

Ministry of Higher Education and Scientific Research

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**A Study the levels of Magnesium, Potassium and Ferritin in
the Blood Serum of people infected with COVID-19**

**A research submitted to the Department of Biology as part of the
requirements for obtaining a BSc degree in Biology**

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1443 A.H

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا

تَعْمَلُونَ خَبِيرٌ ﴿﴾

صدق الله العلي العظيم

[المجادلة: 11]

Dedication

Praise be to Allah, and enough prayers for the beloved Prophet and his family.
To Shamekh mountain, which was based upon the intensity when the source of wisdom Dad
To those who were the source of compassion and kindness my teacher first and lastMam
To which derive their strength and keep the eyes (my daughter Asenath(
To Cindy in life my husband
To the spirit of my colleague-present in our hearts with her smiles beautiful over the life of the late
..... (Mahdi sacrificed(
Every precious family that support me and still from the brothers and sisters who journey to
boyfriends Kasemna moments.
To every department of life sciences.
To all the martyrs of Iraq to (martyrs of October Revolution (To all of Iraq's scientists and elites
and to the effort to provide me with science and knowledge
To the main supporter in this research accompanied by the most beautiful smile (Dr. Rafal Ahmed
Lilo)
To all who have had an impact on my life and to all of my heart and love them contribute pen.

Ola

Thanks and Appreciation

The first thanks is to God Almighty, then my parents for all their efforts since my birth to these moments, you are everything I love you in God the most. I am pleased to extend my thanks to everyone who advised me, guided me, directed me, or contributed to the preparation of this research by connecting me to the required references and sources at any stage of its stages, and I especially thank my honorable professor, Dr. Rafal Ahmed Lilo. My thanks go to the management of the College of Science at the University of Babylon, Department of Life. All members of the research discussion committee.

Ola

Summary

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the seventh human coronavirus, was discovered in Wuhan, Hubei province, China, during the recent epidemic of pneumonia in January 2020. Since then, the virus has spread all over the world, as of 12 April 2022; there have been 497,960,492 confirmed cases of COVID-19, including 6,181,850 deaths, reported to WHO. SARS-CoV-2 as well as SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) cause severe pneumonia with a fatality rate of 2.9%, 9.6% and ~36%, respectively.

This study aimed to determine the concentration of some mineral elements (Potassium and Magnesium) and determine the concentration of ferritin.

The current study extended for the period between 1/12/2021 to 1/3/2022, during this period, 89 blood serum samples were collected from people infected with the Corona virus, for ages from 4 years to 64 years, for both sexes (the infection was confirmed by laboratory immunoassay).

It was found that 24% had a decrease in potassium concentration in the blood serum, 11% had an increase in potassium levels, and the remaining 65% had normal potassium levels. This study showed that a number of patients infected with the Corona virus suffer from low levels of magnesium in the blood, and their number was 18 people, including 10 women and two men, while the number of patients suffering from high magnesium in blood serum was 18 infected, including 6 women and 12 men.

Summary

It was noted that the percentage of people suffering from a decrease in ferritin protein was 2%, while 3% had a high concentration of this protein in the blood serum, and 94% had a normal level of ferritin.

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List of Abbreviations

(IP3)	Inositol 1,4,5-trisphosphate
(DAG)	Diacylglycerol
NK	Natural killer cells
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
TMPRSS2	Transmembrane protease, serine 2
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
IL-6	InterLeukin-6
TNF-alpha	Tumor necrosis factor
3CLpro	3-chymotrypsin like protease
PLpro	papain like proteases
FTH	Ferritin heavy subunit
FTL	Ferritin light subunit

Chapter One

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Mayo Clinic,2020. It was first identified in December 2019 in Wuhan, China, and has resulted in an ongoing pandemic (Hui DS et al,2020; WHO). The first confirmed case has been traced back to 17 November 2019(Josephine,2020). Traces of the virus have been found in December-2019 wastewater that was collected from Milan and Turin(Kate,2020). As of 21 June 2020, more than 8.8 million cases have been reported across 188 countries and territories, resulting in more than 464,000 deaths. More than 4.37 million people have recovered (Johns Hopkins University,2020).

Minerals are inorganic substances, present in all body tissues and fluids. Their presence is required to maintain certain physical and chemical processes which are essential to life..(Bruulsema *et al*, 2013)

Patients infected with SARS-CoV-2 have been shown to have anomalies in sodium, potassium, chloride, and calcium levels. These alterations in electrolyte balance may be caused by extended hospitalization and the use of various drugs, changes in food habits, multiple organ failure, drug-related adverse effects, and hyperventilation (Alfano *et al*, 2021). There is a strong correlation between electrolyte abnormalities and poor clinical outcomes in Covid19 patients (Ezcan *et al*, 2020; Lippi et al,2020; Sarvazad *et al* ,2020; Wu et al,2020; and arvalho *et al*, 2021).

In confirmed Covid19 instances, potassium problems are the most prevalent electrolyte abnormalities. Both high and low blood potassium concentrations have been discovered to be critical determinants that impact the prognosis of illness independently. Clinical results may be improved if the main cause of potassium homeostasis disturbance is discovered and remedied. (Noori *et al*, 2022). Stabilizing mastocyte membranes in alveolar septa and the submucosal airways

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limit their degranulation, therefore inhibiting their release of many mediators. Toll-like receptor 4/NF- κ B axis is hampered by magnesium, which limits neutrophil priming and oxidative burst and also modulates neutrophil and macrophage activity (Maier *et al*, 2020).

Magnesium regulates IP3 and DAG levels in lymphocytes, which are essential second messengers triggered when B and T cell receptors are engaged (Brandao *et al*, 2013).

The cytotoxic activity of T lymphocytes and natural killer (NK) cells is dependent on appropriate intracellular magnesium concentrations, which is why magnesium protects against viral infections (Minton,2013). SARS-CoV-2 infection may be influenced by magnesium levels in the early stages of the disease's progression. Angiotensin-converting enzyme 2 (ACE2), which is expressed in various organs, serves as an entrance receptor for the viral spike (S) protein and is responsible for the pulmonary and extra-pulmonary symptoms of COVID-19 (Gupta *et al*,2020).

Proteolytic cleavage of the S protein by host cell proteases is required for the fusion of viral and cell membranes to occur. Inhibiting the activity of these enzymes may be accomplished in part by the use of magnesium. TMPRSS2 transcription is hampered and, as a result, the enzyme's expression is decreased by magnesium therapy, according to preliminary studies. (Fan *et al*,2021; and Valentina *et al*, 2022).

CRP, ESR, and other inflammatory markers (e.g., IL-6, TNF-alpha, etc.) are elevated in individuals with COVID-19, as are pro-inflammatory cytokines such as IL-6 and TNF-alpha. It has been postulated that the high ferritin level in COVID-19 is connected with the release of cytokines that are involved with inflammation (e.g. IL-6, IL-1 β , and TNF-alpha), cellular damage, metabolic acidosis, ROS production, and secondary tissue damage. In individuals with COVID-19, serum ferritin has been found to correlate with disease severity and its surrogates,

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according to individual investigations (CRP) (Lin *et al* 2020; and Karanvir *et al.*,2022)

Aim of this study:-

The study aims to determine the relationship of the Corona virus with a number of elements (potassium, magnesium) and to determine the concentration of ferritin during infection with the virus.

Chapter Two

Review of Literature

Review of literature

The current coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which is a member of the Coronavirinae virus subfamily, has exerted an unprecedented global impact. As of a January 13, 2022 update, the world Health Organization COVID-19 Dashboard had reported (312.173) million cases and (5.501) million deaths worldwide (WHO, 2022).

However, when considering “excess deaths” (the standard method of tracking changes in total mortality), 19.2 million (95% confidence interval 12.0–22.4 million) people worldwide are estimated to have died as a consequence of the COVID-19 pandemic (The Economist, 2022). The pandemic catalyzed an extraordinary effort to develop vaccines to induce specific adaptive immunity, based on virus-neutralizing antibodies and T-cell responses. Since their rollout in late 2020, vaccines have been highly effective in preventing clinically significant COVID-19 morbidity/mortality. Not with standing this effect, mutations that have given rise to new variant strains with increased infection transmission rates have emerged to trigger breakthrough infections, even in fully vaccinated individuals (Glatman-Freedman *et al.*,2021; Antonelli *et al.*,2022).

This development has markedly altered the landscape for the management of COVID-19, prompting both renewed “lockdowns” that disrupt local and global economies and the revision of estimates of the level of herd immunity necessary to quell disease.(Kim *et al.*,2021; Niyas *et al.*, 2021) Pressure has also increased on healthcare systems, because of both the reduced efficacy of currently approved vaccines to stop virus transmission, and the chronic disabling symptoms and residual fibrosis in the lungs, kidneys, and cardiovascular system that affect some individuals who have recovered from acute COVID-19 infection—so called “long

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COVID.” (Bergwerk *et al.*, 2021; Nalbandian *et al.*, 2021) Consequently, a prudent and, indeed, essential course is to adopt a multipronged strategy that does not place sole reliance on vaccines but instead implements multiple complementary approaches to quell the impact of disease. (Morley and Murray, 2022)

Coronavirus (CoV) structure

The CoV family has a sizable homogeneous “spike protein” (Figure 1). The role of the spike (S) protein, which is composed of 1300 amino acids (Elfiky, 2020b), is to interact with the host cells, such as the pulmonary and parabronchial epithelial cell, and assists the coronavirus to enter through the epithelial cell membrane (Boopathi *et al.*, 2020; Xia *et al.*, 2020). Moreover, the alveolar epithelial cells have ample expression of angiotensin-converting enzyme 2 (ACE2), which is an aim by the virus. The detection of ACE2 by the S protein of the virus permits the invasion of the coronavirus into the human circulation system (Belouzard *et al.*, 2009).

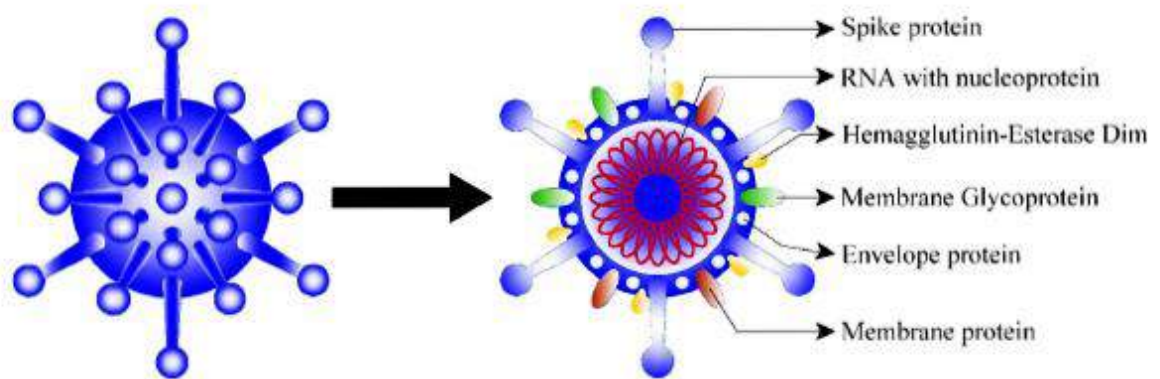
Single-strand RNA (22–26 kilobases) viruses such as the coronavirus family reproduce the virus genomes by capitalizing on host cells. For instance, after coronavirus comes near the ribosome of the epithelial cells or other host cells, it utilizes the ribosome of the host cell to replicate poly-proteins. The replication and ensuing procedures of precursor poly-proteins can arise in the epithelial cells (Hoffmann *et al.*, 2020; Wahedi *et al.*, 2020). After the coronavirus polyproteins are exhibited, two enzymes, coronavirus main proteinase (3CLpro) and the papain-like protease (PLpro) are believed to be involved in cleaving the polyproteins into smaller products used for replicating new viruses. In order to produce the daughter RNA genome, the coronavirus exhibits an RNA-dependent RNA polymerase

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(RdRp), which is an important replicase that catalyzes the synthesis of a complementary RNA strand using the virus RNA (Wrapp *et al.*, 2020).

CoVs gather together near intracellular membranes within the Endoplasmic reticulum- Golgi intermediate compartment (or ERGIC) after infection. Here they bud within the lumen and eventually carried outside the cell through ‘exocytosis’ within vesicles (Gupta *et al.*, 2020). Replication of SARS-CoV-2 depends on the viral RNA dependent RNA polymerase (RdRp) (Elfiky & Azzam, 2020) which is the most probable target of the investigational nucleotide analogue **remdesivir (RDV)** (Agostini *et al.*, 2018; Jordan *et al.*, 2018; Siegel *et al.*, 2017; Tchesnokov *et al.*, 2019).



Fig(1-1) Remdesivir in the treatment of coronavirus disease 2019 (COVID-19): a simplified summary

Symptoms

Common symptoms include fever, cough, fatigue, shortness of breath, and loss of smell and taste (Hopkins, 2020). While the majority of cases result in mild symptoms, some progress to acute respiratory distress syndrome (ARDS) possibly precipitated by cytokine storm (Ye *et al.*, 2020), multi-organ failure, septic shock, and blood clots (Bikdeli *et al.*, 2020). The time from exposure to onset of symptoms

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is typically around five days, but may range from two to fourteen days (Velavan and Meyer, 2020)

Spread

The virus is primarily spread between people during close contact, most often via small droplets produced by coughing, sneezing, and talking. The droplets usually fall to the ground or onto surfaces rather than travelling through air over long distances. However, research as of June 2020 has shown that speech-generated droplets may remain airborne for tens of minutes. Less commonly, people may become infected by touching a contaminated surface and then touching their face. It is most contagious during the first three days after the onset of symptoms, although spread is possible before symptoms appear, and from people who do not show symptoms (CDC, 2020)

Diagnosis

The standard method of diagnosis is by real-time reverse transcription polymerase chain reaction (rRT-PCR) from a nasopharyngeal swab. Chest CT imaging may also be helpful for diagnosis in individuals where there is a high suspicion of infection based on symptoms and risk factors; however, guidelines do not recommend using CT imaging for routine screening (Salehi *et al.*, 2020)

Micronutrients

As the world grapples with COVID-19 and the paucity of clinically meaningful therapies, attention has been shifted to modalities that may aid in immune system strengthening.

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Taking into consideration that the COVID-19 infection strongly affects the immune system via multiple inflammatory responses, pharmaceutical companies are working to develop targeted drugs and vaccines against SARS- CoV-2 COVID-19.

A balanced nutritional diet may play an essential role in maintaining general wellbeing by controlling chronic infectious diseases. A balanced diet including vitamin A, B, C, D, E, and K, and some micronutrients such as zinc, sodium, potassium, calcium, chloride, and phosphorus may be beneficial in various infectious diseases. A deficiency of vitamins and minerals in the plasma concentration may lead to a reduction in the good performance of the immune system, which is one of the constituents that lead to a poor immune state.

Potassium

Hypokalemia can increase ARDS and acute cardiac injury risk, which is considered the most commonly occurring complication in COVID-19. The literature demonstrated that SARS-CoV-2 binds to ACE2 and reduces its expression; consequently, angiotensin-II increases, which subsequently leads to hypokalemia. (Alwaqfi and Ibrahim 2020). COVID- 19 patients showed increased concentration of plasma angi- otensin-II, possibly responsible for acute lung injury and as confirmed in SARS-CoV animal models (Zemlin and Wiese 2020).

A pooled analysis reported that potassium concentration is significantly lower in severe COVID-19 patients than non-severe patients with substantially less heterogeneity than observed for sodium. As with low sodium, reduced plasma potassium levels may be a marker of SARS-CoV-2 infection (Lippi *et al.* 2020).

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Magnesium (Mg)

Magnesium is the forgotten cation. Mg supplementation might reveal very useful in managing the stress triggered by the pandemic and the post-traumatic stress disorder that will plague COVID-19's survivors, health professionals, and common people. It also plays a significant role in immune function by regulating various functions such as immune cell adherence, immunoglobulin synthesis, binding of Immuno- globulin M (IgM) lymphocyte, antibody-dependent cytotoxicity, and macrophage response towards lymphokines (Ni *et al.* 2020). However, some in vitro and in vivo studies suggest that magnesium plays a vital role in the immune response against viral infections (Jayawardena *et al.* 2020). In Singapore, a cohort study reported that the combination of vitamin D, magnesium, and vitamin B12 (DMB) could reduce the progression rate in older patients with COVID-19. Vitamin B12 (1000 IU) and magnesium (150 mg) have a protective effect against respiratory tract infection and reduce pro-inflammatory cytokines. (Tan *et al.* 2020).

Iron (Fe)

Biological function Iron plays a versatile role in the biological system. Despite being an oxidant, iron plays a significant role in hemoglobin and red blood cell production. Role in COVID-19 Recent evidence reveals that apart from pulmonary involvement and elevation in IL-6, COVID- 19 patients display a broader spectrum of hyper-inflammatory syndromes distinguished by cytokine release syndrome (CRS), such as secondary haemophagocytic lymphohistiocytosis (sHLH). Hyperferritinemia is the primary feature of these syndromes,

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which plays a significant role in inflammation. These findings support the theory that the acute phase of SARS-CoV-2 infection induces ferritin production associated with the rapid onset of inflammation. Hence, ferritin's immunomodulatory effects contribute to the formation of reactive oxygen species (ROS) and lead to tissue damage. With this contrast, iron chelation therapy is represented as the novel approach against COVID-19. Iron chelation therapy is the most effective approach in a wide spectrum of diseases associated with iron overload. Therefore, iron chelation therapy is considered an appropriate approach to improve survival in COVID-19 patients. A randomized, clinical trial should be considered (da Silva *et al.*,2018).

Structure, Regulation and Biological Functions of Ferritin

Ferritin is a hollow, globular protein of 480 kDa molecular weight. Classical ferritins consist of 24 subunits. Larger forms of ferritins consisting of 36 subunits are present in the heart and skeletal muscles, and microferritin with 12 subunits is produced by bacteria (Andrews *et al.*,2003; Macedo *et al.*, 2003) Mitochondria and nucleus also contain ferritin, which consists of only FTH (da Silva *et al.*,2018)

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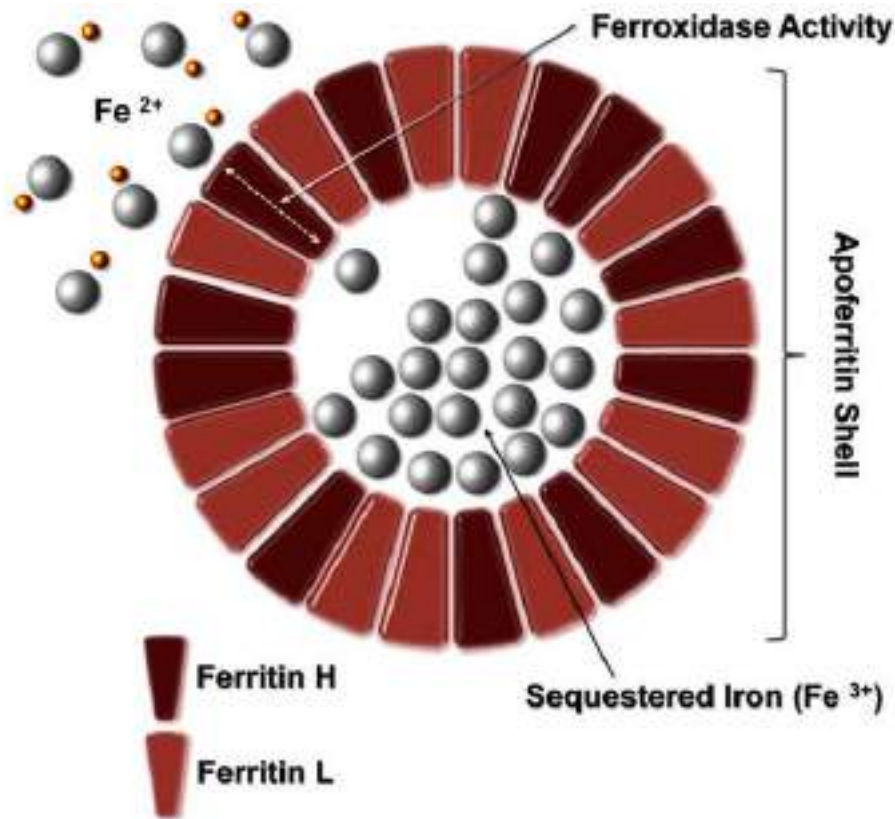


Fig. 1-2. Ferritin structure: apoferritin forms a roughly spherical container within which ferric iron is stored as a ferrihydrite mineral. Apoferritin refers to the iron-free form of the protein; the iron-containing form is termed holoferritin or simply ferritin. The apoferritin shell is composed of 24 subunits of two types, termed H and L, the ratio of which varies widely depending on tissue type and inflammation. Iron is toxic in cellular systems because of its capacity to generate reactive species (shown as yellow spheres) which can directly damage DNA and proteins.

Biological Functions of Ferritin

The main function of ferritin is to store iron (Tosha *et al.*, 2012). FTH has ferroxidase activity, which converts soluble ferrous iron into storable ferric iron. Then, ferric iron enters the cavity of ferritin and forms an iron core under the action of the nucleation site of FTL. Ferritin rich in FTH can accumulate and release iron faster than that rich in FTL, and has a more active iron transport system. Ferritin rich in FTL has more iron than that rich in FTH, which plays an important role in iron storage (Hasan *et al.*, 2008). Christophe Ferreira examined the embryonic

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lethality of mice that knocked out the FTH gene, and the results showed that FTH^{-/-} embryos died at 3.5–9.5 days of embryonic development (Ferreira *et al.* 2000). And FTH silencing disrupts the formation of S-S bonds, causing miss folding of the protein, and increase in the cellular ROS level (Zolea *et al.*,2015). However, FTL^{-/-} mice showed systemic and brain iron dyshomeostasis (Li *et al.*, 2019). This indicated that the expression of the FTH could compensate the function of the FTL to sequester iron in vivo. In addition to iron storage, ferritin functions as a protective agent preventing iron overload, decreasing oxidative stress. It has been reported that NF- κ B antagonizes the tumor necrosis factor- α (TNF- α)-induced apoptosis by upregulating the expression of FTH. The protective effect of ferritin is achieved through iron sequestration and suppressing ROS accumulation, thus inhibiting C-Jun N-terminal kinase (JNK) signaling (Antosiewicz *et al.*,2007); And ferritin is also reported as an acute phase protein, which is part of a general pattern of the systemic inflammation (Slaats *et al.*,2016 ; Moreira *et al.* 2020).

In the early stage of inflammation, pro-inflammatory cytokines such as interleukin-1 (IL-1) stimulates the expression and secretion of ferritin, which reduces the level of iron and inhibits the apoptosis induced by IL-1 (Festa *et al.* ,2015). Studies have shown that the function of ferritin is more dynamic than previous depicted. Ferritin also acts as an immuno-suppressor. Alterations of H-ferritin/T cell immunoglobulin and mucin domain-containingprotein-2 (Tim-2) binding/signaling might be involved in thepathogenesis of autoimmune diseases. Therefore, the detection of ferritin concentration is not only an important indicator to diagnose diseases with iron overload or iron deficiency, but also a marker of inflammatory conditions or autoimmune disorders (Recalcati *et al.*, 2008).

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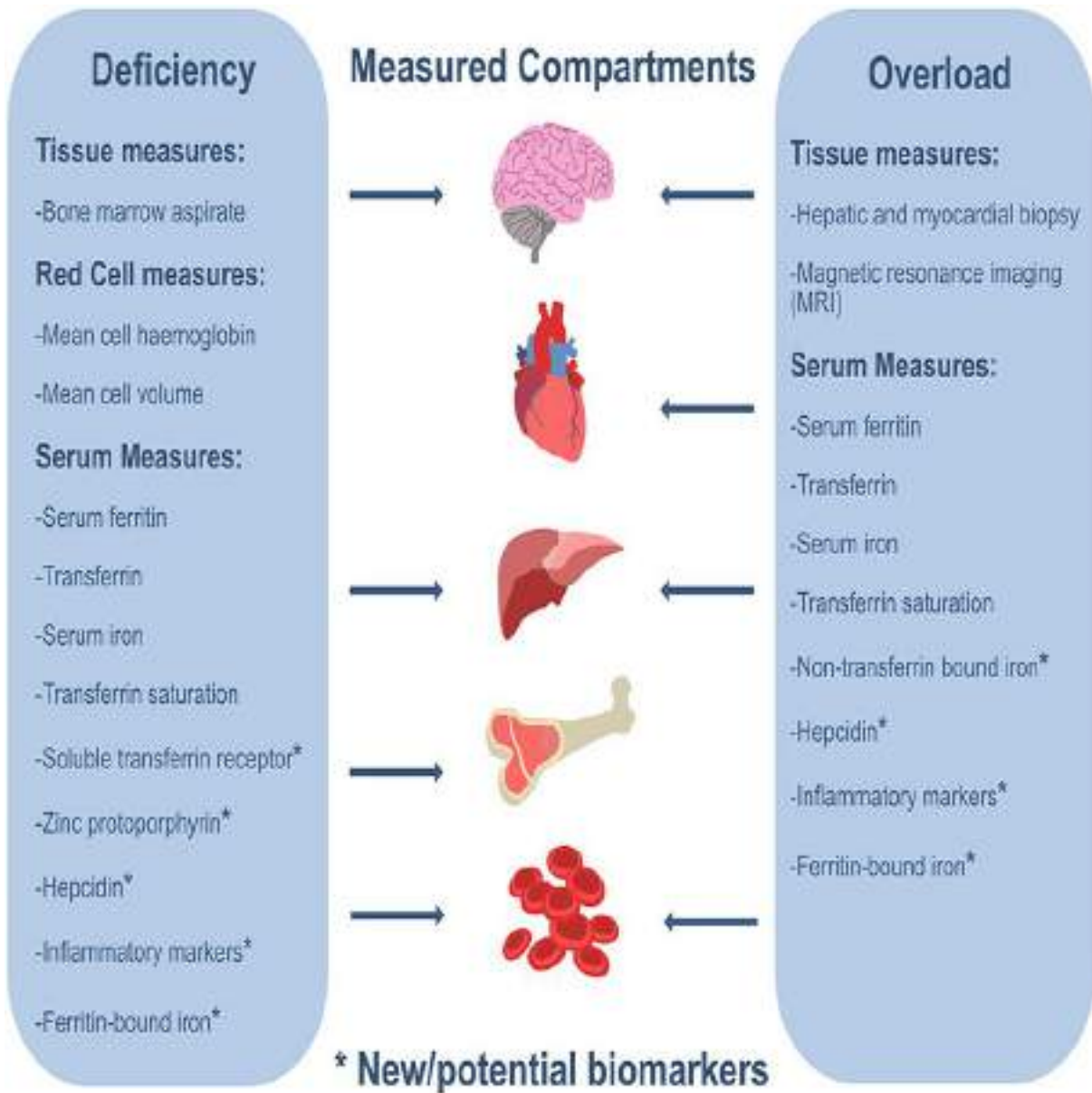
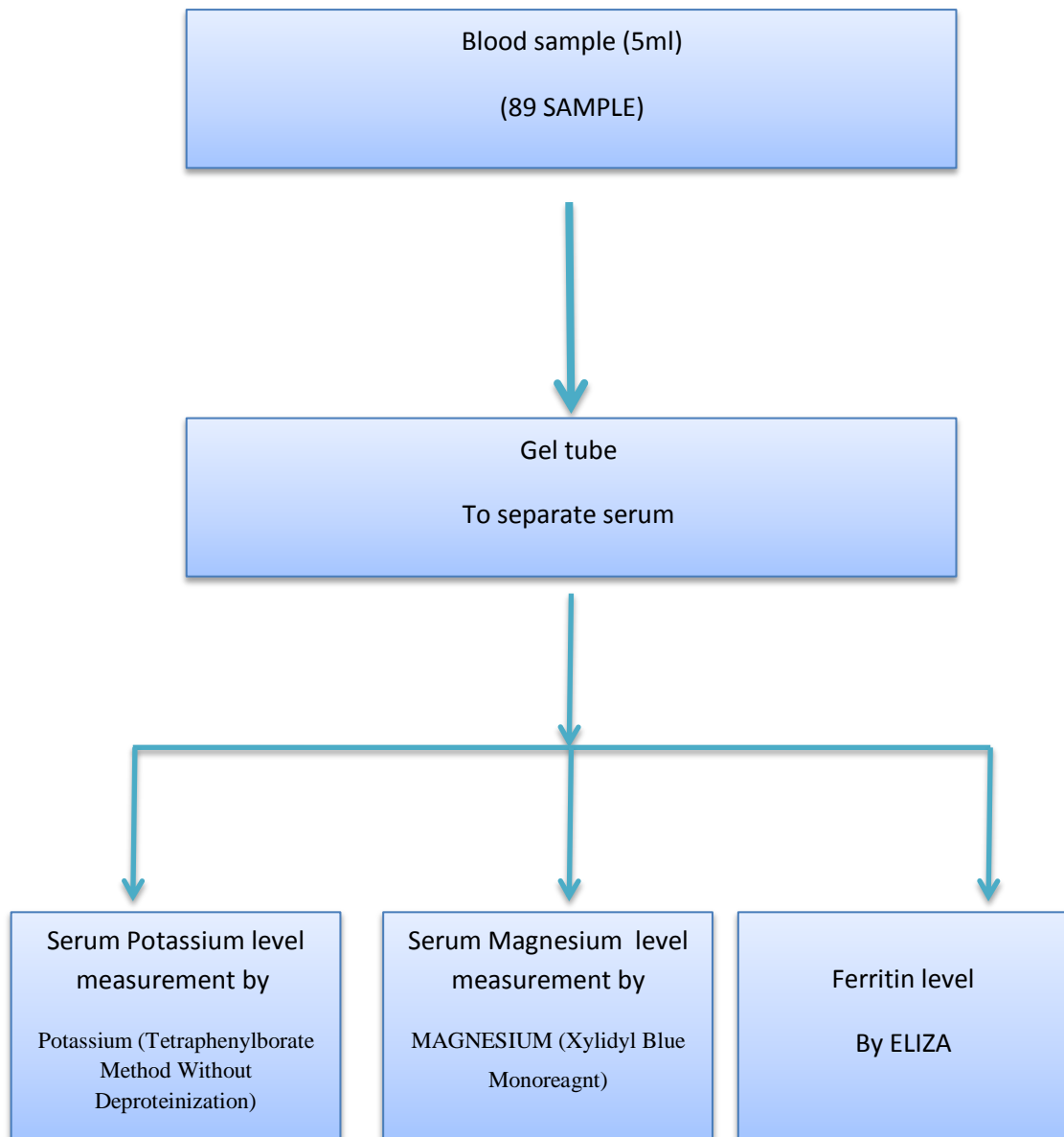


Fig (1-3) Role of ferritin in different place in human body

Chapter Three
Material and Methods

Experimental design



Chapter Three
Material and Methods

❖ Materials :-

A- Devises :-

Devises used in this study listed in table (3-1)

Table (3-1) Devises used in this study.

No.	Apparatus	Company	Country
.1	centrifuge	Hettich	Germany
.2	Different size Micropipettes μ l	Dragon	German
.3	Different size tips	Biobaseic	Canada
.4	Different size tube	Biobaseic	Canada
.5	ELISA reader & washer	Biotek_USA	USA
.6	Gel tube		
.7	Spectrophotometer		
.8	Syringe	Meheco	China

B- Kit

Kits used in this study listed in table (3-2)

Table (3-2) Kits used in this study.

No.	KIT	CATALOG NO.	COMPANY
1.	Potassium (Tetraphenylborate Method Without	298 002	spectrum

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Material and Methods

	Deproteinization)		
2.	MAGNESIUM (Xylidyl Blue Monoreagnt)	285 002	spectrum
3.	Human Ferritin Heavy Chain, FTH1 ELISA Kit	E6766Hu	BT LAB

❖ Methods

1- Samples Collection:-

Blood samples (5ml) by sterile medical syringe were collected from people infected with Corona virus, their ages ranged between 4-64 years and of both sexes. All volunteers were subjected to a number of questions as indicated in the information form in the appendix. The samples were kept in special gel tubes to separate the blood serum. The tubes were left at room temperature for half an hour, after which they were centrifuged with a centrifuge device to separate the blood serum and stored at a temperature -20°C .

2- Potassium (Tetraphenylborate Method Without Deproteinization)

In order to measure the potassium concentration in the blood serum, the following steps were taken:

- a- Dissolve the serum in a water bath at 30°C .
- b- The reaction mixture was prepared according to the table (3-3)

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Table (3-3) Reaction mixture for serum Potassium concentration

	Reagent Blank	Standard	Sample
Reagent R	1mL	1mL	1mL
Standard	20µl
Sample	20µl

- c- The mixtures were mixed, and incubated for 3 minutes at 37 °C.
- d- Mixed again thoroughly and read absorbance of sample (A_{sample}) and standard (A_{standard}) against blank on 578nm.
- e- The following equation was used to find the potassium concentration in the blood serum (samples)

$$\text{Sreum Potassium Conc. (mmol/L)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 5$$

3- Magnesium (Xylidyl Blue Monoreagnt) :-

The following steps were taken to measure the concentration of magnesium in the blood serum:

- a- Dissolve the serum in a water bath at 30°C.
- b- The reaction mixture was prepared according to table (3-4)

Table (3-3) Reaction mixture for serum Potassium concentration

	Blank	Standard	Sample
Reagent R	1mL	1mL	1mL
Standard	10µl
Sample	10µl

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Material and Methods

- c- All mixtures were mixed well and let to stand for 10 minutes at room temperature, then absorbance (at 546nm) of sample and standard read against reagent blank.
- d- The following equation was used to find the magnesium concentration in the blood serum (samples)

$$\text{serum Magnesium Conc. (mg/dl)} = \frac{A \text{ sample}}{A \text{ standard}} \times 2.5$$

4- Human Ferritin Heavy Chain, FTH1 ELISA Kit

- a- Reagents, standard solutions and samples were prepared according to the following steps.
 - i. All reagents were thawed and brought to room temperature before use
 - ii. Standard was Reconstitute the 120ul of the standard (320ng/ml) with 120ul of standard diluent to generate a 160ng/ml standard stock solution. Allow the standard to sit for 15 min. with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (160ng/ml) 1:2 with standard diluent to produce 80ng/ml, 40ng/ml, 20ng/ml and 10ng/ml solutions. Standard diluent serves as the zero standards (0ng/ml). Dilution of standard solutions figure (3-1)

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Material and Methods

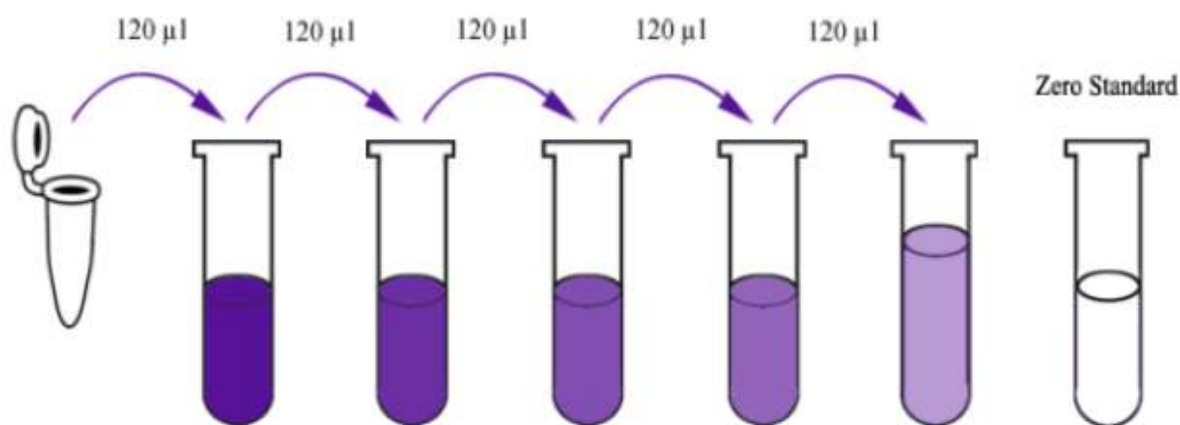


Figure (3-1) Dilution of standard solutions

iii. **Wash Buffer** Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

iv. **Samples preparation**

- Samples were within 5 days (stored at 2-8°C).
- Samples were brought to room temperature before starting the assay.
- Centrifuge to collect sample before use.
- The supernatants were collected carefully.

Before using any reagents, bring them to room temperature. Assays are completed in a room-temperature environment.

b- A 50ul of standard was added to standard well.

c- A 40ul of sample was added to sample wells and then 10ul of Human FTH1 antibody added to sample wells, then 50ul streptavidin-HRP added to sample

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Material and Methods

- wells and standard wells. The Mixtures mixed well, the plate covered with a sealer and incubate for 60 minutes at 37°C.
- d- the sealer Remove and the plate washed 5 times with wash buffer. wells Soaked with 300ul wash buffer for 30 seconds to 1 minute for each wash. the plate dried onto paper towels or other absorbent material.
 - e- A 50ul of substrate solution A added to each well and then 50ul of substrate solution B added to each well. plate covered with a new sealer and Incubated for 10 minutes at 37°C in the dark.
 - f- A 50ul of Stop Solution added to each well, the blue color would change into yellow immediately fig1.

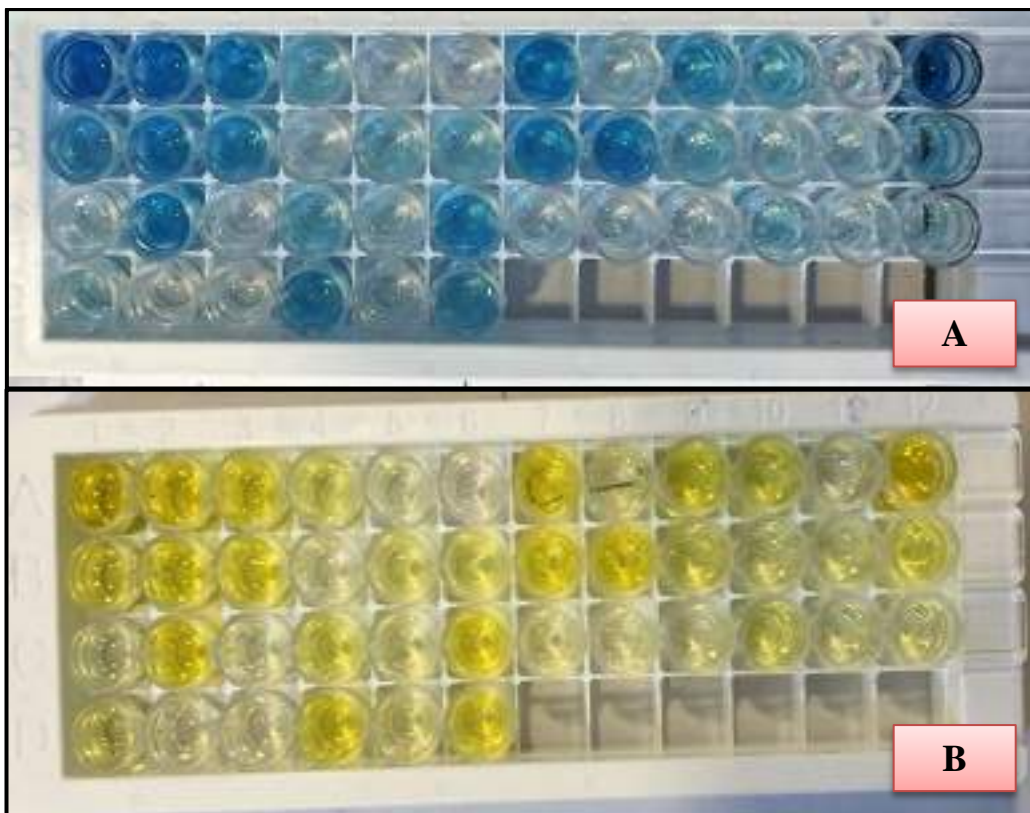


Figure (3-2): A-Before adding stop solution .B- after adding the stop solution

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g- The optical density (OD value) of each well Determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.



Figure (3-3): Human Ferritin Heavy Chain, FTH1 ELISA Kit

1- Patients with COV-19:-

An 89 blood serum samples were taken from people infected with Corona virus from both women and men, for the purpose of studying the concentration of the number of elements (potassium, magnesium and ferritin) that are expected to be affected as a result of infection with the virus.

The number of women was 35(20%) and the number of men was 54 (30%), age range was (4-64 years) as in Figure 1

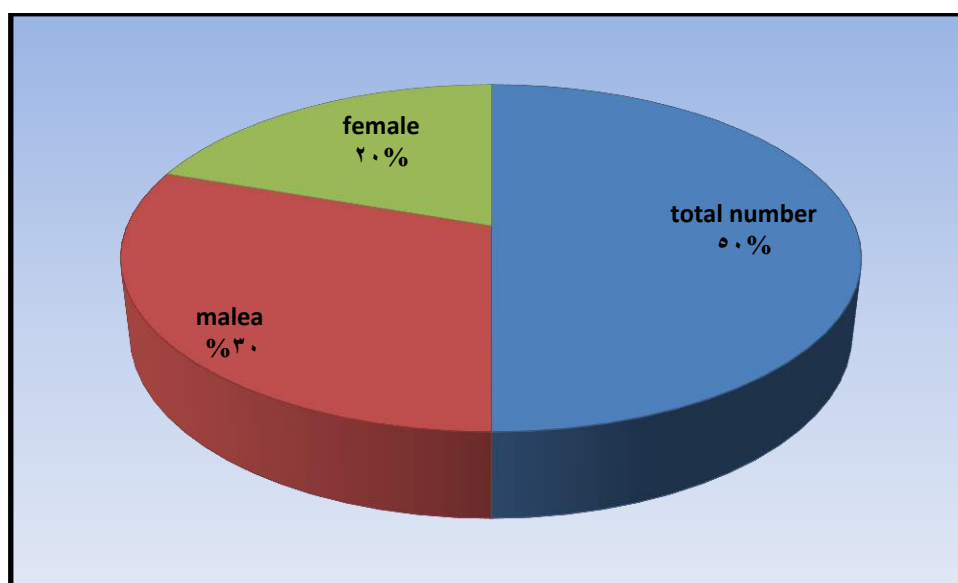


Figure (1):- the percentage of male and female to total number

The subjects included in this study were divided into the following groups according to age as shown in Table (1). A questionnaire (included age, duration, number of infection with COV-19, treatment and other disease) was designed to obtain the information of patient's subjects (depending on AUA score). The laboratory work was carried out in the research laboratory in the Department of Biology, College of Science, University of Babylon.

Table (1): Age of patients with Corona virus-19

Age	No. of cases
1-----15	8
16-----30	24
31-----45	28
46 ----- 60	13
61 ----- 75	17

2- Potassium serum concentration:-

Among the 89 samples studied in this research, it was found that 24% had a decrease in potassium concentration in the blood serum, 11% had an increase in potassium levels, and the remaining 65% had normal potassium concentration , Fig. 2. When comparing the potassium concentration between male and female, it was noted that the increase in potassium was more in male than in female. As for the low potassium concentration, it was more in female than in male than in male Fig.3.

Potassium deficiency is common among Covid-19 patients because this element is excreted in large quantities in the urine, so most patients suffer from potassium deficiency.

Chen *et al.*, 2020, came to the conclusion that people with COVID-19 tend to have hypokalemia. Hypokalemia is hard to fix because the kidneys lose K+ all the time because ACE2 (which is a SARS-CoV-2 receptor) is broken down. The powerful vasoconstrictor angiotensin II is turned into angiotensin by the enzyme Angiotensin-Converting Enzyme 2. Because of this action, ACE2 is involved in

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the renin-angiotensin system. The end of urine K^+ loss is a good sign for the patient's prognosis and may be a reliable, in-time, and sensitive biomarker that shows the end of the bad effect on the RAS system.

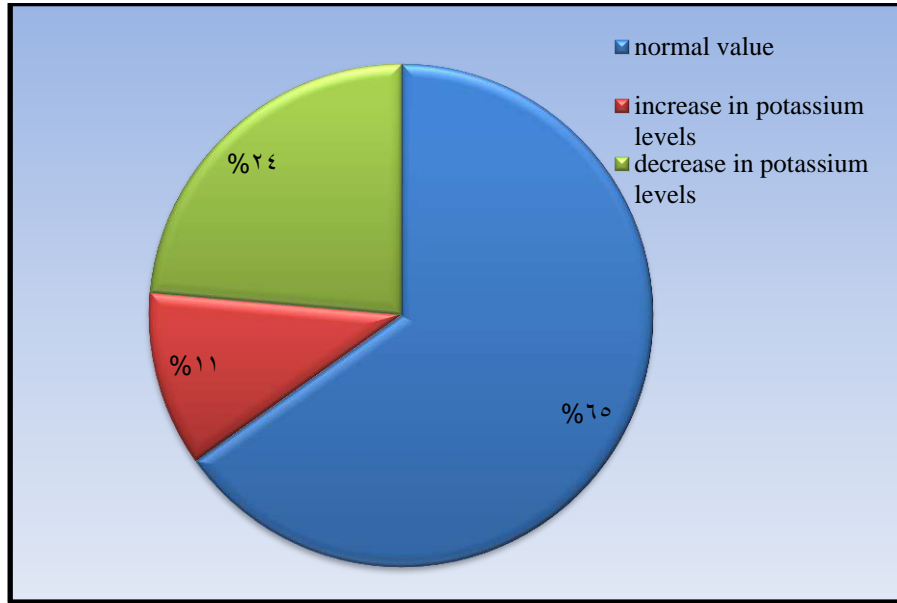


Figure (2):- the concentration of potassium in patient with Covid-19.

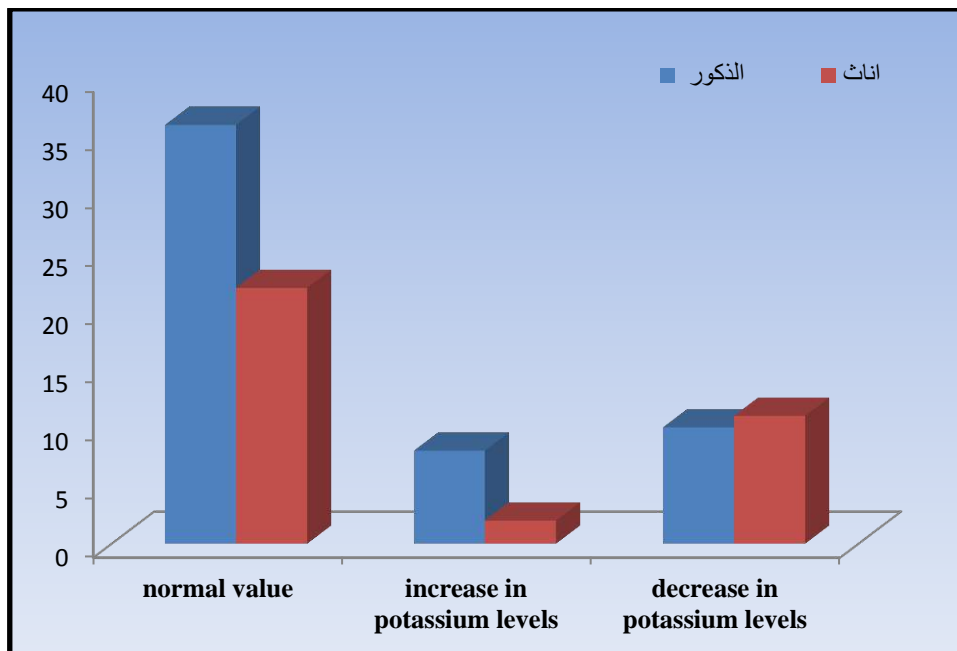


Figure (3):- Compared the concentration of potassium in male and female

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Hypokalemia happened more often than usual in COVID-19 patients who were very sick. A recent Chinese study found that 93 percent of COVID-19 patients had low potassium levels (Chen et al,2020). When ACE2 is turned down in people with COVID-19, it causes a very bad imbalance in the renin-angiotensin system. As biomarkers of hypokalemia, people who were out of balance lost less potassium in their urine and responded more to intravenous potassium (Silhol, et al, 2020).

When people with severe hypokalemia were given 3 g of potassium per day (an average of 34 g over the course of a hospital stay), they got better. Hypokalemia, which is when the potassium level in the blood is below 3.5 mEq/L, can cause problems with fluid and electrolytes in the clinic. 50% of people with clinically confirmed low magnesium levels also had low magnesium levels (Whang et al., 1992). However, the serum level used to define deficiency varies from 0.6 to 0.75 mmol/L, with some being more cautious than others (Huang and Kuo ,2007 ; and Veltri and Mason,2015). Hypomagnesemia can aggravate hypokalemia and induce renal potassium loss. When you don't get enough magnesium, the sodium-potassium ATPase pump doesn't work as well. This lowers the amount of potassium inside the cell. When there isn't enough magnesium in the body, distal potassium release through ROMK channels makes more potassium waste. In magnesium deficiency, increased salt transport to the extremities and aldosterone levels may make potassium loss worse. However, this is not the only thing that can cause hypokalemia (Huang and Kuo ,2007).

Certain medications (e.g., antibiotics, beta 2-receptor antagonists, diuretics, insulin, glucocorticoids, laxatives, etc) may also cause hypokalemia through a variety of mechanisms including intracellular potassium shifting, increased renal loss, and decreased intestinal uptake. Deficiency in magnesium in critically ill patients can not only lead to secondary hypokalemia but also hypocalcemia,

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accompanied by severe neuromuscular and cardiovascular clinical manifestations. Concurrent magnesium deficiency aggravates hypokalemia and renders it refractory to potassium treatment (Veltri and Mason,2015). If the patient is hypomagnesemic or has borderline low-normal serum concentrations, treating hypomagnesemia concomitantly with the hypokalemia is appropriate to prevent serum potassium levels from falling below the normal range after discontinuation of potassium therapy (Wallace,2020).

Magnesium serum concentration:-

This study showed that a number of patients infected with the Corona virus suffer from low levels of magnesium in the blood, and their number was 18 people, including 10 women and two men, while the number of patients suffering from high magnesium in blood serum was 18 infected, including 6 women and 12 men Fig(4).

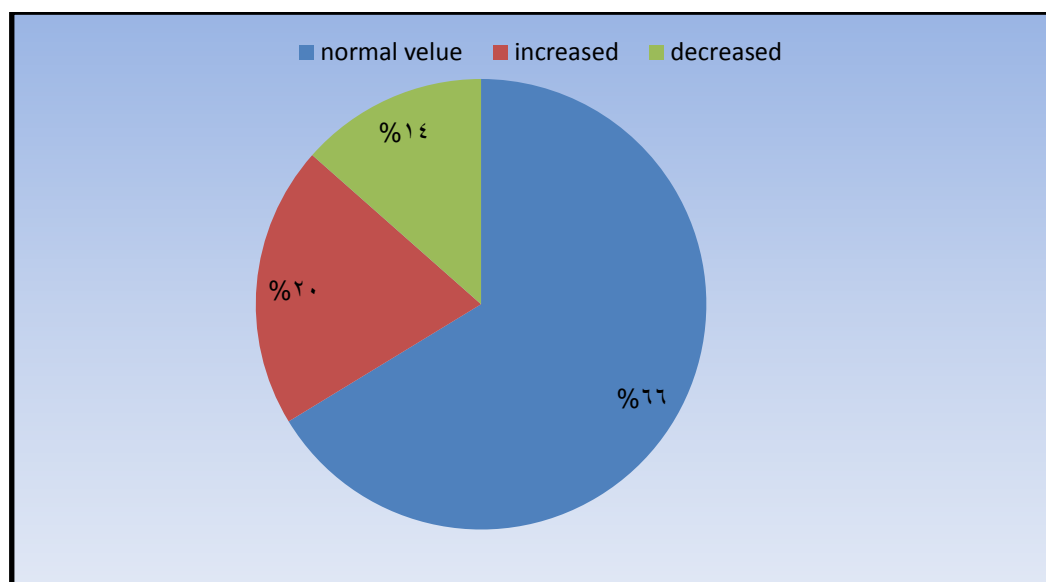


Figure (4):- the concentration of magnesium in patient with COV-19

Among the many essential nutrients, magnesium is the second most abundant intracellular cation after potassium, and involved in >600 enzymatic reactions in

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the body, including those contributing to the exaggerated immune and inflammatory responses exhibited by COVID-19 patients (Wallace,2020).

Magnesium deficiency makes phagocytes more active, raises granulocyte oxidative stress, activates endothelial cells, and raises cytokine levels, all of which lead to inflammation (Wallace *et al.*,2020). Several parts of the COVID-19 are similar to the metabolic changes that have been shown to happen during latent subclinical magnesium deficiency. These changes include a drop in T cells, an increase in the amount of inflammatory cytokines in the blood, and problems with the endothelium. Our data showed that severe COVID-19 patients had significantly lower magnesium levels than non-severe COVID-19 patients. This is in line with the idea by Iotti *et al.*,2021 that a low Mg status might contribute to the change from mild to severe COVID-19 symptoms. It was thought that keeping an eye on the amount of magnesium in the body could be a way to slow or stop the spread of disease (Hao-Long *et al.*, 2021).

Ferritin:-

When measuring the level of ferritin in the blood serum, it was noted that the percentage of people suffering from a decrease in this protein was 2%, while 3% had a high level of ferritin in the blood serum, and 94% had a normal level of ferritin. Figure 5

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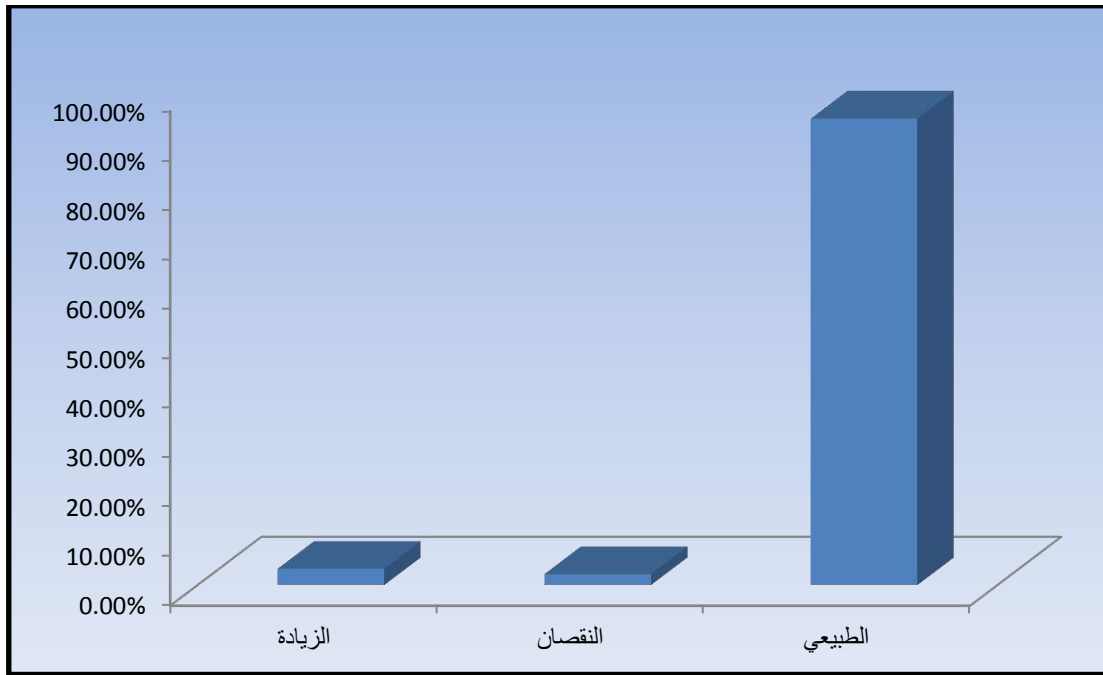


Figure (5):- the concentration of ferritin protein in patient

Most of the research indicates an increase in ferritin concentration in the blood serum when infected with the Corona virus.

Following the global spread of the COVID-19 pandemic and early throughout the pandemic, elevated levels of ferritin in patients with COVID-19 brought the attention of medical staff treating huge numbers of patients (Wang *et al* 2020) as well as researchers by predicting poor outcomes (Huang *et al.*, 2020). The possible correlation between ferritin and worse prognosis however, barely scratched the surface of the hidden elements beneath it which necessitated further investigation. Subsequently, ferritin concentration progressed from a prognostic point of view, to serve as a severity risk factor. More importantly, the dramatic increase in ferritin concentration, alongside lymphopenia, reduced NK cell number activity, abnormal liver function tests, and coagulopathy, had researchers speculating, and later agreeing that COVID-19 might be the newest member in the group of hyperferritinemic syndromes (Colafrancesco *et al.*,2020 and Naim *et al.*, 2022)

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Patients with severe to critical disease had higher ferritin concentration than those with mild to moderate disease, and non-survivors had higher serum ferritin concentration than survivors. Patients requiring ICU and mechanical ventilation had higher serum ferritin concentration than those who didn't require ICU and didn't require mechanical ventilation, according to Kaushal *et al*,2022. COVID-19 patients with renal involvement had a higher concentration of ferritin as well; however, there was heterogeneous data about the relationship between ferritin concentration and COVID-19-associated liver damage. A decreased blood ferritin concentration was seen in patients who did not have COVID-19-related thrombotic events. Serum ferritin is an important indicator of severity in COVID-19 (Kaushal *et al*,2022).

Conclusions and Recommendations

Conclusion:-

- 1- The number of people infected with the virus with low potassium level was more than patients with high potassium concentration.
- 2- More women than men suffer from low levels of magnesium.
- 3- Within the population studied, it was found that patients infected with coronavirus who had an elevated ferritin protein concentration were slightly more than those with a deficiency of this protein.

Recommendations:-

Studying the effect of different vaccines for the Corona virus on minerals and micronutrients in the human body.

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استمارة معلومات

رقم العينة:-

تأريخ جمع العينة:-

الملاحظات	الاسم
	العمر
	الجنس
	مدخن
	يتناول الكحول
	هل يعاني من ارتفاع ضغط الدم
	هل يعاني من مرض السكري
	هل يعاني من امراض اخرى (تذكر)
	عدد المرات التي اصيب بها الشخص بـ COVID

الخلاصة

تم اكتشاف فيروس كورونا 2 (SARS-CoV-2) ، الفيروس التاجي البشري السابع ، في ووهان بمقاطعة هوبي بالصين ، خلال وباء الالتهاب الرئوي الأخير في يناير 2020. ومنذ ذلك الحين انتشر الفيروس في جميع أنحاء العالم اعتبارًا من 12 أبريل 2022 ، كان هناك 492,960,497 حالة مؤكدة من COVID-19 ، بما في ذلك 850,6181 حالة وفاة وتم إبلاغ منظمة الصحة العالمية بها. يتسبب فيروس SARS-CoV-2 وكذلك فيروس SARS-CoV ومتلازمة الشرق الأوسط التنفسية (MERS-CoV) في حدوث التهاب رئوي حاد بمعدل وفيات يبلغ 2.9% و 9.6% و 36% على التوالي.

هدفت هذه الدراسة لتحديد تركيز بعض العناصر المعدنية (البوتاسيوم والمغنيسيوم والفيريتين) في الجسم ودراسة وجود رابط بينها وبين الإصابة بالفايروس.

امتدت الدراسة الحالية للفترة بين 2021/12/1 الى 2022/3/1 حيث تم خلال هذه الفترة جمع ٨٩ عينة مصل الدم من الاشخاص المصابين بفايروس كورونا للاعمار من ٤ سنوات الى ٦٤ سنة ولكلا الجنسين.

وجد أن 24% لديهم انخفاض في تركيز البوتاسيوم في مصل الدم ، 11% لديهم زيادة في مستويات البوتاسيوم ، و 65% المتبقية لديهم مستويات طبيعية من البوتاسيوم. أظهرت هذه الدراسة أن عددا من المرضى المصابين بفايروس كورونا يعانون من انخفاض مستويات المغنيسيوم في الدم ، وكان عددهم 18 شخصا بينهم 10 نساء ورجلين ، فيما كان عدد المرضى الذين يعانون من ارتفاع المغنيسيوم في مصل الدم 18 مصابا بينهم 6 نساء و 12 رجلا. كما تم قياس مستوى الفيريتين لدى الاشخاص المصابين حيث لوحظ أن نسبة الأشخاص الذين يعانون من نقص هذا البروتين كانت 2% ، بينما 3% لديهم مستوى عالٍ من الفيريتين في مصل الدم ، و 94% لديهم مستوى طبيعي من الفيريتين.



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جامعة بابل

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قسم علوم الحياة

دراسة مستويات كل من المغنيسيوم والبوتاسيوم والفرتين في مصل الدم

لاشخاص مصابين بكوفيد-19

بحث مقدم الى قسم علوم الحياة كجزء من متطلبات نيل شهادة البكلوريوس تخصص علوم الحياة
بواسطة

علا ضياء حسين هرير

باشراف

م. رفل أحمد ليلو