECISION

The following data was obtained on an AU640 using 3 serum pools analysed over 20 days.

| n = 80 | Within-run | | Total | |
|---|---|---|---|---|
| Mean µg/L | SD | CV% | SD | CV% |
| 25 | 0.55 | 2.24 | 0.92 | 3.71 |
| 148 | 0.91 | 0.61 | 2.21 | 1.49 |
| 438 | 4.18 | 0.95 | 4.95 | 1.13 |

SENSITIVITY

The lowest detectable level in serum on an AU680 was estimated at 5.47µg/L.

The lowest detectable level represents the lowest measurable level of ferritin that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

CALIBRATION

The calibrator ferritin values assigned to the calibrators are traceable to the 3rd International Standard for ferritin, Recombinant NIBSC

Recalibrate the assay every 30 days, or when the following occur:

Change in reagent lot or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced.

**Quality Control:**

During operation of the Beckman Coulter AU analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate AU User's Guide. Quality control testing should be performed in accordance with laboratory's standard procedure.

CALCULATIONS

The Beckman Coulter analysers automatically compute the ferritin concentration of each sample.

**Reference Ranges:**

**Serum Adults**

| | |
|---|---|
| Adult male: | 20– 250 µg/L |
| Adult female: | 10– 120 µg/L |

**Serum/Plasma Children**

| | |
|---|---|
| Up to 1 month: | 6– 400 µg/L |
| 1 to 6 months: | 6– 410 µg/L |
| 6 to 12 months: | 6– 80 µg/L |
| 1 to 5years: | 6– 60 µg/L |
| 6 to 19years: | 6– 320 µg/L |

LINEARITY

The test is linear within a concentration range of 8.0–450 µg/L.

LIMITATIONS

This assay has been specifically designed to substantially reduce the risk of interference from HAMA or Heterophilic antibodies; however as with all immuno-assays there is always a small risk from such interferences

Samples with extremely abnormal optical characteristics, especially turbidity, may produce atypical results. Such samples must be serially diluted and results compared to ensure that no such interference exists.

**Interfering Substances:**

Results of studies show that the following substances interfere with this serum ferritin procedure

Lipemic: Interference less than 10% up to1000 mg/dL Intralipid

Haemolysis: Interference less than 10% up to 5 g/L haemoglobin

Bilirubin: Interference less than 5% up to 40 mg/dL

Rheumatoid Factor: Interference less than 3% up to 500 IU/mL RF

## Declaration

I, undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the candidate: Raji Tesfaye (BSc)

Signature___________

Place: Addis Ababa University, Department of Medical laboratory Science, Ethiopia

Date of submission_____/_______/___________

This thesis has been submitted with my approval as university advisor.

Name of advisors:

Aster Tsegaye (MSc, PhD) Signature___________________

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission______/______/_________