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Role of Vascular Adhesion Protein-1, E-Selectin and Omentin-1 in Endothelial Dysfunction and study of Nano particles as lipid-lowering agent among Children with Nephrotic Syndrome

A thesis

Submitted to the Council of the College of Medicine, University of Kerbala, in Partial Fulfillment of the Requirements for the Degree of Master in clinical chemistry and Biochemistry By

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We, the examiners committee, certify that we have read this thesis entitled " **Role of Vascular Adhesion Protein-1, E-Selectin and Omentin-1 in Endothelial Dysfunction and Study of Nanoparticles as lipid-lowering agent among Children with Nephrotic Syndrome** " and have examined the student (***Ahmed Dawood Salman***) in its contents. In our opinion, it is accepted as a thesis for the degree of master in (***Clinical Chemistry***) with appreciated (***Excellent***).

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Summary

Nephrotic syndrome is a kidney disorder that causes the body to excrete too much protein in the urine. The most common causes are genetic factors. Other causes can include diseases that affect other parts of the body, infections and certain medications. The symptoms include too much protein in the urine, and low levels of albumin in the blood (hypoalbuminemia) swelling of the body (edema).

Nephrotic syndrome in children causes many complications in many organs of the body and in some cases may cause death. This study was aimed to find chemical parameters that can predict endothelial dysfunction in children with nephrotic syndrome due to high levels of lipids as well as other factors which included Estimation the level E-selectin, Omentin-1 and Vascular adhesion protein-1 (VAP-1). Also Investigated the diagnostic preferences of E-selectin, Omentin-1 and Vascular adhesion protein-1 as a biochemical parameter in nephrotic syndrome using ROC analysis. Furthermore, Prepare and study the structural features of chitosan/iron oxide nanoparticles CS-FeO NPs and Examine the Applications of such nanoparticles as a lipid-lowering agent in children with nephrotic syndrome

Methods: This study was designed as a case-control study, A total of 52 participants with nephrotic syndrome which were collected form Kerbala children teaching hospital, Kerbala health directorate/ Iraq. Serum lipid panel (total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein (LDL) and triglycerides (TG), renal function test (blood urea and creatinine) and albumin in serum and urine were measured using system (Cobas e 411, Roche Diagnostic, Germany), Elisa system was used for the detection of E-selectin, Omentin-1 and vascular adhesion protien-1 level. On other hand In the laboratories of the Faculty of Medicine/ Karbala University, the nanocomposite was prepared from chitosan

and iron oxide. The characterization of Nanoparticle measured in the Ministry of Science and Technology Environment and Water Research and Technology department using scanning electron microscopy (SEM), Fourier transforms infrared spectroscopy (FTIR), UV–Vis spectroscopy, and X-ray diffraction (XRD).

The results of the study showed an increasing in the levels of serum cholesterol, triglyceride, LDL, urine albumin, E-selectin, and VAP-1 with highly significant differences. Results were also shown a decreased in the level of serum albumin and Omentine-1 significantly in patients compared to the control group. The performance of chitosan-iron oxide nanocomposites for the lowering of cholesterol was performed. It was found that **CS-Fe_3O_4 NPs** have an ability to lower lipid content indeed reduced the cholesterol accumulation in the pool samples by ~ 85%, reduced the TG level in the pool samples by 84.5% and reduced the LDL level in the pool samples by 81% .

In conclusion, the proposed biomarkers and dyslipidemia were linked successfully in the diagnosis of endothelial dysfunction in children with nephrotic syndrome. Magnetic Fe_3O_4–chitosan nanoparticles were prepared successfully by the covalent binding of CTS on the Fe_3O_4 nanoparticles. These microspheres were applied to the magnetic-field-assisted lipid lowering processes.

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List of Abbreviation:

Abbreviation	Meaning
NS	Nephrotic syndrome
FTIR	Fourier transform infrared
EDX	Energy Dispersive X Ray
APOE	Apo Lipoprotein E
AFM	Atomic Force Microscopy
SD	Stander Deviation
SEM	Scanning Electron Microscopy
T C	Total Cholesterol
TEM	Transmission Electron Microscopy
TG	Triglyceride
UV	Ultraviolet–visible spectroscopy
WHO	World Health Organization
XRD	X-Ray Powder Diffraction
ELISA	Enzyme-Linked Immunosorbent Assay
HDL-C	High-Density Lipoprotein Cholesterol
LDL-C	Low-Density Lipoprotein Cholesterol
NPs	Nanoparticles
CS	Chitosan

Chapter one

Introduction

And

Literature review

1. Introduction

The nephrotic syndrome (NS) is a kidney disease that increase the permeability of the glomerular membrane. It presents with: nephrotic range proteinuria of urine protein creatinine ratio ≥2g/g, hypoalbuminemia defined as serum albumin levels less than 30 g/L, edema and hyperlipidemia.

Untreated NS can result in anasarca with pleural effusion, ascites, and acute kidney injury. Untreated patients are also at high risk for sepsis, thrombosis, hyperlipidemia and endocrine abnormalities **(Chia-shi Wang, *et* . *al*. 2019)**.

Nephrotic syndrome has a yearly incidence of two to seven new cases and a prevalence of sixteen new cases for every one hundred thousand children. The yearly incidence of nephrotic syndrome in adults is three new cases for every one hundred thousand adults **(Seth Anthony Politano, *et. al*. 2020)**.

1.1. classification of Nephrotic syndrome

- Primary glomerular disease /Idiopathic – causes include
- Minimal change disease
- Membranoproliferative glomerulonephritis
- Focal segmental glomerulosclerosis
- Congenital Nephrotic Syndrome
- Secondary disease/ Non idiopathic
- Henoch-Schönlein purpura (HSP)
- Systemic lupus erythematosus (SLE)

Majority of the cases of nephrotic syndrome are idiopathic of which approximately 80-90% are due to minimal change disease **(Jeffrey B. Hodgin *et. al*. 2022)**.

Approximately 80% of the cases of MCD would response to steroid therapy but 75-85 would develop a relapse and 50% will go on to have frequent relapses The typical age group affected is 1 – 10 years with 2 years the most common age at presentation.

Table (1-1) an inventory of the many factors that might bring on nephrotic syndrome, arranged in accordance with their aetiologias. (Tomo, *et . al*. 2022)

Genetic	Infectious causes	Idiopathic	Others
• Diffuse mesangial sclerosis (DMS) • Epidermolysis bullosa coupled with epidermolysis bullosa • Familial focal segmental glomerulosclerosis (FSGS) • Steroid-resistant nephrotic syndrome	• Cytomegalovirus • HIV-related nephropathy • Congenital diseases such as syphilis, toxoplasmosis, and HIV •Nephropathy associated with HIV	• Membranous glomerulonephritis • Focal segmental glomerulosclerosis • Diffuse mesangial hyper cellularity • Minimal change nephropathy • Membranous glomerulonephritis • Membranoproliferative GN	• Hemolytic uremic syndrome (HUS) • Nephropathy caused by lupus; • Nephropathy caused by immunoglobulin A • Drugs • Cancers

1.2. Pathogenesis

Increased macromolecule filtration across the glomerular capillary wall causes proteinuria in nephrotic syndrome. Fenestrated endothelial cells, the glomerular basement membrane (GBM), and podocytes make up the glomerular filtrati1on barrier. A lamina densa, an electron-dense layer in the middle, an electron-lucent lamina rara interna on the endothelial cell side, and an electron-lucent lamina rara externa on the podocyte side make up the GBM's three-layer structure. **(Ebefors Kerstin, *et . al*. 2021)**

Components of the GBM include heparan sulphate proteoglycans such agrin and type IV collagen, laminin, nidogen, and nidogen. **(Miner,2011)** Foot processes bring lamina rara externa into touch with podocytes, which are highly specialised epithelial cells. **(Nagata, M., 2016)** The slit diaphragm is a membrane with a zipper-like design that maintains the 30-40 nm spacing between the foot processes of neighbouring podocytes. Normally, macromolecules have a hard time passing through the filtration barrier formed by the glomerular capillary wall.**(Hulkko Jenny.2015)**

Polyanions like heparan sulphate proteoglycans provide the endothelial cells and the GBM a net negative charge. This results in a charge barrier that prevents big anions like albumin from being filtered out. The loss of anionic charge detected by light microscopy in minimal change illness, the most prevalent cause of nephrotic syndrome in children, is due to one of two processes that do not involve any structural damage or alteration to the glomerular filtration unit. size and charge selectivity. **(David W. Smith, *et . al*.2022)**

The nephrotic syndrome and damage to the glomeruli may be brought on by a variety of different factors. Proteinuria is brought on by abnormalities in podocyte

function, which are brought on by circulating chemicals in minimal change disease and idiopathic focal segmental glomerulosclerosis (FSGS). These two diseases are responsible for proteinuria. **(Davin, J.C., 2016)** Because of these circulating factors, persons with end-stage renal disease often experience a rapid recurrence of proteinuria shortly after receiving a kidney transplant. Immunological mechanisms might be responsible for the structural alterations in the GBM that are seen in patients with membranous nephropathy. **(Ponticelli, and Glassock., 2014)**Third, structural or functional abnormalities of podocytes may be caused by mutations in podocyte or slit diaphragm proteins. These mutations can occur in hereditary forms of congenital syndrome, in thirty percent of people with steroid-resistant idiopathic nephrotic syndrome, and in some syndromic forms of nephrotic syndrome. **(Gbadegesin, *et . al*.2011)**

1.3. Complications associated with the nephrotic syndrome

A. Infections

Patients diagnosed with Nephrotic syndrome have an infection risk that is much greater than that of the general population as a whole. Although the incidence of new Nephrotic syndrome infections has decreased in countries with higher levels of affluence, the illness continues to be a serious problem in places with lower levels of wealth. **(Gbadegesin, *et . al*.2023)**.

Nephrotic patients are also more likely to have impaired T-cell function. Nephrotic syndrome has the potential to lead to renal failure if it is not addressed **(Kliegman,*et.al*.2007).**

B. Thromboembolism

Those who had severe proteinuria were shown to have a 3.4 times greater chance of developing venous thromboembolism (TE), in comparison to those who did not have nephrotic syndrome. It is also known that there is higher risk of TE in steroid-resistant NS than in steroid-sensitive NS, Proteins that are involved in the suppression of systemic hemostasis may be lost in NS, which may lead to an increase in the synthesis of prothrombotic factors or a local activation of the glomerular hemostasis system, both of which may lead to thrombosis **(Menon S, 2019)**.

alterations in the glomerular hemostatic system, decreased intravascular volume, as well as exposure to CS and diuretics all have a role. **(Ruas, *et* . *al*.,2022)** due to the increased likelihood of thrombosis in the affected artery, nephrotic youngsters should not have their arteries punctured. Doppler ultrasonography or magnetic resonance angiography may be used to identify renal vein thrombosis in children who have nephrotic syndrome if they have gross hematuria. This condition can be identified **(Witz and Korzets., 2017).**

C. Hypovolemic crisis

Nephrotic syndrome often presents with hypovolemic shock **(Menon S, 2019)**. Severely low albumin levels, high dosage diuretics, and vomiting all increase the likelihood of a hypovolemic crisis. Laboratory testing may demonstrate high hematocrit and uric acid levels in addition to the classic symptoms of tachycardia, chilly extremities, poor capillary refill, and moderate to severe abdominal discomfort. To assess fluid balance, the urine sodium excretion rate (UNa) or the fractional excretion of sodium (FENa) may be measured **(Seth Anthony Politano, *et. al*.,2020).** compared to other metrics evaluating renal potassium and salt processing, the ratio of urine potassium to urinary potassium plus urinary sodium

(UK / UNa + UK) was shown to correlate most strongly with log aldosterone. A UK / UNa + UK ratio greater than 0.6 (UK / UNa + UK: >60%) identifies individuals with elevated aldosterone levels and functional hypovolemia in those with renal sodium retention (FENa: 0.5%) **(Matsumoto, *et. al.*,2011).**

D. Anemia

Patients diagnosed with nephrotic syndrome may on occasion be found to have symptoms of mild anemia. Anemia is often characterized by its microcytic and hypochromic characteristics, which are indicative of iron deficiency. Despite this, anemia is resistant to treatment with iron due to the significant loss of serum transferrin that occurs in the urine of certain nephrotic patients **(Audrey D. Kamzan, *et . al*., 2020)**.

EPO-deficiency anemia and transferrinuria are both induced by the loss of EPO in the urine. Hypotransferrinemia and iron-deficiency anemia may be caused by accelerated transferrin catabolism. Subcutaneous injections of recombinant erythropoietin or iron supplements may be used to treat anemias that are caused by a deficiency in either erythropoietin (EPO) or iron, respectively **(Eugene Khandros, *et. al*., 2022)**. However, the best method of correcting these issues will be to treat the underlying proteinuria.

E. Acute renal failure

Rare yet serious, acute renal failure (ARF) is a result of nephrotic syndrome. **(Menon S, 2019)** When albumin levels drop drastically due to severe proteinuria, the plasma's circulation volume drops, leading to circulatory collapse or pre-renal uremia. However, in rare cases, NS without the hallmarks of volume depletion might present with ARF that does not respond to volume replacement and severe diuretic treatment. Possible causes include a drastic drop in filtration surface area and the

near-total eradication of slit pores in visceral epithelial cells caused by a cellular disruption. **(Prasad, *et . al*.,2019)**

F. Edema

Children with nephrotic syndrome often exhibit edema and areas of low tissue pressure. Pericardial effusions are unusual unless heart function is impaired, although ascites and pleural effusions are common. Reduced plasma oncotic pressure and functional hypovolemia come from edema's underlying causes: increased glomerular permeability and hypoalbuminemia. These cause the kidney to retain salt as a secondary effect. **(Jun-Liang Chen**, ***et . al*., 2019)**

Dietary salt restriction and the cautious use of loop-acting diuretics like furosemide and bumetanide are effective treatments for edema. Extreme and unresponsive edema may need treatment with hyper oncotic salt-poor albumin and furosemide. **(Meena, *et. al*., 2020)**

G. Complications of the cardiovascular system

Patients who have NS have a higher risk of developing cardiovascular disease as a result of their hyperlipidemia, which contributes to increased thrombogenesis, and endothelial dysfunction the degree of hypoalbuminemia has been shown to have a substantial correlation with hypercholesterolemia, and both chronic proteinuria and renal insufficiency have been shown to contribute to cardiovascular disease **(Go, Alan S, *et. al*., 2021).**

Children with MCNS who have hyperlipidemia but are sensitive to CS have a low or nonexistent risk of developing cardiovascular disease since their hyperlipidemia is transient and only lasts for a brief period of time. The presence of hyperlipidemia is associated with an increased likelihood of developing atherosclerosis at an earlier age. It would indicate that the length of time nephrotic

hyperlipidemia is present has a significant role in the initiation of vascular damage, and individuals whose proteinuria and albumin levels remain low are at the greatest risk **(Mason, *et al*.,2022).**

The levels of very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and lipoprotein (a) are all shown to be higher in children who have had NS for a prolonged period of time and have experienced repeated relapses. **(Hari, *et. al*., 2020)**

Patients who have elevated levels of both VLDL and LDL are expected to have an increased risk of developing atherosclerosis. The presence of hyperlipidemia is associated with the progression of glomerular and interstitial renal disease. Damage to the endothelium caused by hyperlipidemia may encourage the flow of lipoprotein into the mesangium, which can then lead to proliferation and sclerosis. **(Busuioc RM, *et . al*.,2022)**

1.4. Hyperlipidemia

In 2011, the National Heart, Lung, and Blood Institute of the United States classified hyperlipidemia in children as values beyond the 95th percentile: Triglycerides >130mg/dL; total cholesterol >200mg/dL; at least one of the following: low density lipoprotein >130mg/dL; non-high density lipoprotein cholesterol >145mg/dL. **(Stephen, *et.al*.2020)**

Hyperlipidemia is a common finding in nephrotic syndrome. There is increased total Cholesterol, LDL, VLDL and low or normal HDL. Hyperlipidemia is usually observed during the active phase of disease and disappears with resolution of proteinuria. But in relapsing cases, it may persist and may increase risk of atherosclerosis in later life Hyperlipidemia is an important factor in atherosclerosis **(Stewart, *et.al*.2020).**

1.4.1. Dyslipidemia characteristics

Lipoproteins are the main carriers of lipids in the blood, and they are also engaged in the three major pathways that are responsible for the synthesis and transport of lipids throughout the body Table (1.2). Lipoproteins are the primary carriers of lipids in the blood because they are also involved in the three major routes. These three pathways are the exogenous pathway, the endogenous pathway, and the reverse cholesterol transport pathway Figure (1.1)

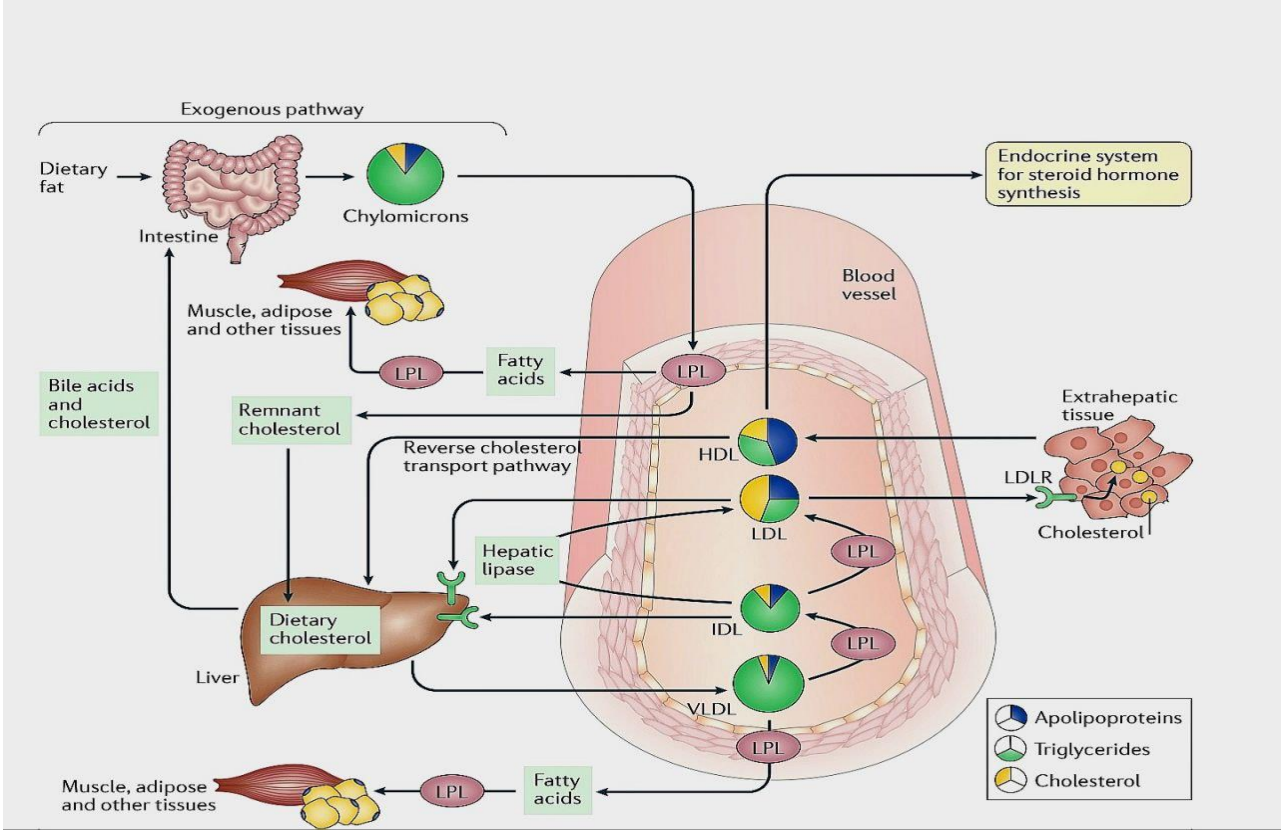


Figure (1-1): Exogenous, endogenous, and reverse cholesterol transport pathways.

Syndrome of nephrotic associated with or not associated with chronic kidney disease (CKD), causes a disruption in the metabolic processes governing lipids and lipoproteins. **(Vaziri, N.D., 2016)**

Proteinuria in nephrotic syndrome patients is proportional to the extent to which lipid metabolism is altered. In nephrotic syndrome, there is an increase in the levels of certain lipoproteins in the blood, including apolipoprotein B (ApoB), cholesterol, and triglycerides. Very low-density lipoprotein, intermediate-density lipoprotein, and lipoprotein(a) are all examples of lipoproteins. HDL cholesterol, or high-density lipoprotein cholesterol, is the quantity of cholesterol carried by HDL **(Vaziri ., 2016).** and the content of ApoA I and ApoA II apolipoproteins. **(Joven, *et* . *al*.,1990)** are quite similar between healthy people and patients with nephrotic syndrome. Cholesterol efflux has been shown to be decreased in individuals with diabetic nephropathy, although its role in creating cholesterol efflux from peripheral tissues has not been investigated in nephrotic syndrome. High blood cholesterol is a hallmark of nephrotic syndrome **(Zhou, *et* . *al*.,2008).**

Patients with nephrotic syndrome have substantial changes in their lipoprotein composition and function, as well as an increase in their total lipoprotein count. ApoA I, ApoA IV, ApoB, ApoC, and ApoE are all up, as is the ratio of ApoC-III to ApoC II, and apoE has also increased. **(Joven, *et* . *al*.,1990).**

Blood lipid and lipoprotein abnormalities in nephrotic syndrome patients are caused by decreased clearance and, to a lesser degree, altered biosynthesis. The kidneys are affected by nephrotic syndrome. Although nephrotic syndrome may affect LDL production, it is not always the case. **(Kaysen, *et* . *al*.,1998)** Because of the reduction in clearance, the levels of virtually all lipoproteins containing ApoB have been changed. **(Garber, *et. al*.,1984)**

Hyperlipidemia in nephrotic syndrome was previously thought to be related to albumin metabolism; however, data from sophisticated studies have challenged the link between hepatic lipogenesis and albumin synthesis. These studies have shown that proteinuria, and not albumin synthesis, is associated with hyperlipidemia in

nephrotic rats. Hyperlipidemia is associated with proteinuria in nephrotic rats. **(Davies, *et . al*., 1990)** In nephrotic syndrome related to CKD, the activity of enzymes like lecithin-cholesterol acyltransferase (LCAT) is decreased, while enzymes like plasma cholesteryl ester transfer protein (CETP) are activated. This can alter the composition of lipoproteins, leading to the production of immature HDL. **(Vaziri, N.D., 2016)**

In patients with nephrotic syndrome, where severe hypertriglyceridaemia is another important lipid abnormality, recent research has identified angiopoietin-related protein 4 (ANGPTL4) and the extent of its sialylation as appealing therapeutic targets for reducing proteinuria and hypertriglyceridaemia. **(Macé and Chugh. 2014).**

There are a number of factors that may contribute to the development of dyslipidaemia in CKD patients, including the presence of other diseases or the use of medications meant to decrease proteinuria and the progression of the disease. Patients with proteinuric diseases may be at greater risk for cardiovascular morbidity and death, hence it is crucial to detect and treat lipid abnormalities in these patients at an early stage of chronic renal disease. **(Keith, *et . al*., 2004)**

Table (1-2) | Classes of lipoproteins:

Lipoprotein	Composition	Apo lipoproteins and enzymes	Function in normal physiology
Low-density lipoprotein (LDL)	Apo lipoproteins (45%) Cholesterol (25%) Triglycerides (30%)	ApoA-I, ApoA-II, ApoC-II, ApoC-III, ApoE, ApoL-I CETP, LCAT	Reverse cholesterol transport from tissues to the liver
Intermediate-density lipoprotein (IDL)	Apo lipoproteins (25%) Cholesterol (45%) Triglycerides (30%)	ApoB-100	Major cholesterol carrier
Very low-density lipoprotein (VLDL	Mostly triglycerides and some cholesterol	ApoB-100	Intermediate between LDL and VLDL
Chylomicrons	Apo lipoproteins (2%) Cholesterol (3%) Triglycerides (93%)	ApoB-48, ApoC-II, ApoC-III, ApoE	Transport of dietary (exogenous) triglycerides to the systemic circulation and to adipose tissue and the liver

ApoA-I, apolipoprotein A-I; CETP, cholesteryl ester transfer protein; LCAT, lecithin-cholesterol acyltransferase.

1.4.2. Dyslipidemia's Clinical Consequences

Patients with nephrotic syndrome are more vulnerable to the many adverse effects of dyslipidemia. Accelerated atherosclerosis, dyslipidemia may lead to an elevated risk of cardiovascular complications including heart attack and stroke. In addition, the elevated risk of thrombosis that has been linked to nephrotic syndrome may be attributable in part to dyslipidemia. One of the most important risk factors for atherothrombotic diseases is dyslipidemia. The risk of thrombosis is raised by atherosclerosis and is made worse by dyslipidemia when platelets are too reactive.

(Jackson and Calkin., 2007) Platelet activation and thrombus development are both aided by LDL oxidation's byproducts. **(Podrez**, ***et. al*.,2007)**

1.4.3. Atherogenic Indices

Dyslipidemia has been considered to be a major risk factor for coronary artery diseases(CAD) **(Yusuf *et al.*, 2004).** In order to predict the probability of developing coronary atherosclerosis and cardiovascular disorders (CVD), a number of lipid parameters have already been used. Most of these consist of plasma lipid and lipoprotein concentrations, while occasional involvement of plasma apolipoproteins. It is interesting that lipid ratios have recently been used as risk indicators for lipids and lipoproteins **(Kim *et al.*, 2017).** Numerous research demonstrate the importance of changing lipid ratios over standard lipid indicators **(Nwagha *et al.*, 2010).**

- Atherogenic coefficient (AC) is the ratio of non-high-density lipoproteins cholesterol (non-HDL-C) to high-density lipoproteins cholesterol (HDL-C) **(Olamoyegun *et al.*, 2016)**. It is a diagnostic alternative, which has been used in predicting the risk of developing *cardiovascular* events.

$$AC = \text{non-HDL-C} / \text{HDL-C}$$

$$\text{Non- HDL-C} = TC - \text{HDL-C}$$

- Atherogenic index of plasma (AIP): is an unconventional lipid ratio representing the logarithm of the molar ratio of TG to HDL-C **(Gómez-Álvarez *et al.*, 2020).** Accumulated evidence showed that AIP is an important predictive index with a positive correlation with *CVD*.

$$AIP = \log (TG/ \text{HDL-C})$$

- Castelli's risk indexes (I & II): (also called cardiac risk indexes) are two lipid ratios, the CRI-I is the ratio of TC to HDL-C, while the CRI-II is the ratio of LDL-C to HDL-C, with notable positive associations with *CVD* risk **(Igharo**

***et al.*, 2020).** many reports assessed and confirmed their positive correlation with CVD **(Tecer *et al.*, 2019)**.

CRI-I= TC/ HDL-C ratio

CRI-II = LDL-C / HDL-C ratio

- Cholesterol index (C-index): is a simple index that predicts the probability of developing CAD with greater accuracy than the other indices **(Ulusoy, 2013).**

C- index= (LDL-C) – (HDL-C)

1.5. Biomarkers of Endothelial Dysfunction

The endothelium of blood vessels helps regulate blood pressure by balancing coagulation with fibrinolysis and atherogenesis. **(Podrez*, et. al*.,2007)** In adults, nephrotic syndrome is a major contributor to the development of atherosclerosis. **(Ordonez*, et. al*.,1993)** Idiopathic nephrotic syndrome (INS) has been linked to atherosclerosis in adults, but its impact on the development of the disease in children has been less evident. **(Lechner, *et. al*.,2004)** Obesity, hypercholesterolemia, increased thrombinogenesis, impaired fibrinolysis, enhanced platelet activation, oxidative stress, insulin resistance, immunological dysregulation, and subclinical inflammation are all risk factors for cardiovascular disease that are linked to INS. **(Tkaczyk, *et . al*.,2008).**

1.5.1. E-Selectin

The adhesion of molecules is the central event in the physiological process, whether it be between cells or between an immune cell and a target biological component of the extracellular matrix. Adhesion molecules are highly expressed glycoproteins that serve as mediators of cell-cell contacts. They play a role in the regulation of cell migration, survival, and apoptosis throughout all kingdoms of life. **(Jaitovich and Etcheverry.,2004)** Integrins, selectins, cadherins, nectins and other members of the

immunoglobulin superfamily, and other adhesion molecules including mucins are the five classes of adhesion molecules. **(Harjunpää, *et . al*.,2019)**

Selectins recognize carbohydrates that are shown on the surface of cells, which enables them to facilitate cell-cell adhesion. **(Taylor and Drickamer., 2007)** The cell membrane glycoproteins known as selectins are responsible for mediating the adherence of hematopoietic and cancer cells to endothelial cells, leukocytes, and platelets in the circulation of blood. **(Ley., 2001)** These adhesion events are very important in the processes of inflammation, infection, cancer, lymphocyte and bone marrow stem cell homing, as well as immune cell monitoring. In both chronic and acute inflammatory disorders, selectins play an important role in the homing of aberrant leukocytes. **(Silva, *et . al*.,2018).**

selectins have been suspected of playing a role in the spread of cancer. (**St Hill, 2011)**. Ca2+ is required for the binding of carbohydrate ligands by selectins, which are members of the C-type mammalian lectin family and are found in mammals. **(Lasky., 1995)**

The selectin subfamily is made up of three different members: the leukocyte (L)-selectin (CD62L), the platelet (P)-selectin (CD62P), and the endothelial (E)-selectin (CD62E). The main sequences of the P-, L-, and E- selectins all have a significant amount of similarity with one another as well as with the selectins found in other species. **(Kansas., 1996)** However, their structure and pattern of cell-type expression is diverse. **(McEver., 2015)**

Platelet granules and endothelial cell Weibel-Palade structures are two of the main storage locations for P-selectin. E-selectin plays a constitutive role in leukocyte rolling and adhesion to endothelial cells, and is abundantly expressed in the endothelia of the bone marrow and skin. Unlike P- and E-selectin, L-selectin is

constitutively expressed on lymphocytes, monocytes, and granulocytes and is cleaved from the cell surface after cell activation. Selectins and their ligands have recently been identified as promising therapeutic targets for the treatment and prevention of a broad variety of diseases. **(Silva, *et . al*.,2018)**

1.5.1.1. Selectins and Their Organization

The selectins make up a group of related molecules that are located in the cell membrane and have a number of structural similarities in common with one another. They have a carbohydrate-recognition domain (CRD) at their N terminus, a domain for epidermal growth factor (EGF), a sequence of consensus repeats (CR) domain, a transmembrane domain, and a short cytoplasmic tail. Additionally, they have a domain for epidermal growth factor (EGF). These domains are all situated inside the area of the protein that is responsible for transport across the membrane. **(Kansas., 1996)** Because selectins are able to bind carbohydrates in a way that is dependent on calcium, they are categorized as C-type lectins. C-type lectins are responsible for the classification of selectins. **(Natoni**, ***et . al*.,2016)**

The N-terminal calcium-dependent lectin domain for carbohydrate recognition (CRD) is one of the five domains that are present in selectins. Other domains include the epidermal growth factor-like (EGF) domain, the series of consensus repeats (CR) domains, the transmembrane domain, and the short cytoplasmic tail (Figure 3). Selectins are glycoproteins that are found on the cell surface and are closely related to one another-Selectin is a glycoprotein that has a molecular weight of 116 kilodaltons and is highly expressed on the external membrane surface of vascular endothelial cells. These cells also release cytokines such as TNF. It has an EGF domain, a unique lectin domain, and six consensus CR domain repeats in its structure. E-selectin contains a 119-residue N-terminal lectin domain that binds

oligosaccharides. It also includes six cysteine-rich consensus repeats. **(Collins, *et . al*.,1991)**

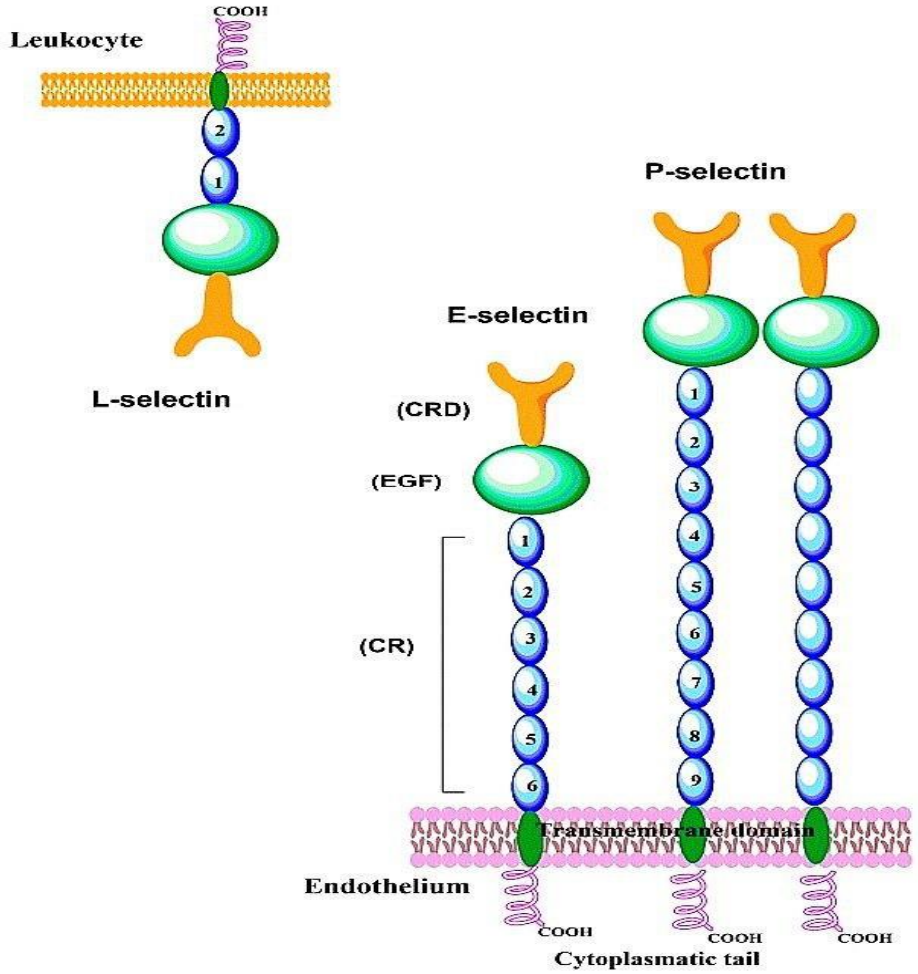


Figure (1-2): Structures of P-, E-, and L- selectins.

The EGF-like domain of E-selectin is linked to the stem of six consensus repeats by a series of single transmembrane helices, and the bottom of the stem is connected to a short C-terminal cytoplasmic domain by another series of single transmembrane helices. The EGF-like domain of E-selectin is also connected to the stem of six consensus repeats by a series of single transmembrane helices. It is only possible for the expression of e-selectin to change as a result of the action of stimuli if de novo transcription is performed. Therefore, it takes between three and four hours for E-

selectin to become visible on the cell surface after stimulation, and it might take anywhere from sixteen to twenty-four hours for it to return to its baseline levels. **(McEver, *et . al*.,2015)**

1.5.1.2. The chemical Role of Selectins

Selectins interact with their counter-receptors to mediate adherence to other cells or matrix components. Selectin binding interactions facilitate leukocyte adherence to endothelium and subsequent extravasation to the site of inflammation or injury, where they can fight off infections and repair tissue damage. **(Ley and Kansas., 2004)**

Despite widespread belief to the contrary, selectins have been found to play a harmful effect in a number of acute and chronic inflammatory disorders. **(Silva, *et . al*.,2018)**

A important factor in the development of atherosclerosis is the abnormal homing of leukocytes into endothelial cells, which is mediated by P- and E-selectins. This factor is a contributor to atherosclerosis. **(Davies, *et . al*.,1993)** Experiments that were carried out on mice revealed much reduced levels of atherosclerotic plaques in animals that lacked P and E selectin. These findings provided evidence for the presence of selectins in this chronic inflammatory disease. **(Collins, *et . al*.,2000)**

1.5.2. Omentin-1

Omentin-1 is a 313-amino-acid novel adipocytokine. It is expressed in mesothelial, vascular, airway goblet, small intestine, colon, ovaries, plasma, and visceral (omental and epicardial) fat. Omentin-1 is anti-inflammatory and atheroprotective properties may make it a risk factor for numerous illnesses and an acute-phase

reactant. Omentin-1-boosting treatments may prevent or treat many diseases. **(American Physiological Society.2017)**

1.5.2.1. Role of omentin-1

It is generally accepted that adipose tissue functions as a big gland that is capable of producing both paracrine and endocrine hormones. There is mounting evidence to support the hypothesis that adipocytes are responsible for the relationship between obesity and cardiovascular diseases (CVD). A significant variety of mediators, which have an effect on metabolism as well as inflammation and coagulation, are produced by adipose tissue. Because of its beneficial effects on inflammation, glucose homeostasis, and cardiovascular illnesses, the new adipocytokine omentin has emerged as one of the most popular topics of discussion in recent years. **(Tan, *et . al*.,2015)**

1.5.2.2. Mechanism of Omentin's protective effects in diverse pathophysiological processes:

Omentin, an essential component that connects organs to adipose tissue, has a protective function in a broad variety of physiological and pathological processes by influencing a large number of cells signaling pathways Figure (1.4), Omentin inhibits TNF-induced cyclooxygenase-2 (COX-2) production in endothelial cells. This is accomplished by activating adenosine 5'-monophosphate-activated protein kinase (AMPK), which in turn stimulates the endothelial nitric oxide synthase (eNOS)/NO pathway. Omentin's anti-inflammatory properties are a result of this process. **(Yamawaki, *et. al*.,2011)** In addition to this, the stimulation of eNOS and NO caused vasodilation in blood arteries that were isolated. **(Maruyama, *et. al*.,2012)** and decreased agonist-induced increase in blood pressure. **(Brunetti, *et. al*.,2014)**

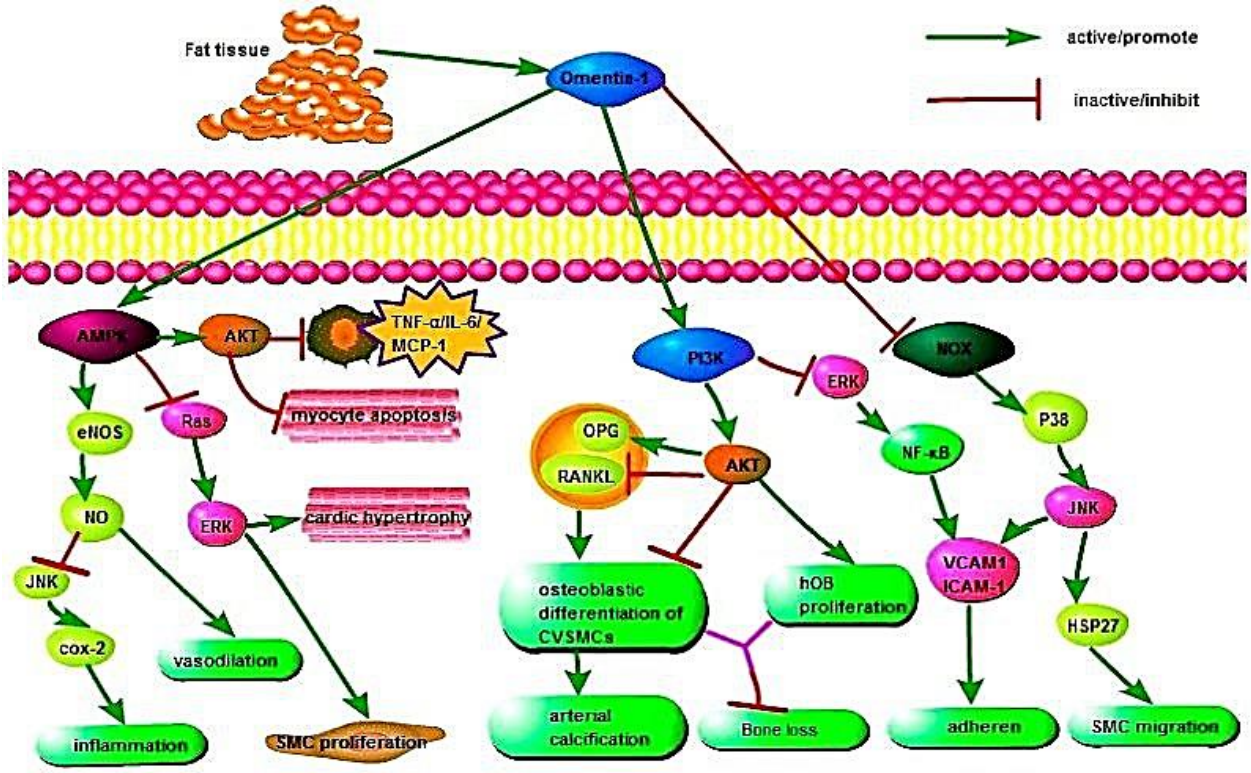


Figure (1-3): Omentin's protective mechanisms in different pathophysiological conditions

1.5.3. Vascular adhesion protein 1 (VAP-1)

Vascular adhesion protein-1 is a dimeric endothelium transmembrane molecule that has a molecular weight of 170 kilodaltons. It is also known as VAP-1. It is most abundantly expressed under normal conditions on the high endothelial venules of peripheral lymph nodes as well as on the endothelia of the liver. It is a glycoprotein that promotes the adhesion of tissue-specific lymphocytes in a manner that is dependent on sialic acid. This effect is brought about by the glycoprotein. **(Kurkijärvi, *et. al*.,1998)**

The molecule known as amino oxidase copper containing 3 (AOC3), also known as vascular adhesion protein-1 (VAP-1), is a multifunctional molecule that promotes inflammation and functions as both an enzyme and an adhesive. VAP-1 is a primary

amine oxidase that belongs to the family of semicarbazide-sensitive amine oxidases (SSAO). It catalyzes the oxidation of primary amines, which is necessary for the production of ammonium, formaldehyde, methylglyoxal, and hydrogen peroxide. Primary examples of cell types that are able to express the VAP-1 gene are endothelial cells, smooth muscle cells, adipocytes, and pericytes. It plays a part in a broad number of biological processes, including vascularization, angiogenesis, and the extravasation of immune cells, amongst others. Vascular inflammatory disorders include conditions such as atherosclerosis, stroke, diabetes, neurovascular disorders (such as Alzheimer's Disease), hepatic disease (such as non-alcoholic steatohepatitis), and skin conditions (such as psoriasis). VAP-1 is being researched as a novel clinical biomarker and as a potential therapeutic target for these conditions. (**Hui. Li, *et* . *al*.,2021)**

1.5.3.1. The involvement of VAP-1 in atherosclerosis

The retention of LDL and dysfunction in endothelial cells are the primary contributors to atherosclerosis. In response to vascular inflammation, VAP-1 is attracted to the luminal surface of endothelium, where it interacts with Siglec-9/10 (sialic acid-binding immunoglobulin-like lectin 9/10) to enhance monocyte infiltration into atherosclerotic lesions. This interaction is necessary for the development of atherosclerosis. Byproducts of VAP-1's enzymatic activity, such as hydrogen peroxide, have the potential to further increase endothelial VAP-1 expression as well as monocyte transmigration. When smooth muscle cells (SMCs) in the media are subjected to inflammation, their phenotype changes from the resting "contractile" state to the more dynamic "synthetic" state, which is characterized by increased proliferation, migration, and collagen synthesis. This occurs when the SMCs transition from the "contractile" state. Through its enzymatic products, the VAP-1 protein found in the plasma membrane of SMCs may be able to prevent the

phenotypic change of these cells by preventing the proliferation of SMCs and collagens. Therefore, VAP-1 is a pathogenic component in plaque instability, which is characterized by a large lipid core rich in monocyte/macrophage-derived foam cells and a thin fibrous crown. VAP-1 plays a role in the development of plaque instability show that in figure (5). **(Hui .Li, *et . al*.,2021)**

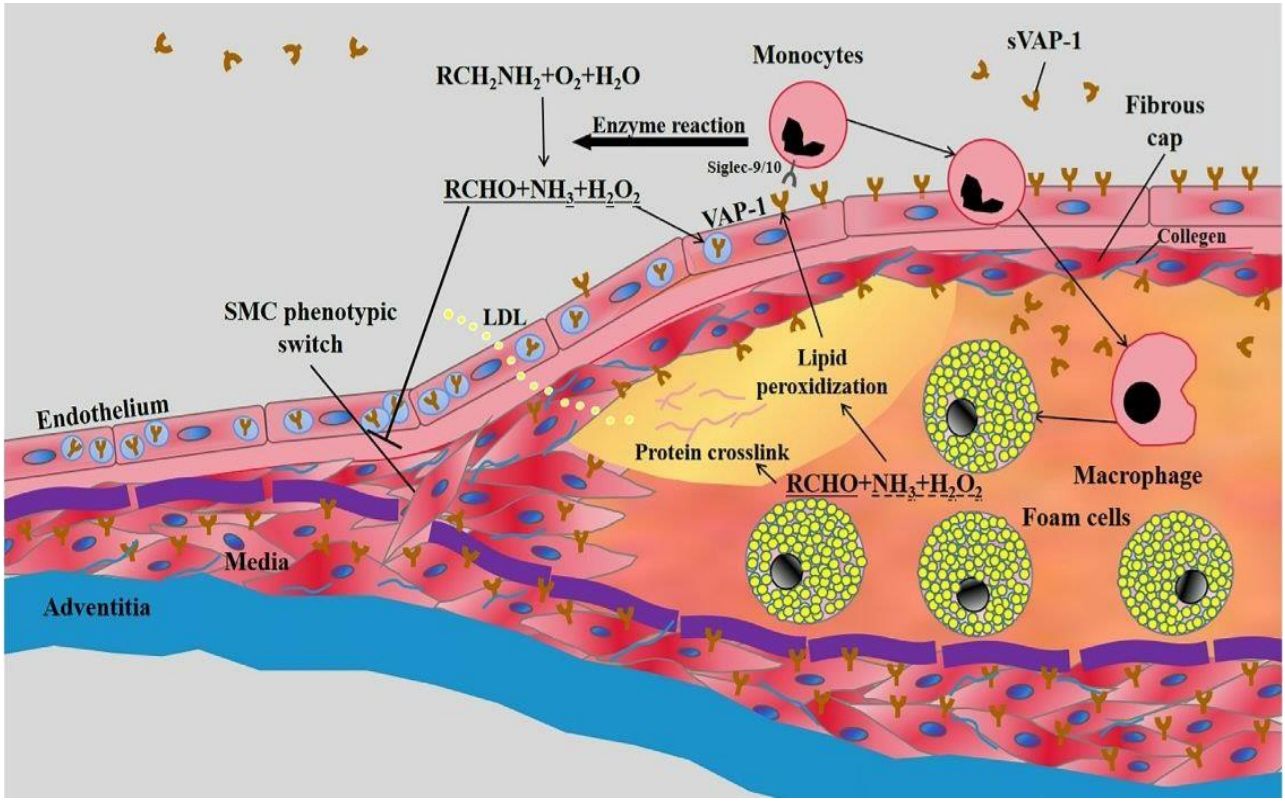


Figure (1-4): Vascular adhesion protein 1's role in atherosclerosis

1.6. Nanomedicine

Nanomedicine is the application of nanotechnology in the field of medicine with a view to enhancing the diagnosis and treatment of various diseases. Nanotechnology is already involved in a range of biomedical applications including drug and vaccine delivery, diagnostic imaging, nano sensor diagnostics, nano-enabled therapies, and tissue engineering **(Lloyd-Parry, *et. al*.,2018)**.

There are several aspects that nanotechnology is researching for disease treatment purposes, for instance: Increasing the efficiency of drug delivery and reducing side effects, looking for specific targeting of the active components in cell/tissues; Improving the properties of pharmacologically active drugs such as stability, solubility, half-life, and tumor aggregation; generating stimuli-responsive drug release; also it could be involve in expanding the area of drugs encapsulated/attached to bio macromolecules such as proteins and/or mRNA; furthermore it might be used for improvement of therapeutic efficiency by delivering multiple active agents to a specific targeted site in order to overcome limitations such as drug resistance; Overcoming biological barriers; Improving the sensitivity of diagnosis and imaging of tumorous sites; Linking anti-cancer active components with imaging molecules in order to attain a real-time assessment of the in vivo efficiency of the drugs; Developing new paths for the manufacture of synthetic vaccines; and also, improving cancer diagnosis and imaging with scaled-down medical devices **(Shi, j, *et . al*.,2017) (Lungu, I. I., *et . al*.,2019)**.

Moreover, due to their small sizes, nanomaterials have the potential to overcome the biological barriers. For example, the smaller capillaries have sizes around 3 micrometers in diameter. Nanomaterials that are specifically engineered to have a maximum size of 200 nm could take advantage of the circulatory system and get transported to a specific site and deliver pharmaceutically active agents. Another advantage of their small size is their very large surface area. Due to their increased surface area, a larger quantity of drug molecules can be attached and delivered. The increased drug loading volume comes as an excellent advantage for cancer drug delivery therapies. Moreover, binding specific targeting ligands increases the chances for effective drug delivery **(Faisal, N., *et . al*.,2019)**.

Numerous types of nanoparticles have been developed with different drug targeting technology as shown in Figure (1.6) in order to attain an efficient drug delivery

system that also has specific functions for diagnostic purposes, apart from its therapeutic effects. Due to their particular properties, both organic and inorganic nanoparticles have been researched for this purpose **(Zhou, Q, *et . al.*,2018)**.

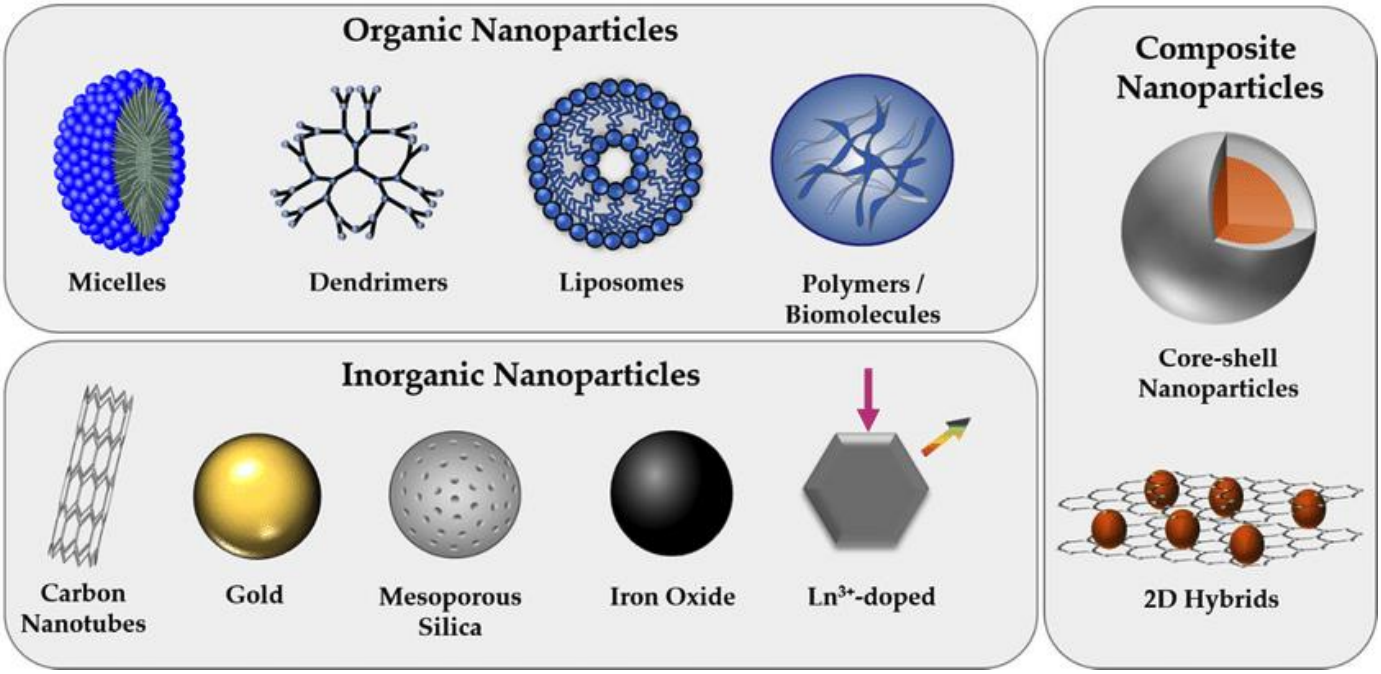


Figure (1-5) A commonly used nanoparticle (NP) types, classified as organic, inorganic, or composite structures.

1.6.1. Synthesis and characterization of chitosan/iron oxide nanoparticles

Researchers have recently been interested in metal oxide nanoparticles covered with bio-organic polymers or ligands due to their potential in a number of fields, including the biomedical and pharmaceutical fields. **(Bharathi, *et . al.*,2019)**

Surface modification of metal oxide nanoparticles using organic polymers can improve the attributes of the particles on several fronts, including the physical, the chemical, and the biological. **(Zhu, *et . al.*,2018)** Among all of the many types of polymers, chitosan has garnered a great deal of attention because to the exceptional traits that it possesses, including low toxicity, biodegradability, biocompatibility,

and antibacterial qualities. **(Rabea, *et . al*.,2003)** Chitosan surface modifications of metal oxide nanoparticles improve both their biocompatibility and their characteristics for use in medical applications. **(Sun, *et . al*.,2014)**

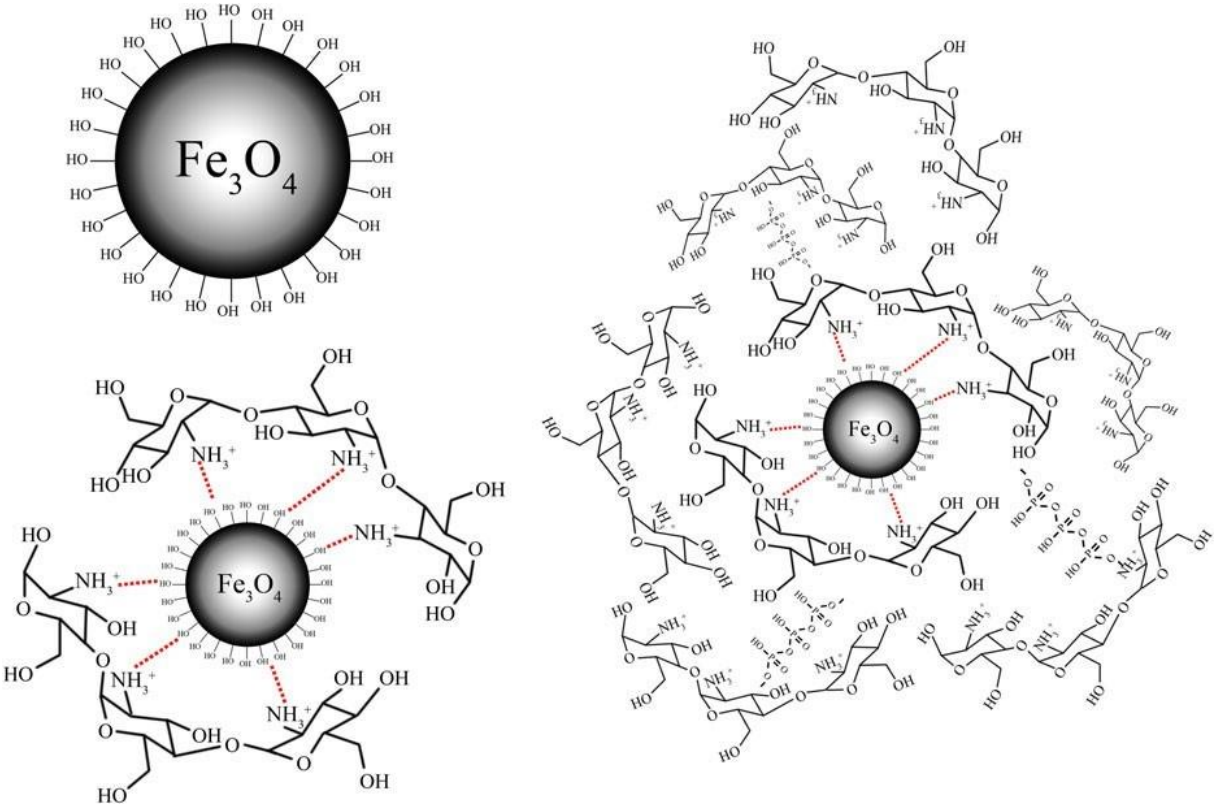


Figure (1-6) Schema of in situ synthesized chitosan-coated magnetic nanoparticles (CS MNPs)

1.6.2. Chitosan:

Chitosan, which is a mucopolysaccharide, has a number of features with cellulose. Chitin is used to produce this chemical by deacetylating the cellulose acetate molecule in the chitin. (Figure 6) illustrates the structure of the chitin deacetylation reaction. The fungus as well as the crustacean Chemical structures of chitin and its fully deacetylated derivative Chitosan. **(Schmitz, *et . al*.,2019)**

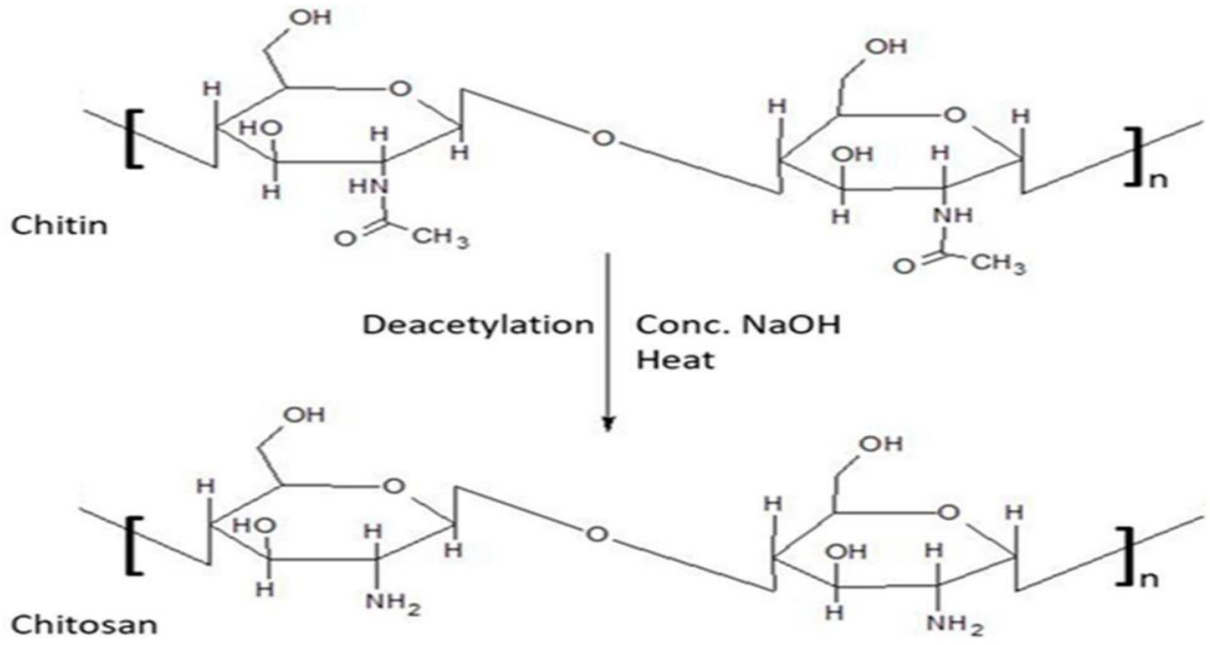


Figure (1-7): Preparation of Chitosan from chitin by deacetylation.

1.6.3. Utilizing Chitosan Nanoparticles

Nanoparticles of chitosan are naturally occurring materials that have exceptional physicochemical, antibacterial, and biological capabilities. Because of these qualities, chitosan nanoparticles are an outstanding ecologically friendly material, and the bioactivity that they contain is absolutely safe for human ingestion. Chitosan nanoparticles have also been shown to have antimicrobial properties. Chitosan nanoparticles are fully safe for eating by humans and shouldn't be avoided. **(Malmiri, *et. al*.,2012).** Chitosan nanoparticles find use in a diverse range of industries as a result of their one-of-a-kind qualities. The following will go through a few of them:

A. Tissue engineering

Either the process of producing biological alternatives for implantation within the body or the process of modifying tissues through an active mechanism may be referred to as tissue engineering. Tissue engineering can also be used

interchangeably. Research in the field of biomedicine encompasses both of these methods in their entirety. In order to carry out the process of tissue engineering, it is necessary to make use of live cells that have been modified in some way, either genetically or as a result of the effect of their extracellular environment. The area of tissue engineering tries to repair, replace, maintain, or improve upon the functionality of an organ or tissue by focusing on that organ's or tissue's function as the primary objective of its research and development efforts. **(Jayakumar, *et . al*.,2010)**

Tissue engineering is either the process of developing biological alternatives for implantation inside the body or the process of remodeling tissues by an active mechanism. Both of these processes fall under the category of biomedical research. The utilization of living cells that have been altered in some manner, either genetically or by the influence of their extracellular environment, is required in order to carry out the process of tissue engineering. The purpose of tissue engineering is to repair, replace, maintain, or enhance the functioning of a certain organ or tissue. **(Peppas, *et . al*.,2004)**

B. Cancer diagnosis

The use of semiconductor nanocrystals, also known as quantum dots, as **fluorescent** probes hold the greatest promise for a wide variety of biological **applications**. Despite the potential benefits of nanocrystals, there is still a concern over the cytotoxicity of the heavy metals that make up their composition **(Jayakumar, *et . al*.,2010)**. The non-toxicity of chitosan nanoparticles is a **major** plus in this regard. Chitosan nanoparticles' anticancer efficacy stems, in **part**, from their diminutive size The very small particle size of chitosan, which **increases** both its specific surface area and its surface to volume ratio, contributes to an increase in the substance's bioavailability. **(Ghadi, *et . al*.,2014)**

C. Drug delivery

Many different kinds of colloidal delivery vehicles have been developed as a consequence of the fact that chitosan nanoparticles may function as carriers. This has led to a greater variety of delivery options. **(Malmiri, *et . al*.,2012)** Chitosan nanoparticles have the power to pass through biological barriers and prevent macromolecules from decaying when they are exposed to biological medium. Chitosan nanoparticles also have the ability to protect themselves from biological degradation. In addition to this, it is possible to affect the controlled delivery of chemicals to the site of choice, such as medications or macromolecules. **(Rajasree and Rahate., 2013)**

D. Enzyme immobilization

Support The presence of an amino functional group in chitosan gives it an attractive possibility for immobilizing enzymes. Chitosan is a suitable substrate for immobilizing enzymes because of its resistance to chemical degradation and protection against metal ion disruption, two of the many desired features that make chitosan a good candidate for this use. Chitosan is widely acknowledged as the optimal substrate for immobilizing enzymes. Chitosan has been used in this capacity for many years. **(Ghadi, *et . al*.,2014)**

E.Antioxidant activity

Chitosan has been shown to have antioxidant effects via many studies. It does this by exchanging the neutralization of free radicals and the chelation of metal ions for the contribution of either a hydrogen atom or a lone pair of electrons. **(Rajalakshmi, *et . al*.,2013)**

F. Antimicrobial agent

By binding to the negatively charged phospholipids of the plasma membrane, chitosan triggers cell death and the leakage of intracellular components. Chitosan may be able to chelate metal ions, which might explain its antibacterial effect. It has been found that nano size silver/chitosan nano formulations (NFs) exhibit fungicidal capabilities, making them useful as an agent for antifungal treatment of seed-borne plant diseases such Aspergillus favus, Rhizoctonia solani, and Alternaria alterneta. It has been shown that chitosan may enter cells, bind to DNA, and block the production of mRNA. **(Kaur, *et* . *al*.,2012)**

1.7. Biomedical applications

The in situ coating approach, which involves the alkaline coprecipitation of Fe (II) and Fe(III) precursors in aqueous solutions of hydrophilic chitosan polymers, is commonly used to synthesize chitosan-coated magnetic nanoparticles (CS MNPs). These polymers slow the iron oxide core development during preparation, stabilize the nanoparticles in aqueous environments through steric repulsions, and lessen opsonization in living organisms. **(Mornet, *et. al*.,2004).** For the purpose of this experiment, an in-situ synthesis approach was used for the production of CS MNPs. This technique consisted of the coprecipitation of iron salts in the presence of chitosan and tripolyphosphate. After being exposed to an acidic environment, the amino groups may become positively charged as a result of the protonation process. Chitosan may thus react with molecules such as the hydroxyl (Fe-OH) groups that are located on the surface of magnetite nanoparticles. These nanoparticles are similarly negatively charged. A polyvalent anion is formed from sodium tripolyphosphate (TPP) due to the presence of three separate phosphate groups. Chitosan polymers that have been thoroughly dissolved are rapidly adsorbed by hydrophilic cores of Fe3O4, which precipitate as a result of the production of nuclei

with high surface energies. The incorporation of TPP results in the formation of cross-links between the molecules of adsorbed chitosan. These cross-links are formed as a result of ionic interactions between the positively charged amino groups of chitosan and the negatively charged TPP. Under these conditions, the formation of homogeneous layers of chitosan polymers by physical cross-linking that was mediated by electrostatic contact with Fe_3O_4 cores served to stabilize each individual nanoparticle. Using this method results in the production of monodispersed CS MNPs that have a uniform coating of low-molecular-weight chitosan polymer on their surface. **(Calvo, *et . al*.,1997)**

1.8. Aims of the study

The aims and objectives of this study were:

- Estimation the level E-selectin, Omentin-1 and Vascular adhesion protein-1 (VAP-1) in children with nephrotic syndrome
- Study the correlation of serum E-selectin, Omentin-1 and Vascular adhesion protein-1 and biochemical with nephrotic syndrome cases
- Investigate the diagnostic preferences of E-selectin, Omentin-1 and Vascular adhesion protein-1 as a biochemical parameter in nephrotic syndrome using ROC analysis.
- Prepare and study the structural features of chitosan/iron oxide nanoparticles CS- Fe_3O_4 NPs
- Examine the Applications of CS- Fe_3O_4 NPs as a lipid-lowering agent in children with nephrotic syndrome

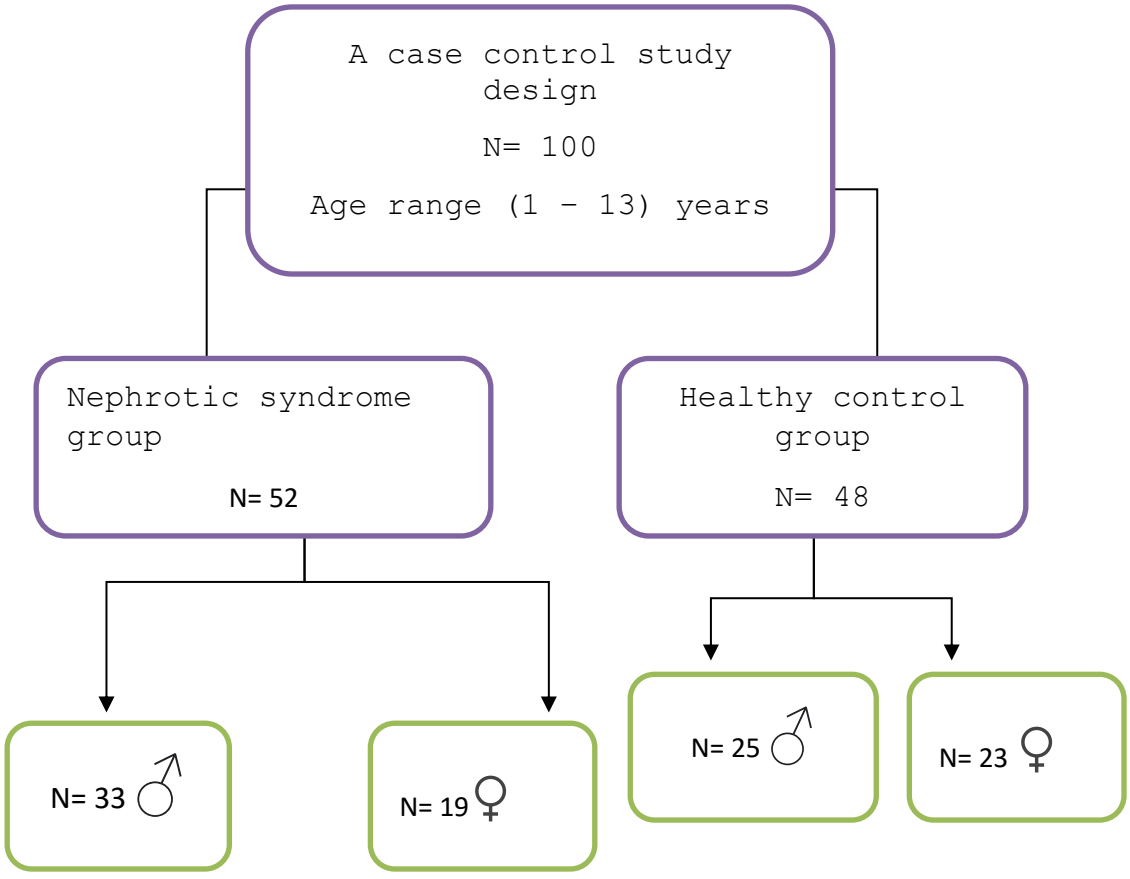
Chapter Two

Materials And Methods

2. Subjects Materials and Methods

2.1. Study Design and Ethical approval

A case-control study design, for a total 100 subjects were collected, study was conducted throughout the period from September / 2022 to August / 2023. The College of Medicine, University of Kerbala, and Kerbala Health Directorate validated the study's ethical approval. Approval also taken from administration of children teaching hospital and from each patient after explaining the nature and purpose of study as shown in Schemed.



Schemed of the Study

2.2. Patients

Whole blood samples of fifty-two nephrotic syndrome patients (33 males 19 female) of children were collected at the kidney diseases consultant of teaching hospital for children, Kerbala health directorate, Iraq with age ranged between 1 - 13 years. An exhaustive interview addressing personal history, family history, demographic information and laboratory examination was performed. A questionnaire was formed to get the data of the patient set which contain the age, weightiness, height, history of disease.

2.2.1. Control group

An apparently healthy 48 subjects (25 males and 23 female) were chosen from well-known volunteers' participants. Blood samples were drawn from the volunteers, participants had no history of diseases. The ages of the participants were also convergent in the whole study group.

2.2.2. Inclusion criteria

The (Pediatric Nephrology criteria - 2009) was assumed to fifty-two nephrotic syndrome children with ages run between (1 – 13 years). It is possible to diagnose patients with any two of the subsequent conditions:

Classical features of nephrotic syndrome include generalized edema, abdominal distension, and ascites. Blood pressure related to hypoproteinemia, or elevated if kidney impairment has developed. In severe forms of nephrotic syndrome, urinary protein >20 g/l, and serum albumin level <10 g/l is noted right after birth. The severity of proteinuria may only manifest after partial correction of hypoalbuminemia with albumin infusions. Microscopic hematuria or leukocyturia

may be present. Profound hyperlipidemia is often present in severe nephrotic syndrome. Microscopic hematuria or leukocyturia may be present. While oliguria is common, blood levels of creatinine and urea levels are variable. Most patients with NPHS1 mutations have normal renal function in the first few months, whereas rapid deterioration of kidney function may occur in other genetic forms.

Exclusion criteria

Children suffered from diseases (acute kidney injury disease, diabetes mellitus, chronic renal failure and malignant diseases) were excluded.

2.2.4. Blood Samples Collection

Disposable syringes and needles were used for blood collection (5 mL). Blood samples were obtained from nephrotic syndrome children and control groups by vein puncture. Each sample obtained will be divided into two parts:

A. The foremost tube contained 3 milliliters of blood, set in gel tubes. Next, it stayed for 10 to15 minutes at room temperature for clotting. Then centrifuged the blood for 10 to 15 minutes at 4000 x g. for separation of serum and divided into 4 parts and put in Eppendorf tubes then stored at - 20 ˚C till examination of the biomolecules in this study.

B. Another portion was applied for the study of nanotechnology. It comprised 2 milliliters of blood specimen that have been placed in gel tube and 2ml in heparin tube. Then this first tube saved freezing at -20 ˚C till investigation of nanotechnology.

2.3. Chemicals and Kits

The kits used in this study are summarized in Table 2.1

Table 2.1: Chemicals and kits are used in this study and their suppliers

NO.	Chemicals and Diagnostic Kits	Manufactured by	Origin
1	Blood urea	Cobas	Germany
2	Serum creatinine	Cobas	Germany
3	Cholesterol	Cobas	Germany
4	Triglyceride	Cobas	Germany
5	low density lipoprotein	Cobas	Germany
6	Iron oxide Nano powder (Fe3O4)	Qualkems	India
7	Chitosan	Sigma	India
8	Xylene	Scharlau	SPIN
9	acetic acid	Scharlau	SPIN
10	Thiamin pyrophosphate (TPP)	Qualkems	India
11	Ethanol 95%	Scharlau	SPIN
12	Human SELE(E-Selectin) ELISA Kit	Elabscience	USA
13	Human(Intelectin 1/Omentin) ELISA	Elabscience	USA
14	Human VAP-1(Vascular Adhesion Protein 1) ELISA Kit	Elabscience	USA

2.4. Instruments and Lab Equipment

The instruments and laboratory tools used in this study are summarized in Table 2.2

Table 2.2: Instruments and Lab Equipment that used in this study

NO.	Apparatuses and Equipment	Company	Origin
1	Roche diagnostics /Cobas c111 Auto analyzer	Cobas	Germany
2	Auto sample changer	Shimadzu	Japan
3	Balance sensitive	A&D	Japan
4	Centrifuge	Hettich	Germany
5	Deep freezer	Fisher Scientific	USA
6	Distillator (Water distiller)	Gfl	Germany
7	ELISA instrument system	Biotek	USA
8	Mechanical stirrer	Labtech	Korea
9	Micropipettes	Bioasic	Canada
10	Oven	Binder	USA
11	pH meter	Inolab	Germany
12	Refrigerator	Concord	Lebanon
13	Ultra-sonication bath	Labtech	Korea
14	Vortex mixer	Gemmy	Taiwan
15	Water bath	Memmert	Germany

2.5. Methods

2.5.1. Roche diagnostics /Cobas c111 Auto analyzer/ Chemistry System /Germany for determination Total cholesterol, triglyceride, high density lipoprotein, serum urea, serum creatinine, serum albumin, c-reactive protein

Principle:

The cobas c111 main instrument uses absorption photometry for determining the amount of absorbance in a fluid. The absorbance is used to calculate the concentration in the solution. Loading the sample. The operator identifies the sample, places it on the instrument, and defines the order.

Measuring process: The measuring process for each test consists of forty regular cycles, each lasting 18 seconds. In each of these cycles, a measurement is taken, irrespective of what other actions take place during this cycle. The application definitions determine what is done in which cycle, and they also define which results are taken into account for the result calculation.

2.5.2. Enzyme-Linked Immunosorbent assay (ELISA) tests.

2.5.2.A. E-Selectine level determination

A. principle of the test

This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human E-Selectin. Samples (or Standards) are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human E-Selectin and Avidin Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are

washed away. The substrate solution is added to each well. Only those wells that contain Human E-Selectin, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human E-Selectin. You can calculate the concentration of Human E-Selectin in the samples by comparing the OD of the samples to the standard curve.

B. Kit components

1. Micro ELISA Plate (Dismountable)
2. Reference Standard
3. Concentrated Biotinylated Detection Ab (100×)
4. Concentrated HRP Conjugate (100×)
5. Reference Standard & Sample Diluent
6. Biotinylated Detection Ab Diluent
7. HRP Conjugate Diluent
8. Concentrated Wash Buffer (25×)
9. Substrate Reagent
10. Stop Solution
11. Plate Sealer

C. Reagent preparation

1. Bringed all reagents to room temperature (18-25℃) before use.

2. **Wash Buffer:** Diluted 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepared 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warmed it in a 40℃-water bath and mixed it gently until the crystals have completely dissolved.

3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Added 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and inverted it gently several times. After it dissolved fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 5000 pg/mL

Then maked serial dilutions as needed. The recommended dilution gradient is as follows: 5000, 2500, 1250, 625, 312.500, 156.250, 78.13, 0 pg/mL. Dilution method: Tacked 7 EP tubes, added 500uL of Reference Standard & Sample Diluent to each tube. Pipetted 500uL of the 5000 pg/mL working solution to the first tube and mix up to produce a 2500 pg/mL working solution. Pipetted 500uL of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don’t pipetted solution into it from the former tube. Gradient diluted standard working solution should be prepared just before use.

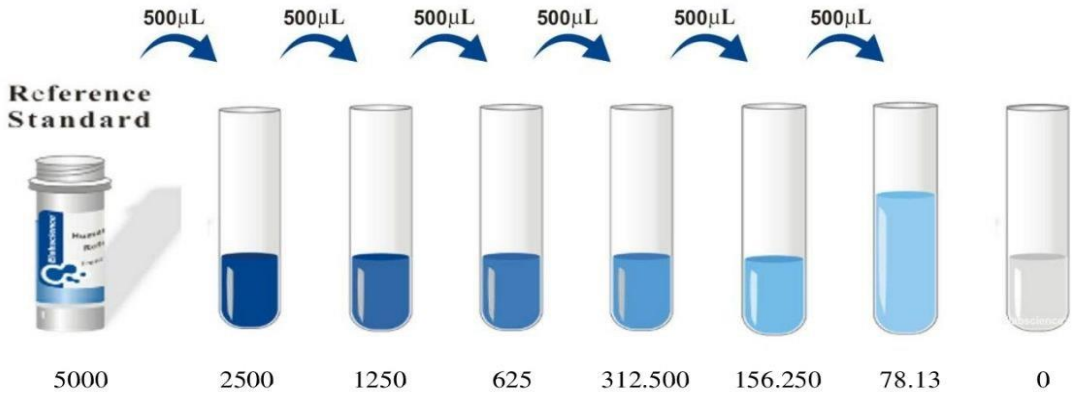


Fig. (2.1): Serial Dilution method for E-Selectin standard.

4. **Biotinylated Detection Ab working solution:** Calculated the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then diluted the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent (Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99).The working solution was prepared just before use.

5. **Concentrated HRP Conjugate working solution:** HRP Conjugate is HRP conjugated avidin. Calculated the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then diluted the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99).The working solution was prepared before use.

D. Assay procedure

1. Determined wells for **diluted standard, blank** and **sample**. Added 100 µL each dilution of standard, blank and sample into the appropriate wells

Covered the plate with the sealer provided in the kit. Incubated for 90 min at 37℃.

2. Immediately added 100 μL of Biotinylated Detection Ab working solution to each well. Covered the plate with a new sealer. Incubated for 1 hour at 37°C.

3. 350 μL of wash buffer was Added to each well. Soaked for 1 min and aspirate or decant the solution from each well and pat it dried against clean absorbent paper. Repeated this wash step 3 times. Maked the tested strips in use after the wash step.

4. 100 μL of HRP Conjugate working solution was Added to each well. Covered the plate with a new sealer. Incubated for 30 min at 37°C.

5. wash process repeated the for 5 times as conducted in step 3.

6. 90 μL of Substrate Reagent was added to each well. Covered the plate with a new sealer. Incubated for about 15 min at 37°C. Protectd the plate from light.

7. 50 μL of Stop Solution was added to each well.

8. Determined the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

E. Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log axis, with standard concentration on the x-axis and OD values on the yaxis. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution.

The actual concentration is the calculated concentration multiplied by the dilution factor.

2.5.2.B. Vascular Adhesion Protein-1 level determination

A. Test principle This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human VAP-1.

B. Kit components

Same the kit components used in E-selectin kits.

C. Reagent preparation

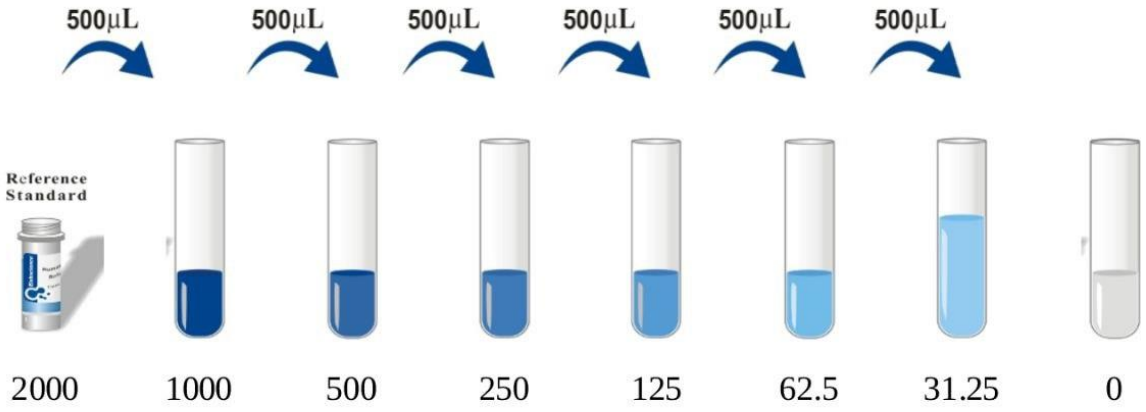


Fig. (2.2): Serial Dilution method for VAP-1 standard.

2.5.2.C. Omentine-1 level determination

A. Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human ITLN1. Standards or samples are added to the micro-ELISA plate wells and combined with the specific antibody.

B. Kit components

Same the kit components used in E-selectin kits.

C. Reagent preparation

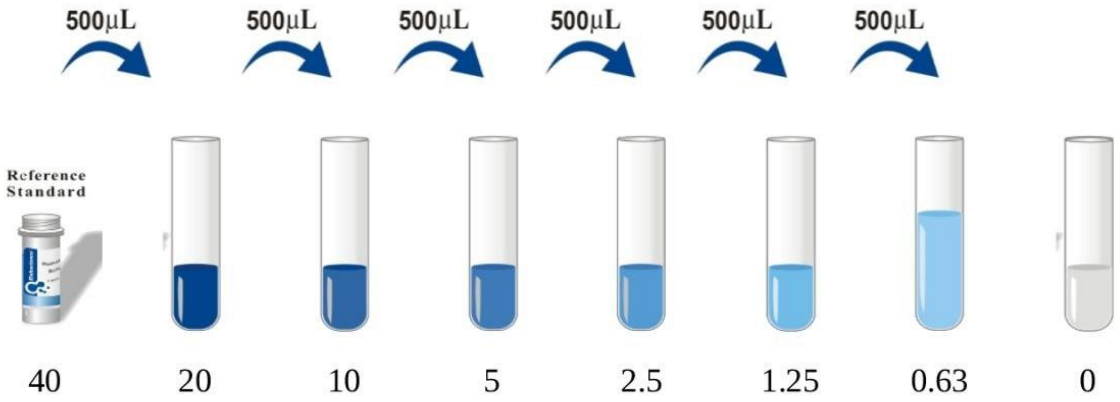


Fig. (2.3): Serial Dilution method for Omentine-1 standard

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2.5.3. Synthesis of chitosan- Cs-adduct

Chitosan takes place using Dean–Stark (Clevenger) apparatus in presence of xylene until the theoretical amount of water was separated. Chitosan product was separated by filtration, washing several times with methanol, hot distilled water, ethanol and then dried in an electric oven at 50◦C and weighed.

2.5.4. Synthesis of chitosan-iron (Cs- Fe_3O_4)NPs

Cs- Fe_3O_4 NPs (5 mg/ml) was dissolved in acetic acid solution (1% w/v) until the solution is clear then, TPP solution was added to Cs- Fe NPs solution with ratios; 1:2.5 (w/w %) with continuous stirring at ambient temperature for 6h. The

production of Cs- Fe_3O_4 NPs /TPP nanoparticles started via the TPP initiated ionic gelation mechanism. These nanoparticles were separated, washed several times then supernatant layer was removed and the precipitate re-suspended in water.

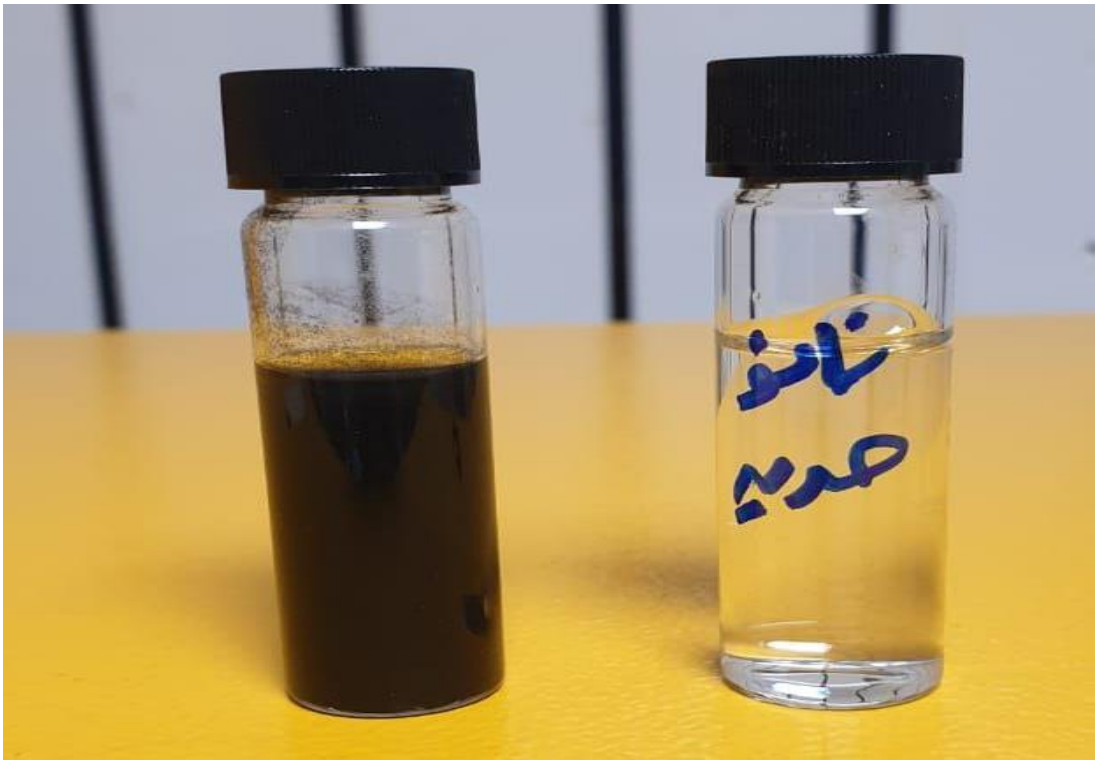


Figure 2.4 : Photograph of a Cs-Fe_3O_4 in deionized water (left one is Fe_3O_4 and the right is Cs-Fe_3O_4)

2.5.5. Characterizations of chitosan iron oxide nanoparticles

UV –Visible Spectrophotometer, atomic absorption, FTIR, Zeta potential, XRD, FEM, and EDX have been used for Chitosan- iron oxide nanoparticles characterization.

A. UV-visible Analysis. The UV- visible absorbance spectral analysis was done by using UVVIS spectrophotometer (SHIMADZU1900, Japan) after diluting a small amount of the sample into distilled water at wave length 330 - 700 nm.

B. Zeta potential. The surface zeta potentials of the magnetic nanoparticles and nanocomposite were measured using a (Zetasizer Nano-ZS DLS particle size analyzer, Malvern)

C. X- Ray diffraction. The phase and crystallinity were analyzed using powder X-ray diffraction (XRD) analysis obtained by an X-ray diffractometer (pw1730, philips, Holland) in the range of 2θ=20–80°. The mean crystallite size of g-Fe3O4 nanoparticles was quantitatively determined from XRD data by employing Debye Scherer's equation: $\mathbf{D = k\lambda/\beta cos\theta}$ Where D is the average crystallite size, K=0.9 is the shape factor, λ is the X-ray wavelength (in nm), θ is the Bragg diffraction angle, and β is the full width at half maximum (FWHM) of the intense peak (in radians).

D. Transmission electron microscope (TEM) Transmission electron microscope (TEM) technique is used to investigate the structure and measure the particle size of the nanocomposite. TEM, Zeiss-EM10C-100 KV, Germany was used.

2.5.6. Applied the Nano particles on pooling serum and whole blood samples

A. 5 serum samples were mixed to prepare pooling samples.
B. 5 whole blood samples were mixed to prepare pooling samples.
C. A series of different concentrations of the Nano particles were prepared.
D. The Nano particles was added to the prepared samples under body temperature conditions.
E. The mixture of Nano particles and samples was incubated for 30 minutes and 60 minutes.
F. The mixture was exposed to a magnetic field after incubation to separate the nanomaterial from the samples.

Statistical Analysis:

- Information from the questionnaire from all participants were entered a data sheet and were assigned a serial identifier number. Multiple entry was used to avoid errors. The data analysis for this work was generated using The Statistical Package for the Social Sciences software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA) and the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. Copyright (2013 – 2020) (1).
- Descriptive statistics was performed on the participants' data of each group. Values were illustrated by n (%) for categorical. The distribution of the data was checked using Shapiro-Wilk test as numerical means of assessing normality.
- The association between the analyzed factors were estimated using odds ratios (ORs) and 95% Confidence Interval Range which calculated by a non-conditional logistic regression.
- Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests with p- values <0.05 (two-side) were considered to be statistically significant.
- The optimal threshold with high specificity and sensitivity for critical cases was detected using receiver operating characteristic (ROC) analysis. It was found out that all the values of P were two-sided, and a $P < 0.05$ was considered to be statistically significant.

Chapter Three

Results

3. Results

3.1 Demographic and clinical characteristics

A total of 100 participated were included in this study, with n=48 samples taken from normal cases acting as controls and patient groups n=52. patients groups were divided into subgroups based on Age, sex, and Duration of disease. The clinical demographic characteristics and laboratory parameters of the study groups were summarized in Table 3.1 Out of n=52 cases n=33 63.5% were males and n=19 36.5% were females. The mean age of the cases was 7.67 years. The age range of participants was between 1-12 years old, 11% of the patient was within 1- 4 years, while 17% of the patient were within the age range 5-8, and 24% of the patients were in the age range 9-12 years.

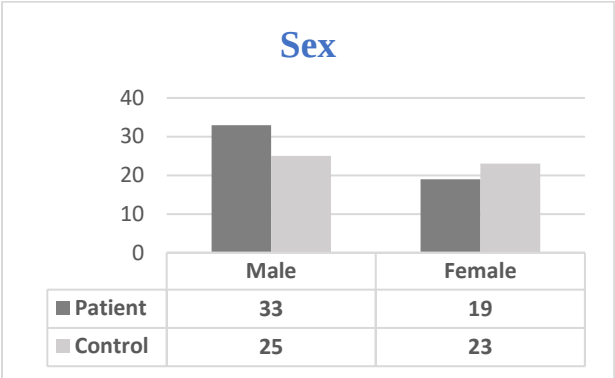
Also, the analysis of data illustrated that most patients 38.46% had having duration of disease of 4 - 8 Years, 34.62% having duration within < one Year , and 26.92% of the patients group had having duration 1-3 Years.

Table 3.1: Description demographic characteristics of sample

Variables	Mean ±SD
Age (Years)	7.67±3.61
Duration of disease (Years)	3.15±2.51

Table 3.2: Descriptive of the demographic characteristics of the study population (N=100).

Variable	Groups	Patient N=52	Control N=48
Age. Groups	1 - 4 Years	11	14
	5 - 8 Years	17	20
	9 - 12 Years	24	14
Sex	Male	33	25
	Female	19	23
Duration of disease	0	0	48
	< one Years	18	0
	1 - 3 Years	14	0
	4 - 8 Years	20	0



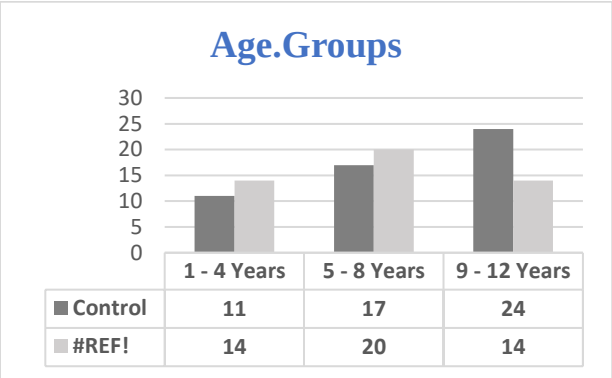


Figure 3.1: Descriptive of the demographic of the patient population for Gender and Age groups (n= 100)

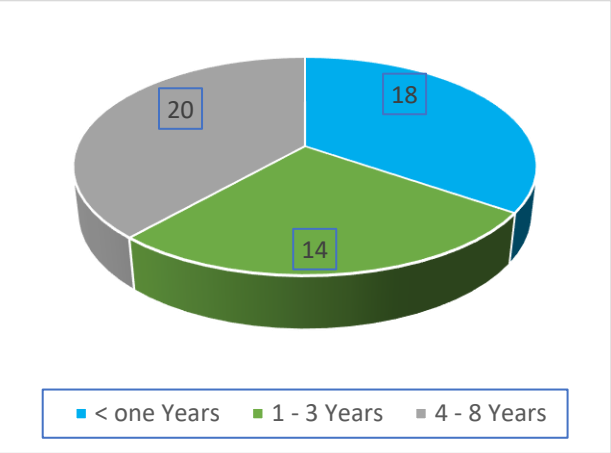


Figure 3.2: Descriptive of the demographic of the patient population for Duration of disease groups (n= 52)

3.2 Examination the level of Selectin, VAP-1 and Omentine-1 in nephrotic syndrome group compared to control

Generally, patients with nephrotic syndrome were shown an increasing range level of the Selectin, and VAP-1 when comparing to the healthy control groups, while the range level of Omentine was decreased compared to healthy control

Results were indicating a highly statistically significant difference in Selectin, and VAP-1 level among groups, The means and standard deviations were presented in (table 3.2). The mean levels of Selectin and VAP-1 in patients were (1967.13±434.75) & (42.27±68.89) pg/dl which was significantly higher than for Control group (1479.13±394.42) & (13.61±3.91) pg/dl,($p \leq 0.001$). The Distribution of serum level of Selectin, VAP-1 and Omentine in patients compared to healthy control group was presented in Figure (3.3)

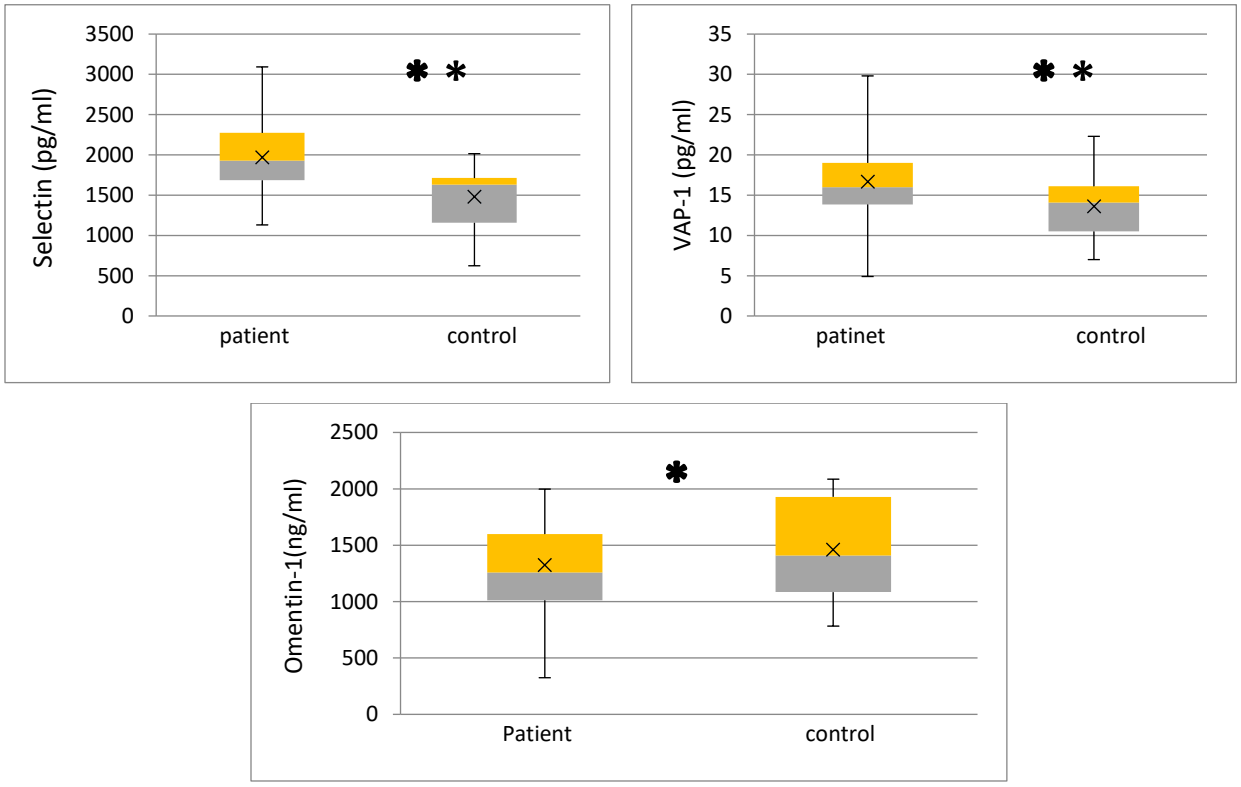


Figure 3.3: Boxplot of the Distribution of serum level of Selectin, VAP-1 and Omentine-1 in nephrotic syndrome group compared to the control (: significant; *= Non-significant)**

Table 3.3: Difference between mean levels of biochemical in nephrotic syndrome with patients and control groups.

Biomarkers	Groups	Mean	SD	SE	Max-Min	P value
Selectine1 (pg/ml)	Patient	1967.13	434.75	60.29	1967.13-434.75	**<0.001 [**]**
	Control	1479.13	394.42	56.93	1479.13-394.42	
VAP-1 (pg/ml)	Patient	42.27	68.89	9.55	42.27-68.89	**0.004 [**]**
	Control	13.61	3.91	0.56	13.61-3.91	
Omentin-1 (ng/ml)	Patient	1354.60	407.38	56.49	1354.60-407.38	0.07 **[*]**
	Control	1516.17	476.42	68.77	1516.17-476.42	
Serum. Albumin (g/l)	Patient	2.62	0.62	0.09	1.2-3.4	**<0.001 [**]**
	Control	4.32	0.36	0.05	3.8-4.9	
Urea (mg/dl)	Patient	27.76	23.60	3.27	1-114	**0.001 [**]**
	Control	16.50	2.50	0.36	12- 22	
Creatinine (mg/dl)	Patient	0.55	0.51	0.07	0.2-3.6	0.34 **[*]**
	Control	0.48	0.15	0.02	0.2-0.8	
Total Cholesterol (mg/dl)	Patient	260.83	122.78	17.03	90-600	**<0.001 [**]**
	Control	126.02	13.52	1.95	90-142	
TG (mg/dl)	Patient	231.98	130.02	18.03	500-519	**<0.001 [**]**
	Control	98.83	15.61	2.25	71-134	
HDL (mg/dl)	Patient	47.02	22.03	3.05	16-136	**0.003 [**]**
	Control	56.75	4.27	0.62	51-65	
LDL (mg/dl)	Patient	167.41	104.24	14.46	42-500.80	**<0.001 [**]**
	Control	49.50	14.40	2.08	8-67	
T -test was *: significant at $p \leq 0.05$ SD: standard deviation; standard error ; **: significant; *= Non-significant.						

3.3 Examination the level of Lipid profile in nephrotic syndrome group compared to control

In particular, the plasma concentrations of cholesterol, triglycerides and apolipoprotein B (ApoB)-containing lipoproteins (including very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) are all elevated in nephrotic syndrome. Results were indicated a significant difference ($P < 0.05$) of all Lipid profile in nephrotic syndrome group compared to control as shown in Figure 3.3, Table 3.3.

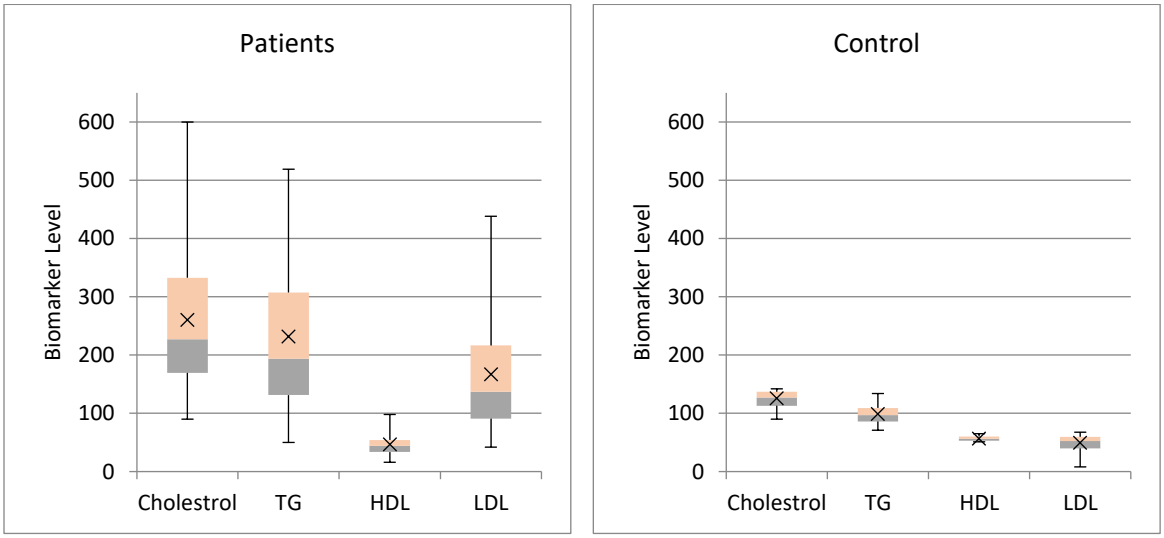


Figure 3.4: Boxplot of the Distribution of serum level of Lipid profile in nephrotic syndrome group compared to control

3.4 Examination the level of atherogenic indices in nephrotic syndrome group compared to control

The risk of dyslipidemia complication among children with nephrotic syndrome need to be considered, this study was focus on the detection of atherogenic indices in patient group and compered to healthy control. Results indicated that there were a massive increased and significant differences in the mean level of Atherogenic coefficient (AC), Atherogenic index of plasma (AIP), Castelli's risk indexes (CRI) (I & II), and Cholesterol index (C-index) that measured in the patient group when compared to healthy control, as presented in Figure 3.5 & 3.6

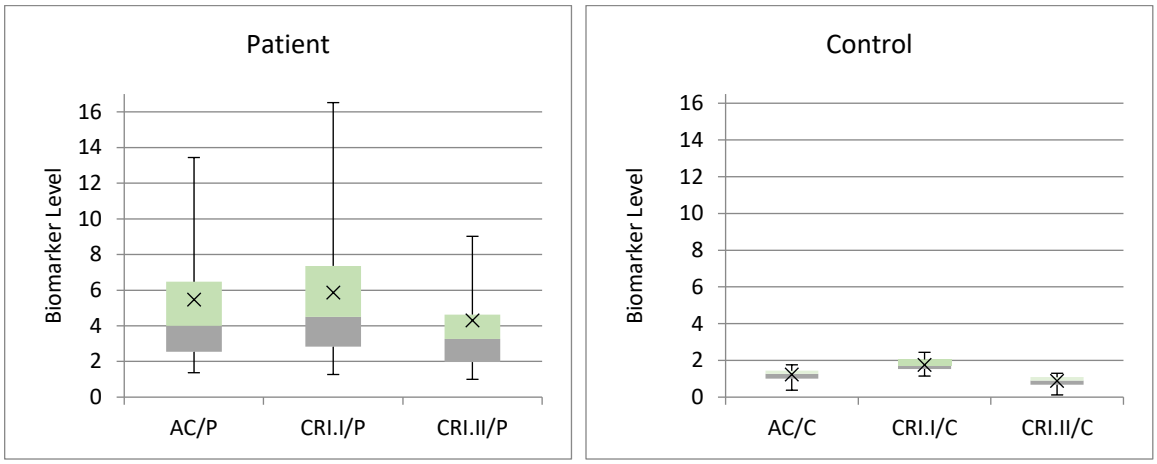


Figure 3.5: Boxplot of the Distribution of serum level of Atherogenic coefficient (AC) & Castelli's risk indexes (I & II) in nephrotic syndrome group compared to control

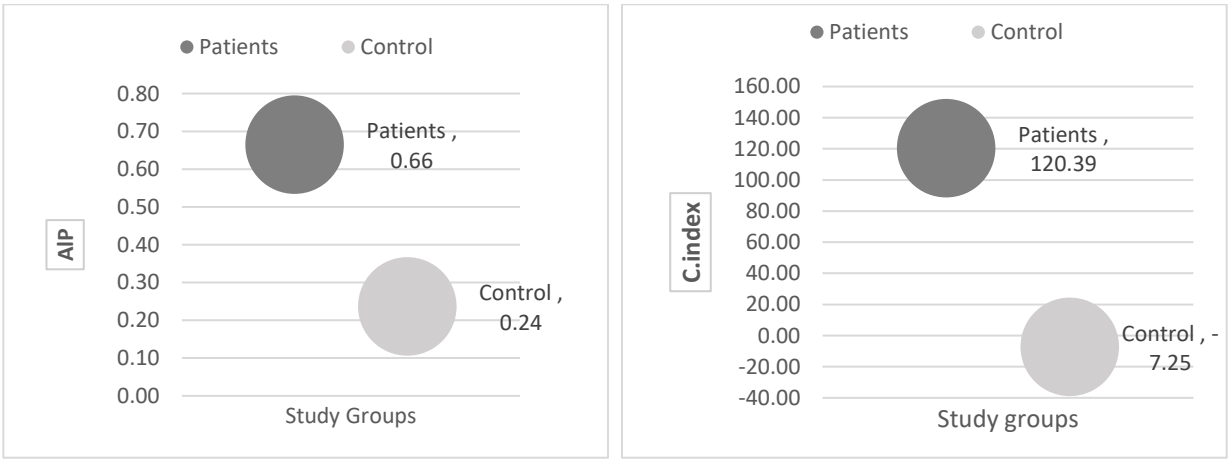


Figure 3.6: Mean differences of serum level of Atherogenic index of plasma (AIP) & Cholesterol index (C-index) in nephrotic syndrome group compared to control

3.5 Difference between the level of biochemical in nephrotic syndrome with the duration of disease.

The biomarkers levels were examined based on the duration of nephrotic syndrome. Generally, there was insignificant differences in the mean level of Selectin, VAP-1, and Omentine-1. For selectin, the mean level was increased slightly (20.58pg/dl) in the duration of nephrotic syndrome through (1-3 Years) compare to the group who were their duration less than one year and the group having duration of nephrotic syndrome (4-8Years), but all biomarkers were insignificant p value > 0.05, as shown in figure (3.6)

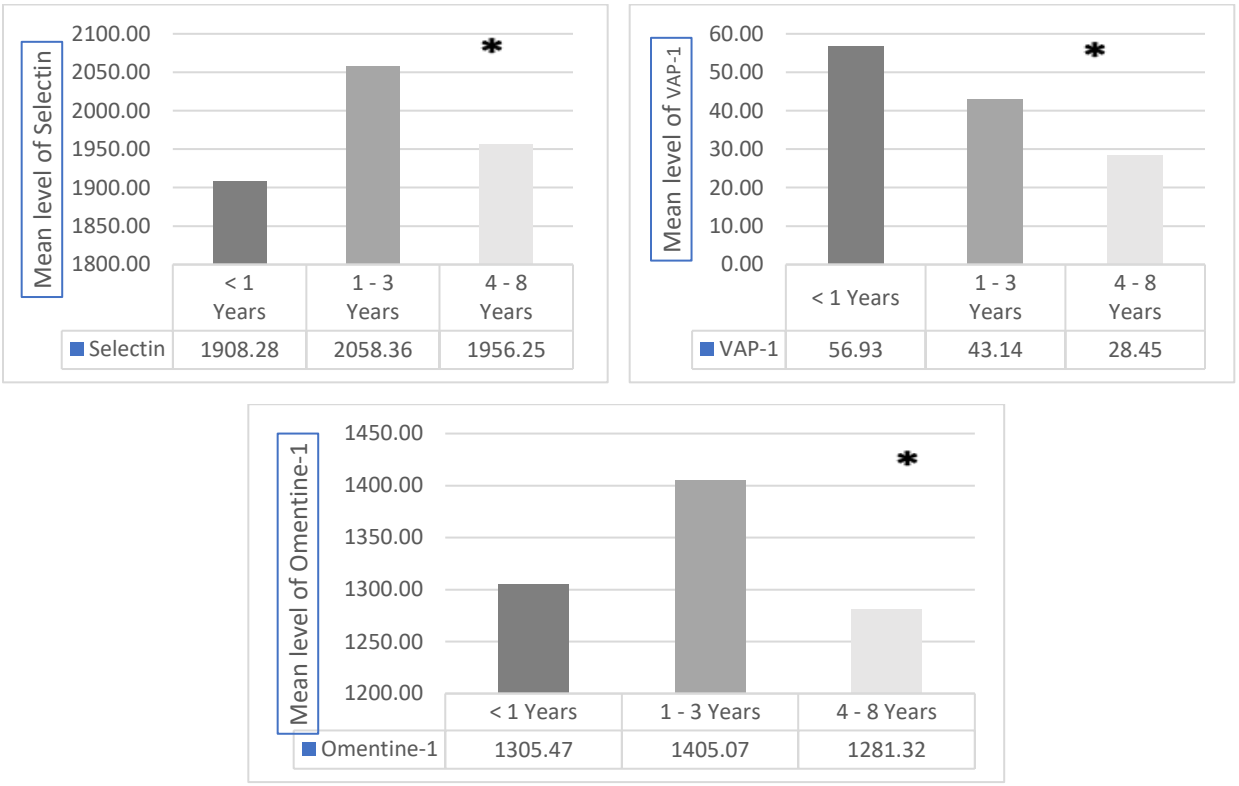


Figure 3.7: Difference between mean levels of biochemical in nephrotic syndrome with duration Group 1 (< one Years), Group 2 (1 -3 Years), Group 3 (4 - 8 Years), (ANOVA-test was **= significant at p ≤ 0.05, * = Non significant).

3.6 Difference between the level of lipid profile in nephrotic syndrome with the duration of disease.

In this study, the lipid profile levels were also examined based on the duration of nephrotic syndrome. Generally, patients with nephrotic syndrome were shown an increasing level of all lipid panel, only levels of cholesterol and LDL were increased significantly with increasing the duration of diseases as presented in figure (3.8).

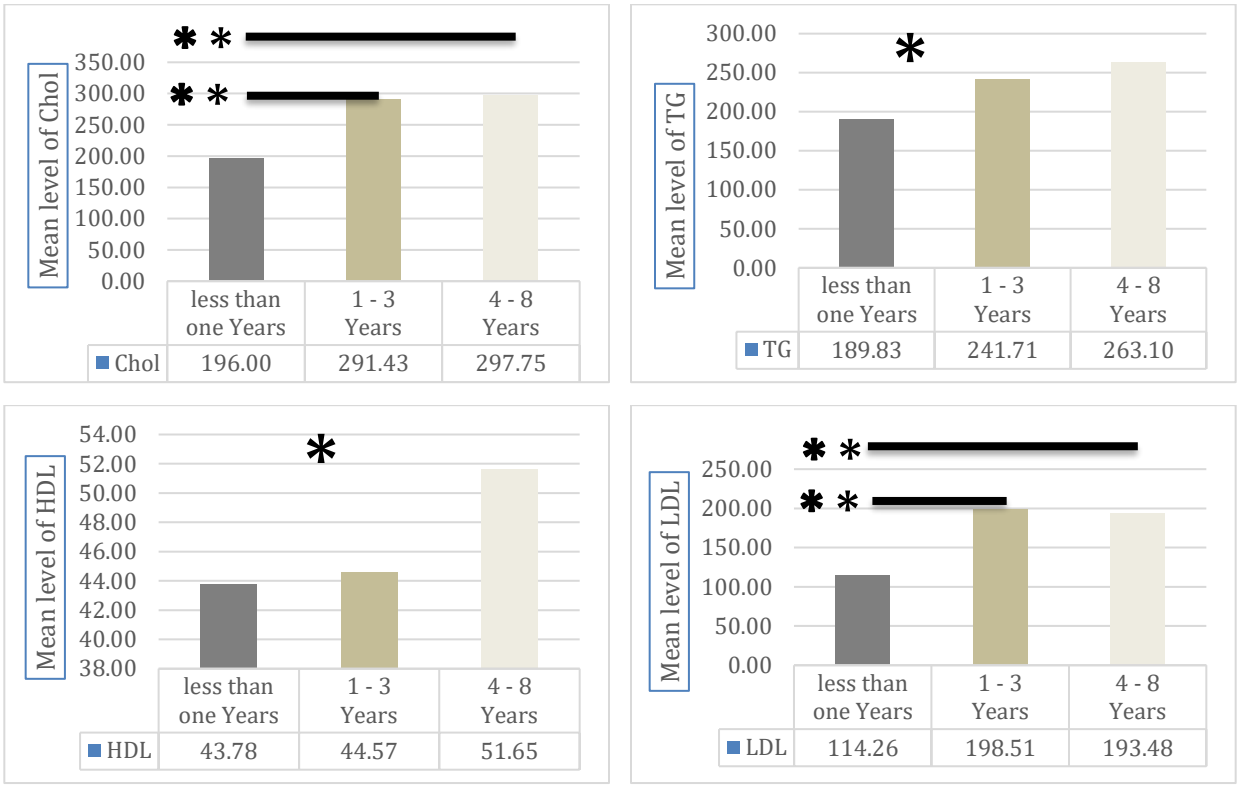


Figure 3.8: Difference between mean levels of lipid profile in nephrotic syndrome with duration Group 1 (< one Years), Group 2 (1 -3 Years), Group 3 (4 - 8 Years), (ANOVA-test was **= significant at $p \leq 0.05$, * = Non significant).

3.7 Difference between the level of atherogenic indices in nephrotic syndrome with the duration of disease.

This study was examined the atherogenic indices based on the duration of nephrotic syndrome. Generally, patients with nephrotic syndrome were shown a marked differences in the mean level of AC, AIP, CRI.I, CRI.II and C.index when comparing the duration of disease. Nephrotics are at an increased risk for

cardiovascular disease due to the hyperlipidaemic state. The risk varies depending on the type of lipid abnormalities. **(Yamagata, *et.,al.* 2007)**.

Results was indicated that C. index were increased significantly when compared patient's groups who have duration less than on year with group who have duration 1-3 years, P value less than 0.05 as presented in figure (3.9)

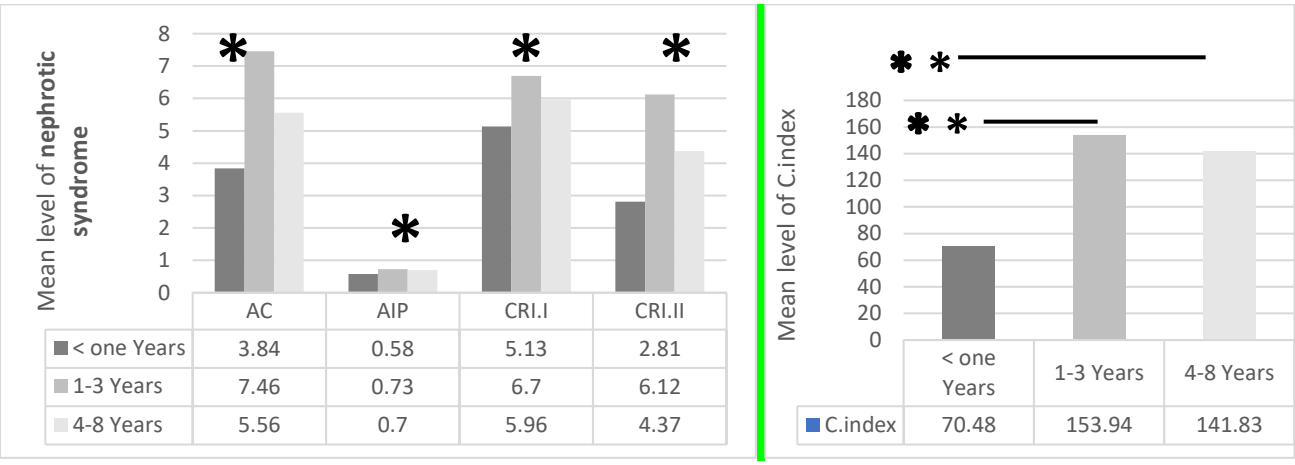


Figure 3.9: Difference between mean levels of atherogenic index in nephrotic syndrome with duration Group 1 (< one Years), Group 2 (1 -3 Years), Group 3 (4 - 8 Years), (ANOVA-test was **= significant at p ≤ 0.05, * = Non significant).

3.8 Difference between the level of biochemical, lipid profile and atherogenic index in nephrotic syndrome based on gender groups.

Table (3.3) illustrated the mean level of the biochemical parameters in the patients and control groups according to the gender. Results were shown that male patients were illustrated a significant decreased in the mean levels of Omentine compared to control, p values were <0.05, while female patients were insignificantly different compared to control group, P value >0.05.

results were also shown that the levels of E-Selectin & VAP.1 which increased markedly in both gender of patients group in compared to healthy control, p values were <0.05. few data are available regarding these risks in children.

Therefore, this study was also examined the lipid profile panel based on gander groups. Results were indicated that cholesterol, TG and LDL were increased significantly in both male and female children compared to healthy control, p value <0.001. as presented in Table (3.3)

Consequently, the dyslipidaemia of nephrotic syndrome is probably often under-treated, particularly in children, of whom very few would have pre-existing hyperlipidaemia from other causes.

Therefore, our study was also focus on the estimation of Atherogenic coefficient (AC) , Atherogenic index of plasma (AIP) , Castelli's risk indexes (I & II) , and Cholesterol index (C-index) and investigated their differences based on gender groups. Results were shown a massive increased in the mean levels of atherogenic indices in both gender compared to health control group as presented in Table (3.3)

Table 3.4:The effect of gender with the biochemical parameters according to the Patients and control groups.

Biomarker	Male			Female		
	Patients N=33	Control N=25	P value	Patients N=19	Control N=23	P value
Seletine	20.18±4.55	15.97±2.57	**<0.001 ****	18.78±3.92	13.51±4.77	**<0.001****
VAP.1	15.98±5.34	13.22±3.87	**0.033 ****	20.77±8.53	14.03±3.99	**0.002****
Omentine	1299.24±386.21	1568.52±490.13	**<0.001****	1450.73±435.50	1459.26±465.12	**<0.001****
T -test was *: significant at p ≤ 0.05 SD: standard deviation; **: significant; *= Non-significant.						

Biomarker	Male			Female		
	Patients N=33	Control N=25	P value	Patients N=19	Control N=23	P value
cholestrol	244.45±107.93	125.36±11.67	**<0.001 ****	289.26±143.74	126.74±15.51	**<0.001****
TG	224.70±126.37	98.36±16.78	**<0.001****	244.63±138.71	99.35±14.58	**<0.001****
HDL	45.09±18.09	55.76±4.10	**0.002****	50.37±27.83	57.83±4.27	0.26 *

LDL	154.42±97.01	49.93±11.75	**<0.001****	189.97±114.94	49.04±17.08	**<0.001****
T -test was *: significant at p ≤ 0.05 SD: standard deviation; **: significant; *= Non-significant.						

Biomarker	**Male**			**Female**		
	Patients N=33	**Control N=25**	**P value**	**Patients N=19**	**Control N=23**	**P value**
AC	5.41±4.66	1.26±0.31	**<0.001****	5.58±4.30	1.21±0.35	**<0.001****
AIP	0.66±0.33	0.24±0.09	**<0.001****	0.67±0.27	0.23±0.08	**<0.001****
CRI.I	6.06±4.65	1.78±0.36	**<0.001****	5.54±3.38	1.73±0.33	**<0.001****
CRI.II	4.20±3.97	0.91±0.26	**<0.001****	4.47±3.84	0.86±0.32	**<0.001****
C.index	109.33±102.05	-5.83±14.69	**<0.001****	139.60±112.41	-8.78±19.71	**<0.001****
T -test was *: significant at p ≤ 0.05 SD: standard deviation; **: significant; *= Non-significant.						

3.9 Difference between the level of biochemical parameters in nephrotic syndrome based on age groups

In Figure (3.10) a comparison of serum level of Selectin in different age groups was performed. The level of Selectin increasing in case compered to control within the age groups ((1-4), (5-8) and (9-12)) years with a mean value of 2058.27, 2002.29 and 1900.46 (pg/ml), A highly statistically significant differences were found in all age groups

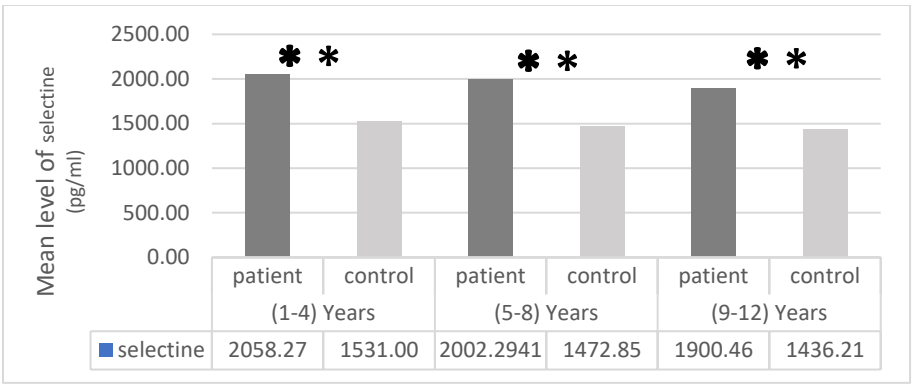


Figure 3.10: Mean differences of the measured Selectine in children compared to control based of Age group, (T-test was **= significant at p ≤ 0.05, * = Non significant)

In Figure (3.11) a comparison of serum level of VAP-1in different age groups was performed. The level of VAP-1 increasing in case compered to control within the age groups ((1-4), (5-8) and (9-12)) years with a mean value of 56.75, 69.12 and 16.61 (pg/ml), A highly statistically significant differences were found in (5-8)and (9-12) age groups

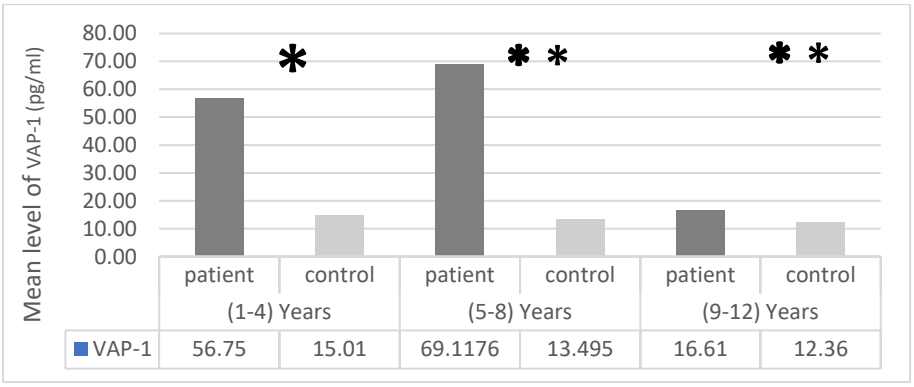


Figure 3.11: Mean differences of the measured VAP-1 in children compared to conreol based of Age group, (T-test was **= significant at p ≤ 0.05, * = Non significant)

In Figure (3.12) a comparison of serum level of omentine-1 in different age groups was performed. The level of omentine-1 decreasing in case compered to control within the age groups ((1-4), (5-8) and (9-12)) years with a mean value of 1311.10, 1343.3125 and 1316.88 (pg/ml), finally, found in all age groups no-significant

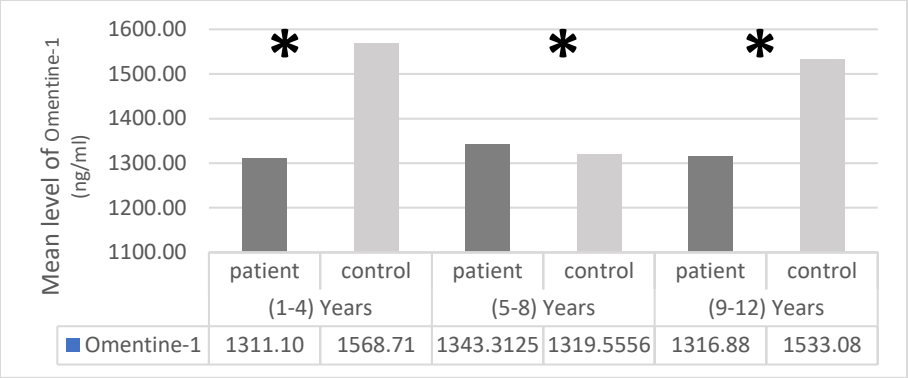


Figure 3.12: Mean differences of the measured Omentine-1 in children compared to control based of Age group, (T-test was **= significant at p ≤ 0.05, * = Non significant).

3.10 Difference between the level of atherogenic index in nephrotic syndrome based on age groups

In Figure (3.12) a comparison of serum level of AC in different age groups was performed. The level of AC increasing in case compered to control within the age groups ((1-4), (5-8) and (9-12)) years with a mean value of 5.80, 5.310 and 5.59 (pg/ml), A highly statistically significant differences were found in all age groups

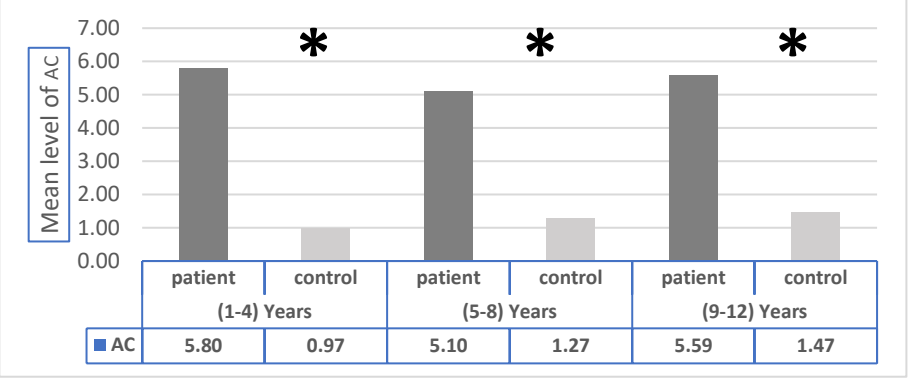


Figure 3.13: Mean differences of the measured AC in children with nephrotic syndrome compared to control based of Age group, (T-test was **= significant at p ≤ 0.05, * = Non significant).

In Figure (3.13) a comparison of serum level of AIP in different age groups was performed. The level of AIP increasing in case compered to control within the age

groups ((1-4), (5-8) and (9-12)) years with a mean value of 5.80, 5.310 and 5.59 (pg/ml), A highly statistically significant differences were found in all age groups

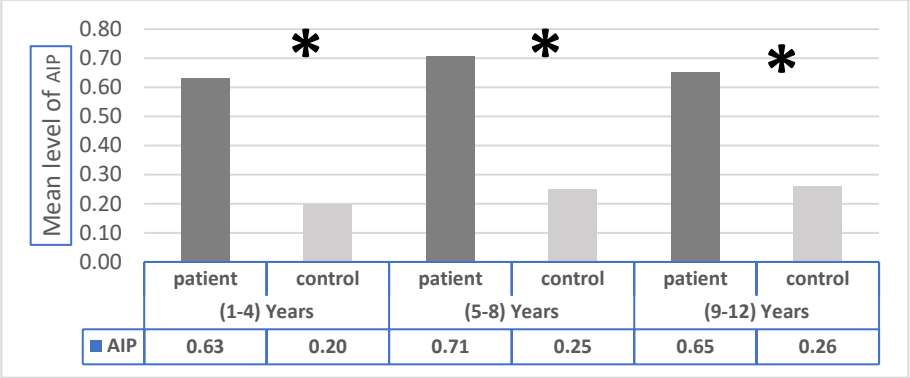


Figure 3.14: Mean differences of the measured AIP in children with nephrotic syndrome compared to control based of Age group, (T-test was **= significant at p ≤ 0.05, * = Non significant).

In Figure (3.14) a comparison of serum level of AC in different age groups was performed. The level of AC increasing in case compered to control within the age groups ((1-4), (5-8) and (9-12)) years with a mean value of 5.88, 6.06 and 5.73 (pg/ml), A highly statistically significant differences were found in all age groups

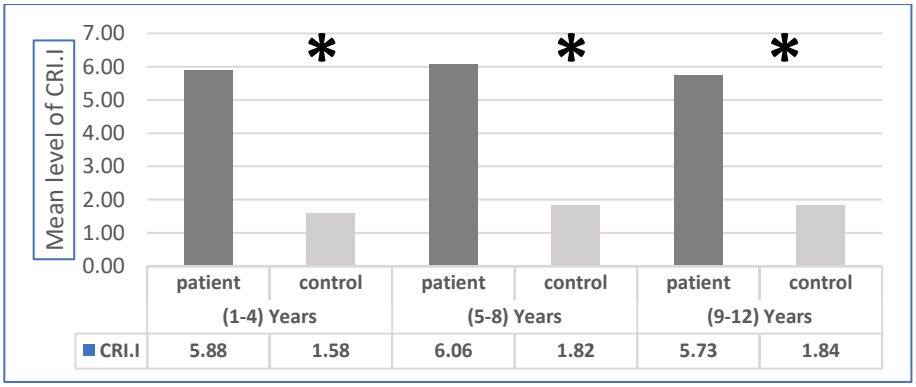


Figure 3.15: Mean differences of the measured CRI.I in children with nephrotic syndrome compared to control based of Age group, (T-test was **= significant at p ≤ 0.05, * = Non significant).

In Figure (3.15) a comparison of serum level of CRI.III in different age groups was performed. The level of CRI.III increasing in case compered to control within the age groups ((1-4), (5-8) and (9-12)) years with a mean value of 4.63, 3.89 and 4.44 (pg/ml), A highly statistically significant differences were found in all age groups

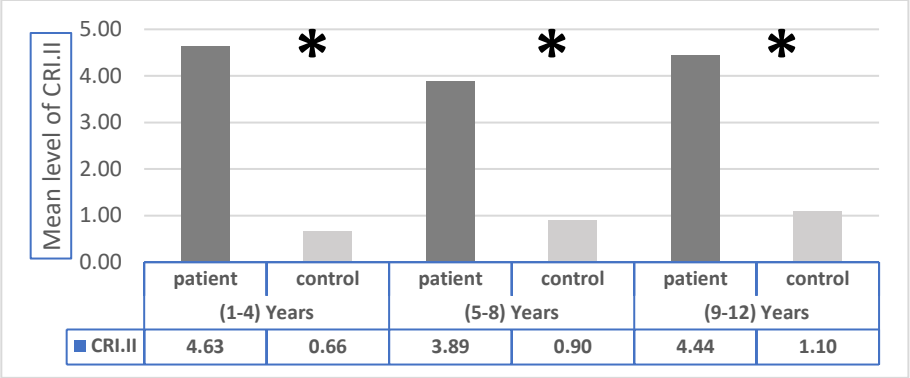


Figure 3.16: Mean differences of the measured CRI.II in children with nephrotic syndrome compared to control based of Age group, (T-test was **= significant at p ≤ 0.05, * = Non significant).

In Figure (3.16) a comparison of serum level of C.index in different age groups was performed. The level of C.index increasing in case compered to control within the age groups ((1-4), (5-8) and (9-12)) years with a mean value of 99.65, 101.80 and 143.07 (pg/ml), A highly statistically significant differences were found in all age groups

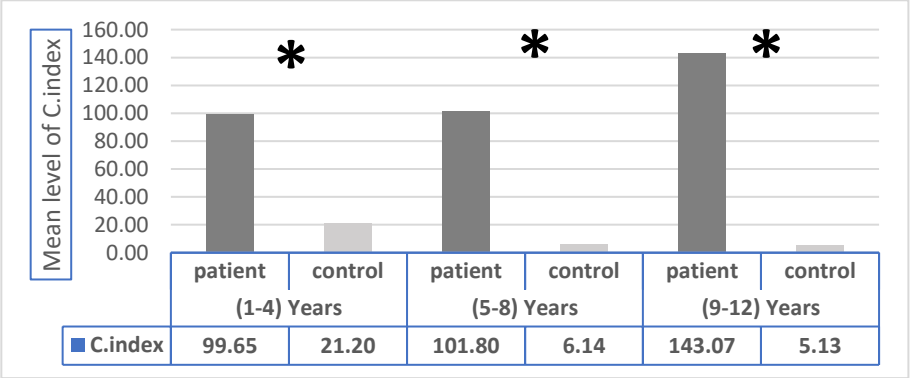


Figure 3.17: Mean differences of the measured C.index in children with nephrotic syndrome compared to control based of Age group, (T-test was **= significant at p ≤ 0.05, * = Non significant).

3.11: Study the association of biomarkers with patients' groups

Binary logistic regression was performed to analyze the associating of the Selectin VAP-1 and Omentine-1 with the nephrotic syndrome. The biomarkers (Selectin, VAP-1 and Omentine) showed a highly significant differences in patient and represented as a risk factor (OR: 1.004; 95% CI: (0.998-1.023), OR: 1.002; 95% CI: (1.000-1.004) and OR: 1.003; 95% CI: (1.002-1.005) respectively, as shown in table (3.4).

Table 3.4: Estimation the Associated of analyzed factors (Selectin, VAP-1 & Omentine-1) in nephrotic syndrome Patients Compared to control group

Biomarkers	OR(Lower–Upper)	P value
Selectin	1.004(0.998-1.023)	<0.001 * *
VAP-1	1.002(1.000-1.004)	0.003 * *
Omentine-1	1.003(1.002-1.005)	<0.001 * *
Results are presented [**]; Significant, [*]; Non significant, OR: Odds Ratio, CI; Confidence Interval,		

Binary logistic regression was performed to analyze the associating of the AC, AIP, CRI.I, CRI.II and C.index with the nephrotic syndrome. It was found that the biomarkers (AC, AIP, CRI.II and C.index) shown a highly significant differences in patient and represented as a risk factor (OR: 115.65, OR: 62.817, OR: 89.041 and OR: 110.266) respectively, as shown in table (3.5).

Table 3.5: Estimation the Associated of analyzed factors (AC, AIP, CRI.I, CRI.II and C.index) in nephrotic syndrome Patients Compared to control group

Biomarkers	OR	P value
AC	115.65	<0.001 * *
AIP	62.817	<0.001 * *
CRI.I	1.22	0.271 *

CRI.II	89.041	<0.001 * *
C.index	110.266	<0.001 * *
Results are presented [**]; Significant, [*]; Non significant, OR: Odds Ratio, CI; Confidence Interval,		

3.12: Receiver Operating Characteristic Analysis

ROC curve and AUC analysis for the Selectin and Omentine for Patients compared to control group were performed. Results of the receiver operating curve (ROC) curve and AUC analysis for the Selectin and VAP-1 diagnostic parameters were shown that Selectin and VAP-1 have a good performance for prediction nephrotic syndrome patients, data are presented in Figures (3.17) and table (3.6) & (3.7)

For Selectin levels: (sensitivity = 76.9%, specificity 97.9%) at a level = 675.5 , VAP-1 levels (sensitivity 64.4%, specificity 70.8%) at a level = 15.05, the p-values of the AUC were <0.01 and highly statistically significant. results confirmation of the Sensitivity & Specificity were confirmed using Youden's J statistics to the parameters.

Table 3.6: Receiver operating characteristic curve showing sensitivity and specificity of biomarkers of Selectin & Omentine in patients compared to control group

Variable	AUC	Sensitivity %	Specificity %	P value	Cut-off points	Youden index	CI (95%)
Selectin	83%	76.90%	97.90%	0.007 * *	675.5	0.748	0.789-0.948
VAP-1	77.2%	64.4%	70.80%	0.001* *	15.05	0.362	0.599-0.802
Omentine.1	36.5%	97%	15.4%	0.181 *	827.5	0.154	0.145-586
Results are presented [**]; Significant, [*]; Non significant							

Table 3.7: Receiver operating characteristic curve showing accuracy, positive & negative predictive values of biomarkers in patients compared to control group

Variable	P value	PPV	NPV	Accuracy
Selectin	<0.001 * *	95.24%	79.31%	86%
VAP-1	<0.001 * *	70.80%	65.40%	68%
Results are presented [**]; Significant, [*]; Non significant, PPP= Positive protective value, NPV= Negative protective value				

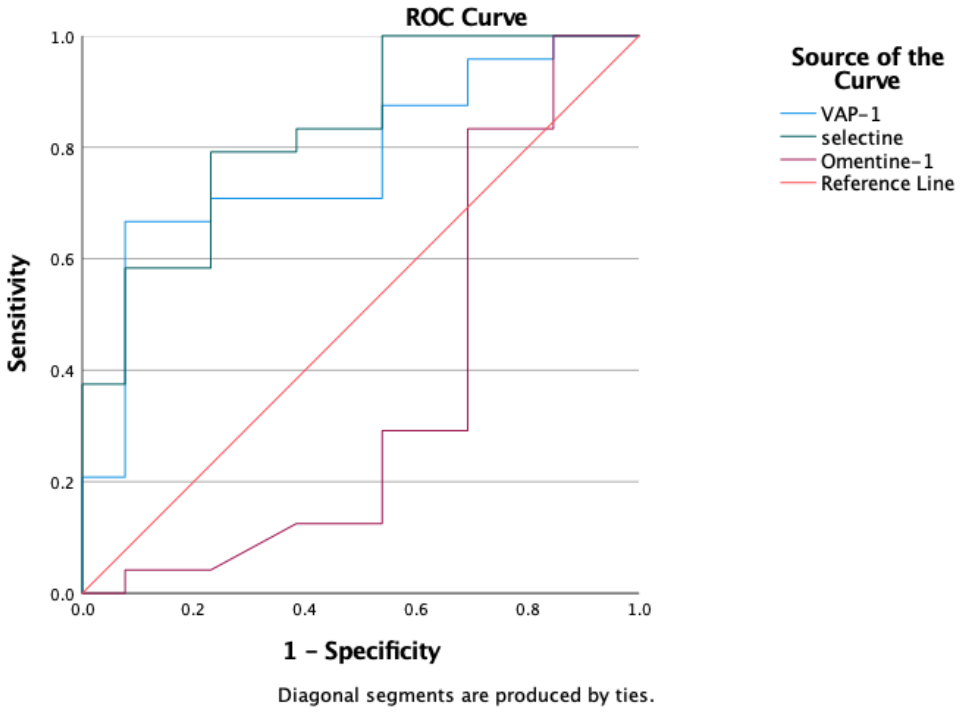


Figure 3.18: Receiver operating characteristics (ROC) curve analysis of S.Selectine and Omentine levels in Patient and Control, The area under ROC curve: 80% and 72% respectively.

Furthermore, the analysis of the optimal diagnostic points for predicting a complication of dyslipidemia in nephrotic syndrome cases was performed.

Diagnostic thresholds point of the Atherogenic indices were presented in Table (3.8) & (3.9), results were indicated that most of the measured indices were shown a high Sensitivity & Specificity toward the study case, p values were < 0.001. To the best of our knowledge, this is a detailed study about the analysis of the optimal diagnostic points for predicting nephrotic syndrome cases.

Table 3.8: Receiver operating characteristic curve showing sensitivity and specificity of Atherogenic indices in patients compared to control group

Variable	AUC	Specificity %	Sensitivity %	P value	Cut-off points	Youden index	CI (95%)
AC	98.3%	98 %	94.20%	<0.001* *	1.7976	0.942	(0.962-1.000)
AIP	90.4%	97 %	82.70%	<0.001* *	0.3872	0.827	(0.835-0.973)
CRI.I	90.4%	99 %	82.70%	<0.001* *	2.4388	0.827	(0.835-0.973)
CRI.II	97.8%	96 %	90.40%	<0.001* *	1.3042	0.904	(0.955-1.000)
C.index	98%	95%	92.30%	<0.001* *	18.9	0.923	(0.957-1.000)
Results are presented [**]; Significant, [*]; Non significant							

Table 3.9: Receiver operating characteristic curve showing accuracy, positive & negative predictive values of Atherogenic indices in patients compared to control group

Variable	P value	PPV	NPV	Accuracy
AC	<0.001 * *	99%	94.12%	97%
AIP	<0.001 * *	91.5%	83%	87%
CRI.I	0.271 * *	53.93%	63.64%	55%
CRI.II	<0.001 * *	95.92%	90.2%	93%
C.index	<0.001 * *	99%	96%	96%
Results are presented [**]; Significant, [*]; Non significant, PPP= Positive protective value, NPV= Negative protective value				

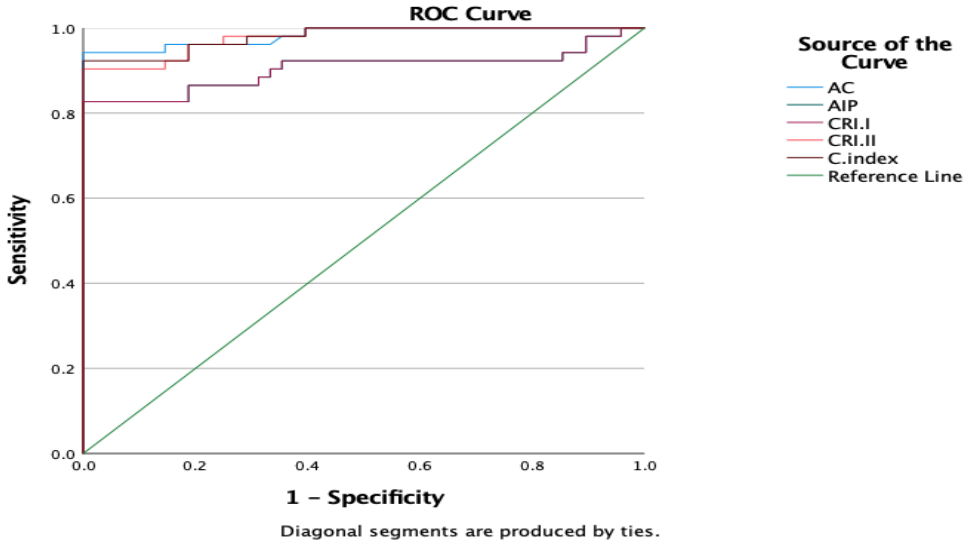


Figure (3.19): ROC curves for, Atherogenic indices in nephrotic syndrome patients to analyse the optimal diagnostic points for predicting such cases compared to control group.

3.13. Preparation and study the structural features of chitosan/iron oxide nanoparticles CS-Fe3O4 NPs

Magnetic Fe_3O_4–chitosan nanoparticles were prepared by the covalent binding of chitosan (CTS) onto the surface of magnetic Fe_3O_4 nanoparticles. Interesting characteristics of chitosan include its polycationic nature, biodegradability, biocompatibility, bioactivity, and nontoxicity were the main motivation behind this work. This study was also including the preparation and study the structural features of chitosan/iron oxide nanoparticles CS-Fe3O4 NPs and examine the applications of chitosan nanoparticles as a lipid-lowering agent in children with nephrotic syndrome

The Iron oxide-loaded chitosan nanoparticles (CS- Fe_3O_4 NPs) were synthesized according to a previous study related to **(El- Ghaffar, *et., al*.2010)**. The nanoparticles were produced by interactions between the positively charged chitosan and the negatively charged phosphate groups of TPP in the ionic gelation technique.

The microstructures of synthesized nanoparticles were investigated by SEM, as can be seen in Fig. (3.20), CS MNPs structures were successfully fabricated and within the particle sizes around (33-55) nm. Scanning electron microscope analysis of prepared chitosan nanoparticles shows the distribution and nanoparticles size. The results of SEM images show that synthesized CS- Fe_3O_4 NPs were smooth, spherical particles, singular or in aggregates with particle sizes in the range of (35.85), (36.99), and (55.91 nm).

The formation of Fe_3O_4 NPs and CS- Fe_3O_4 nanocomposite was monitored by UV-Visible spectroscopy. The spectral analysis was carried out in the range of 200-1000 nm. The synthesized Fe_3O_4 NPs exhibited spectral absorbance shift at 268 nm. It was reported earlier that the spectral absorbance around 250 nm is a characteristic feature of Fe_3O_4 NPs **.**

The interaction and structural changes of functional groups that used to prepare the nanoparticles were examined by FTIR spectroscopy and the results are shown in Fig. (3.22) and (3.23). The FTIR spectrum of Fe_3O_4 NPs and CS-Fe_3O_4 nanocomposite indicating that the CS-Fe_3O_4 was synthesized purely.

In the (CS-Fe_3O_4 **)** nanocomposite FTIR spectrum, the highest FTIR peaks obtained at 3437 cm-1 attributed to O-H and C-H stretch vibrations of phenols and alkynes. FTIR spectral bands at 1643, 1366, 1512, 1126, 906 and 839 cm-1 respectively corresponds to N-H bend, N-O, C-H, O-H and C=O stretching of amino (-NH2) , nitro compounds, alkyl groups phenols (O-H stretch) and carboxylic (–COOH) groups***)*** **(S. Vasantharaj, *et., al*.2018)**.

The other short peaks observed from 500 to 800 cm^{-1} were assigned to the presence of metal-oxygen (Fe-O) **(P. Rajiv, *et., al*.2017)**. The Presence of other functional groups like amino, nitro and alkyl groups may be derived from chitosan ($C_{56}H_{103}N_9O_{39}$) (Fe-O) **(D. Bharathi, *et., al*.2019)**.

The surface charges of the prepared drug delivery systems were determined by zeta potential measurements.

While the synthesized iron oxide nanoparticles exhibit negative charged surfaces with zeta potentials, the surface charge was dramatically changed to positive values (+ 18.3 mV). This confirms the presence of -NH2 groups on the surface of CS-Fe3O4 nanocomposite in protonated form, and thus establishing the presence of chitosan on the prepared nanocomposite fig (3.24) **(D. Bharathi, *et., al*.2019)**.

Similar findings were documented by Shi et al. who have reported positive zeta potential surface charge of CS- Fe_3O_4 NPs **(S.F. Shi, *et., al*.2012)**.

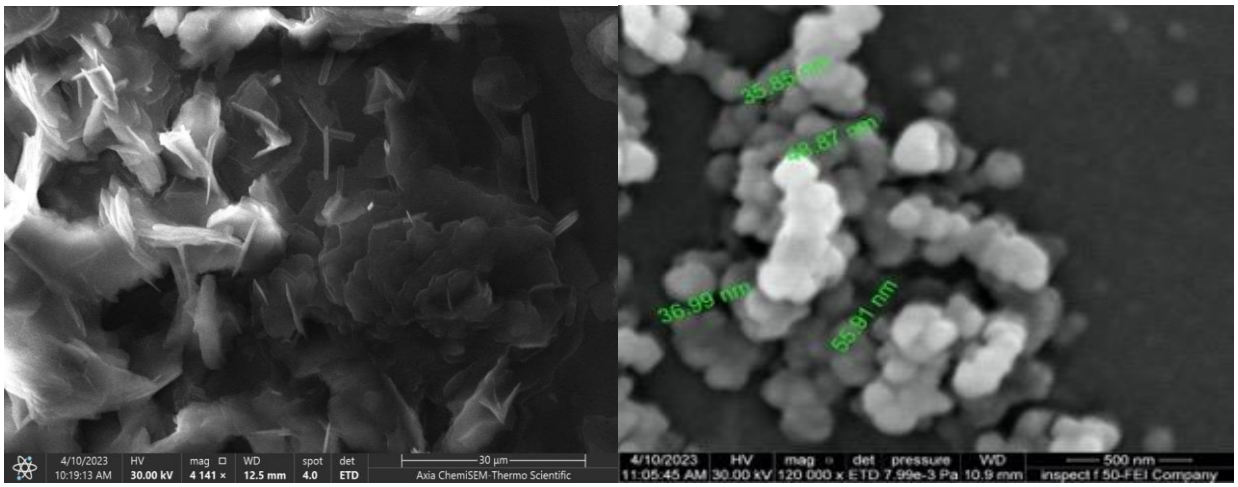


Figure 3.20: Scanning electron microscopy (SEM) shows the morphology (the left fig.) and size (the right fig.) of biogenic nanoparticles (CS-Fe_3O_4 NPs)

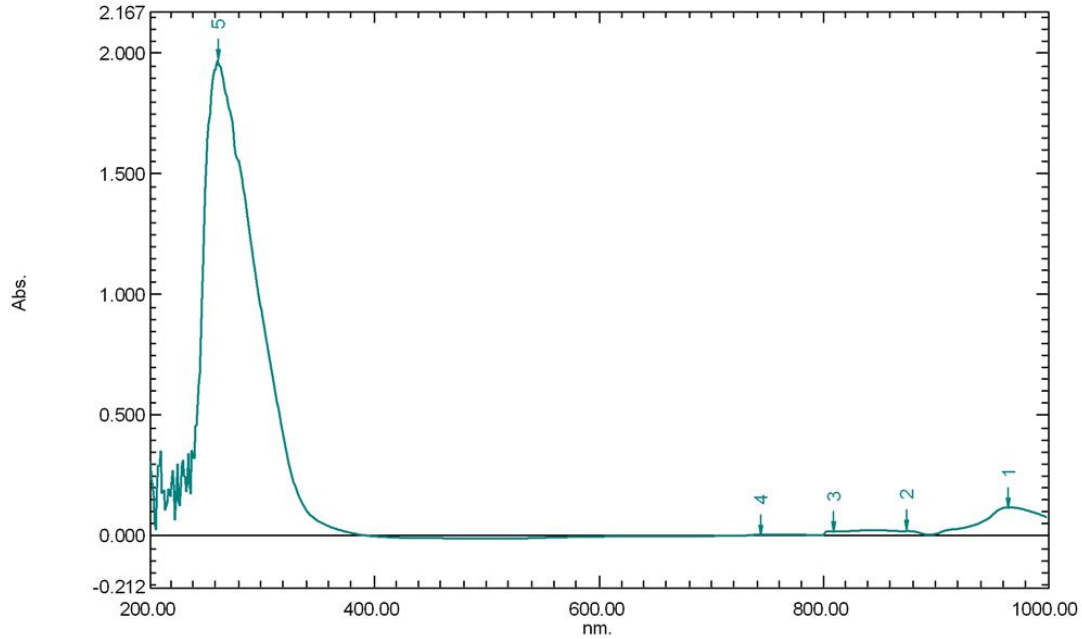


Figure (3.21) spectrum peak of chitosan iron oxide in UV-Vis

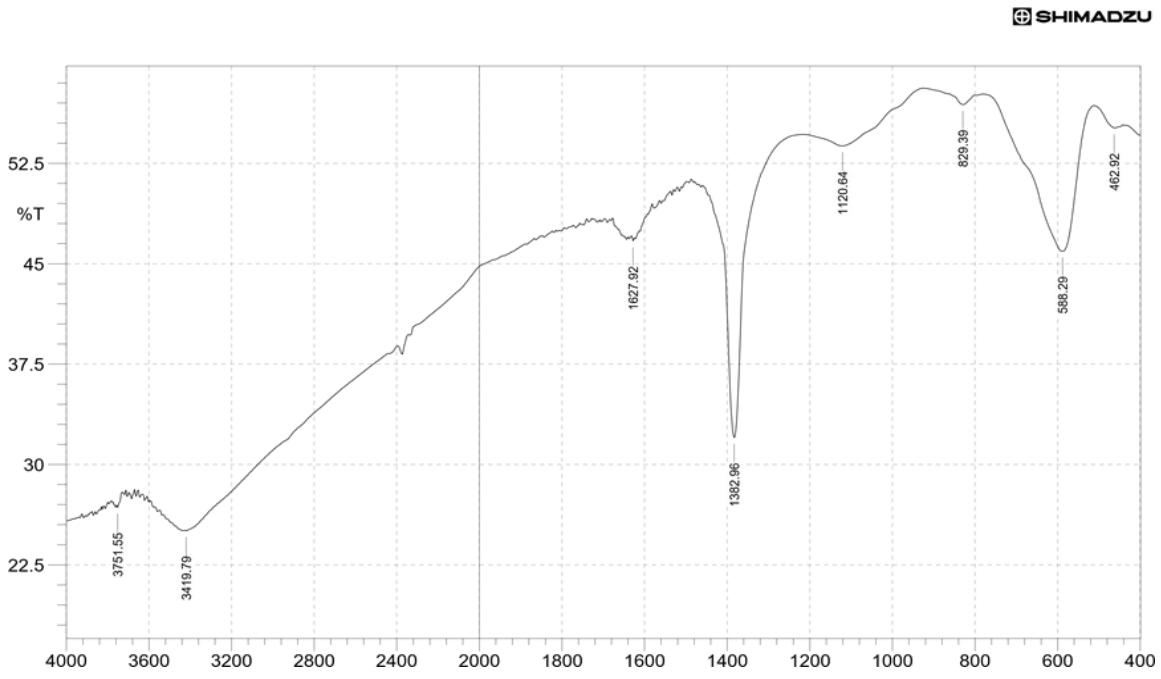


Figure (3.22) FTIR of (Fe_3O_4) nanoparticle

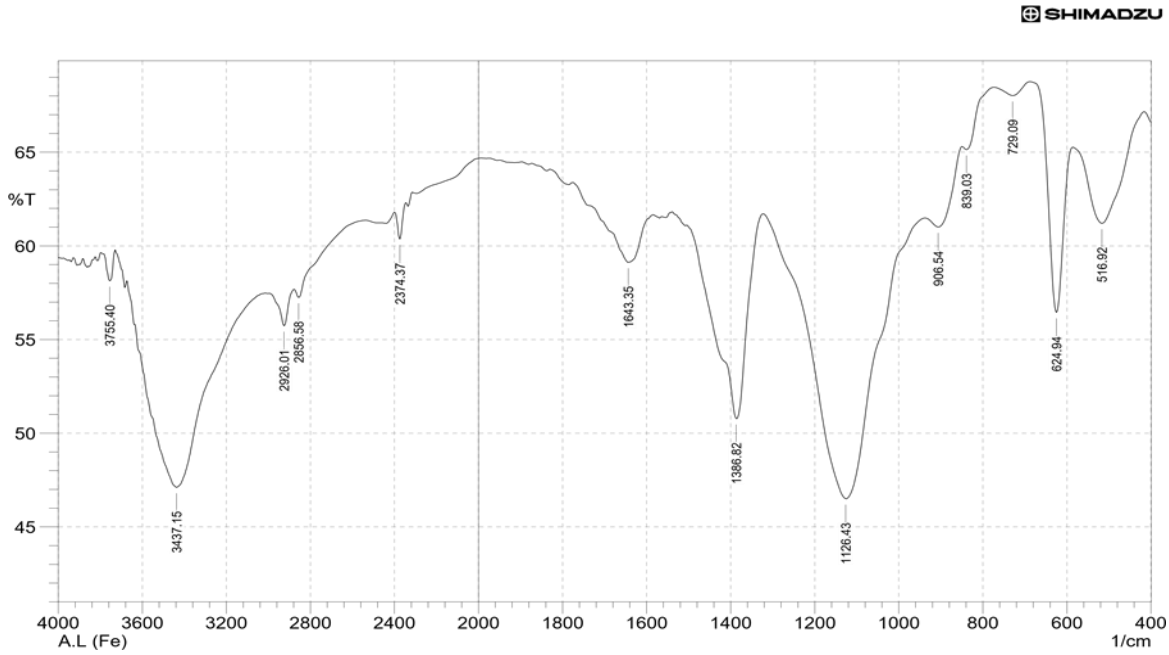
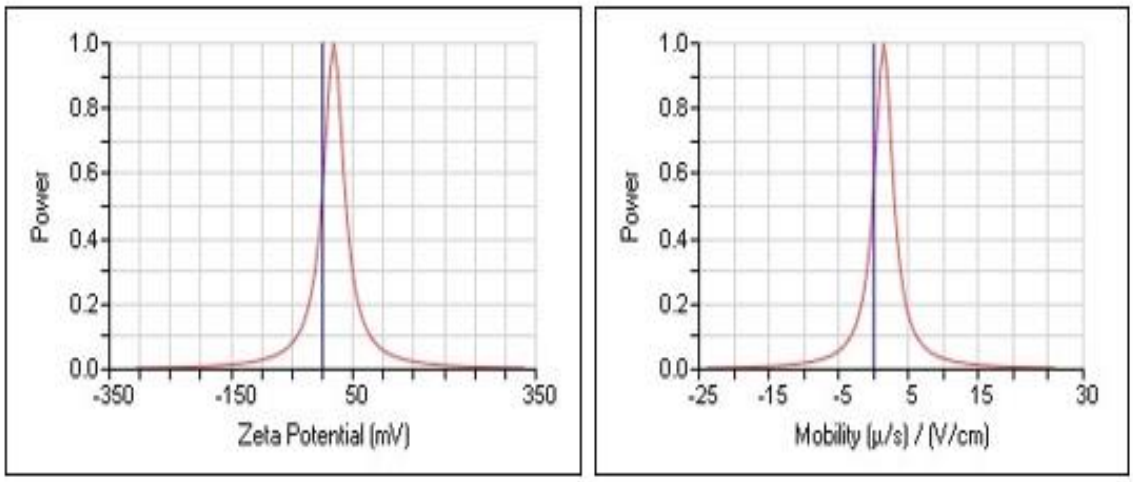
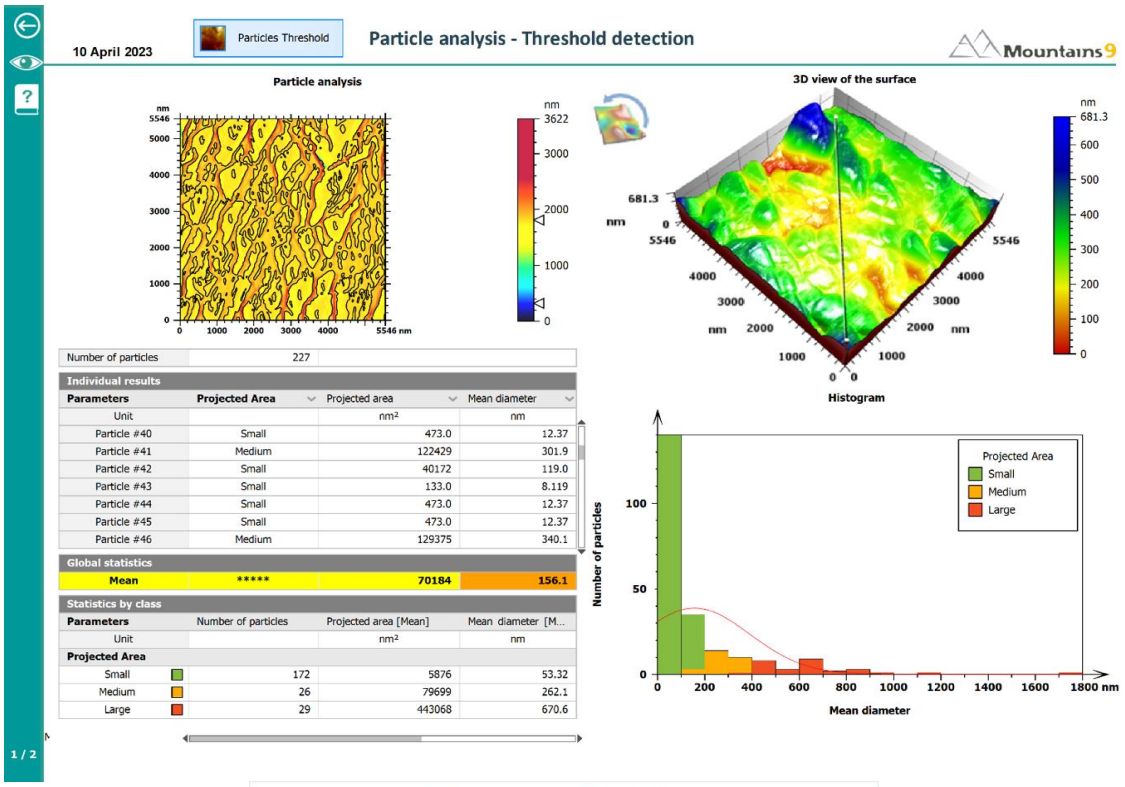


Figure (3.23) spectrum peak of FTIR for (CS-Fe_3O_4 nanopartical)

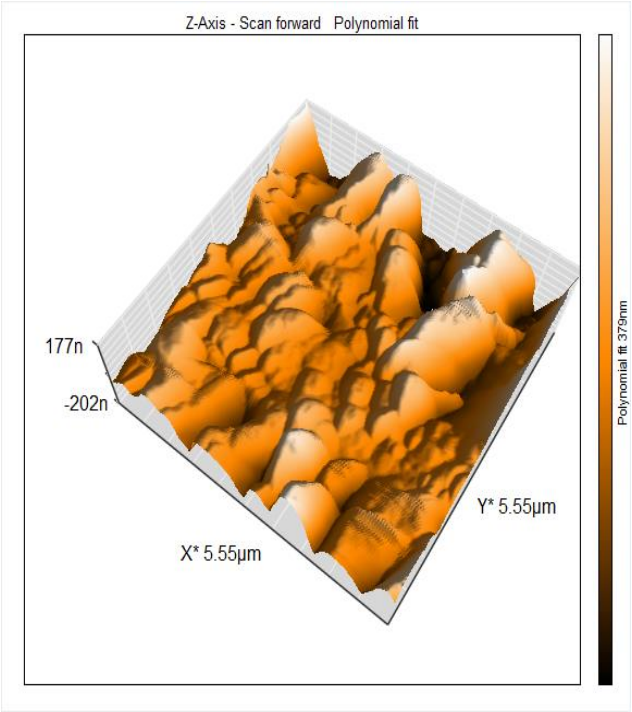


Figure(3.24): Zeta Potential of (CS- Fe_3O_4**)**

Atomic Force Microscopy (AFM) analysis was also used to provide images with near-atomic resolution for measuring surface topography. It was used for producing the size distribution as shown in Fig(3.25) . AFM analysis of CS- Fe_3O_4 showed that the nanoparticles were well dispersed and the large ratio for small size (53%) below 56 nm.

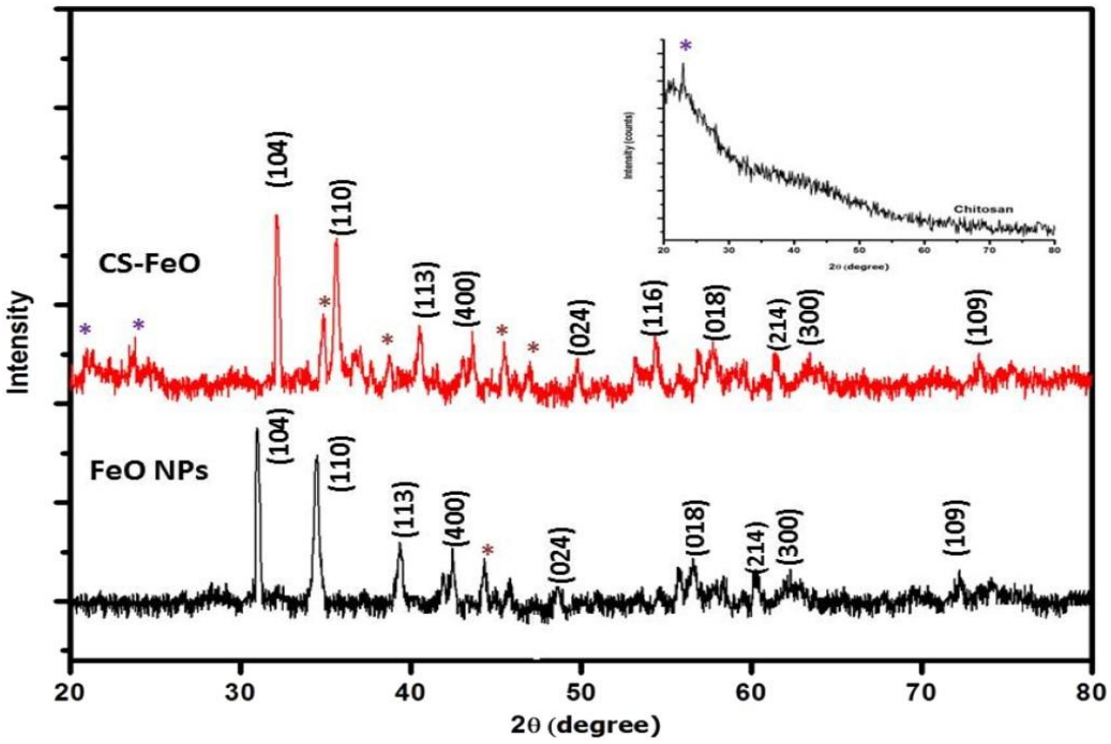


Number of particles	227		
Individual results			
Parameters	**Projected Area**	Projected area	Mean diameter
Unit		nm²	nm
Particle #40	Small	473.0	12.37
Particle #41	Medium	122429	301.9
Particle #42	Small	40172	119.0
Particle #43	Small	133.0	8.119
Particle #44	Small	473.0	12.37
Particle #45	Small	473.0	12.37
Particle #46	Medium	129375	340.1
Global statistics			
Mean	*****	70184	156.1
Statistics by class			
Parameters	Number of particles	Projected area [Mean]	Mean diameter [M...
Unit		nm²	nm
Projected Area			
Small	172	5876	53.32
Medium	26	79699	262.1
Large	29	443068	670.6



Figure(3.25) : Atomic force microscopy (Cs- Fe_3O_4**)**

The prepared chitosan iron oxide nanoparticles were examined by XRD The **(CS-Fe_3O_4)** pattern, the characteristic diffraction peaks observed around 2θ = 32º, 36º, 41º, 50º, 54º, 57º, 62º, 64º, and 74º respectively corresponding to (104), (110), (113), (024), (116), (018), (214), (300), and (109) orientation planes and were indexed to a rhombohedral crystalline structure, Similar finding were reported by. [10] The 2θ peak obtained at near 23º in the given XRD data may be due to the crystallization of chitosan phase in the prepared CS- Fe_3O_4 nanocomposite and the other extra peaks (*) may be due to the crystallization of bioorganic phase in the prepared Fe_3O_4 NPs and CS- Fe_3O_4 nanocomposite. The average crystallite size of Fe_3O_4 NPs was found to be 46 nm whereas the crystallite size of CS- Fe_3O_4 nanocomposite was calculated to be 55 nm.



Figure(3.26) : XRD for chitosan iron oxide nanoparticle

The SEM images of CS- Fe_3O_4 nanocomposite also revealed agglomerated spherical shaped grains. EDX spectrum of prepared CS- Fe_3O_4 nanocomposite showed the

presence of Fe, O and N. The presence of nitrogen (N) was supposed to arise from the amine (-NH2) group of chitosan. The detection of N in the spectrum also supports the presence of chitosan polymer on prepared nanocomposite.

3.14. Application CS- Fe_3O_4 NPs as a lipid lowering agent

Estimation of mean levels of determination pool of serum lipid profile with and without the prepared nanoparticles (**CS-** Fe_3O_4 **NPs**) pediatric nephrotic syndrome patients was illustrated in **Figures (3.27)**

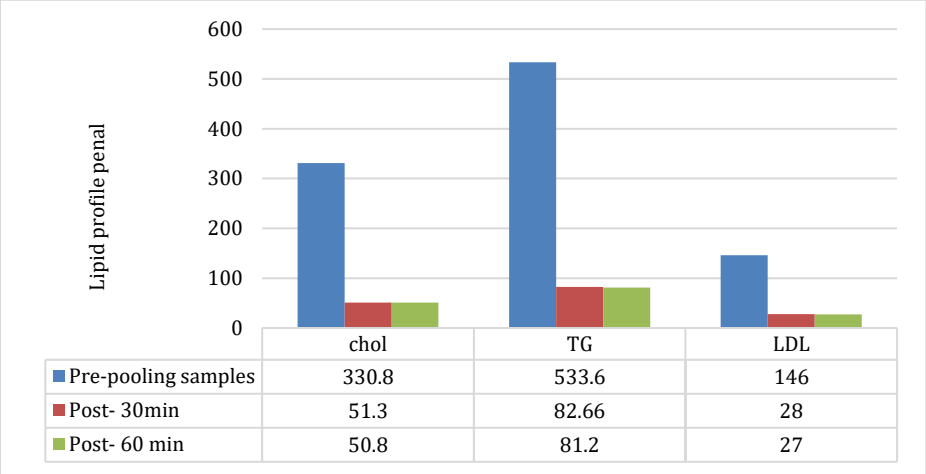
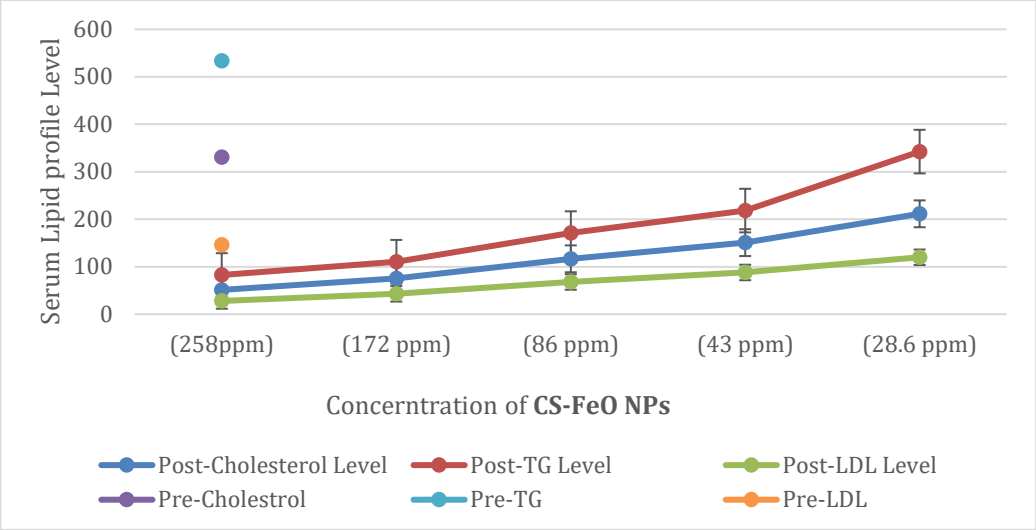


Figure (3.27) Mean level of pre-post test of serum lipid profile with and without the prepared nanoparticles (CS- Fe_3O_4 **NPs)**

CS- Fe_3O_4 **NPs** have shown a good effect as a lipid lowering agent. The pool level of serum and blood lipid profile in the presence **CS-** Fe_3O_4 **NPs** was decreased positively with increasing concentration of the **CS-** Fe_3O_4 **NPs** after incubation at $37c^o$ as compared to the pre-test, data were presented in Figure (3.28) & (3.29)



Pool sample +(Cs- FeO_3) NPs	Conc.1 (258ppm)	Conc.2 (172 ppm)	Conc.3 (86 ppm)	Conc.4 (43 ppm)	Conc.5 (28.6 ppm)
Cholesterol Level	51.3	75.8	116.7	150.8	211.5
TG Level	82.66	110.6	170.9	218.3	342.5
LDL Level	28	43	68	88	120

Figure (3.28) Estimation plot of determination pool level of serum lipid profile with and without different concentration of the prepared nanoparticles (CS- Fe_3O_4 NPs) in sera patients of pediatric nephrotic syndrome

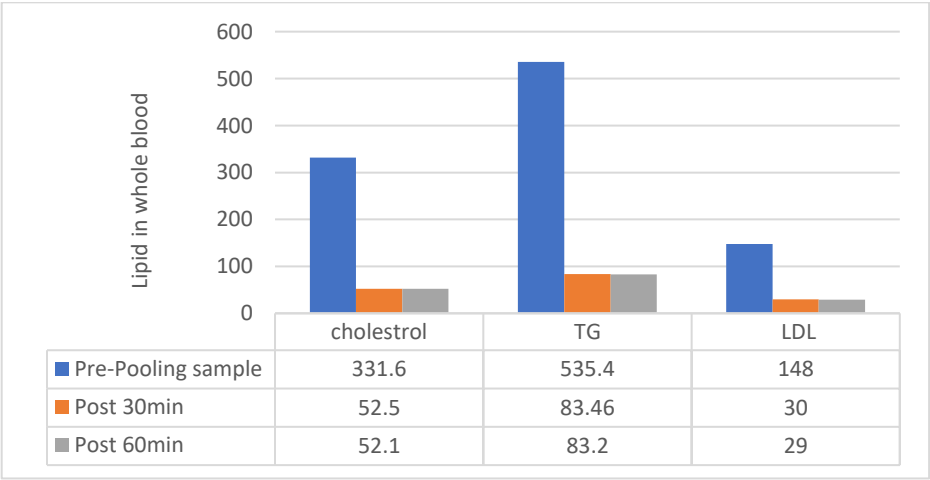
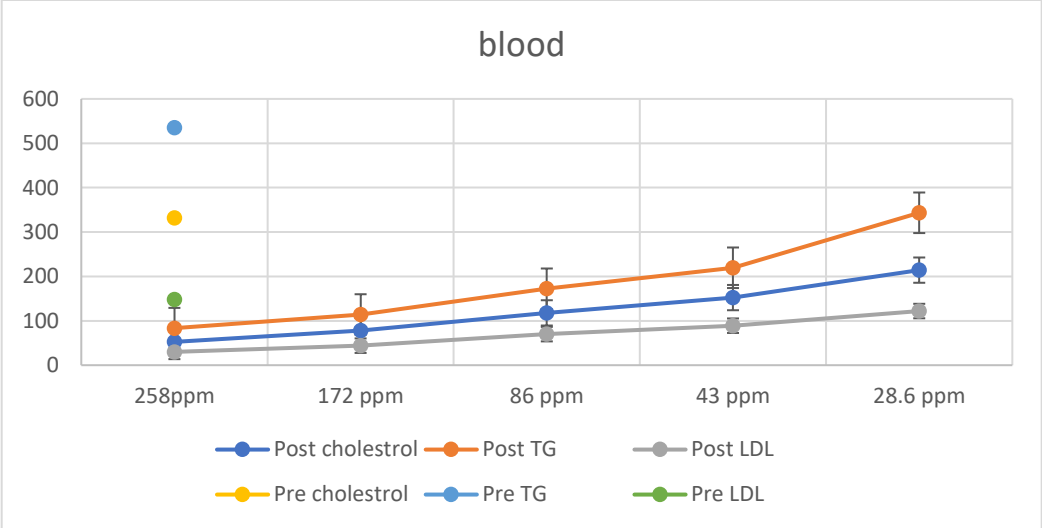


Figure (3.29) Mean level of pre-post test of <u>whole blood</u> lipid profile with and without the prepared nanoparticles (CS- Fe_3O_4 NPs)



Cs Fe3O4	258ppm	172 ppm	86 ppm	43 ppm	28.6 ppm
Post cholestrol	52.5	77.8	117.9	152.3	214.2
Post TG	83.46	114.2	172.2	219.5	343.4
Post LDL	30	44	70	89	122

Figure (3.30) Estimation plot of determination pool level of <u>whole blood</u> lipid profile with and without different concentration of the prepared nanoparticles (CS- Fe_3O_4 NPs) in sera patients of pediatric nephrotic syndrome

It was found that **CS-** Fe_3O_4 **NPs** have an ability to lower lipid content indeed reduced the cholesterol accumulation in the pool samples by ~ 85%, reduced the TG level in the pool samples by 84.5% and reduced the LDL level in the pool samples by 81% . These results confirmed that **CS-** Fe_3O_4 **NPs** might be used as cholesterol sinks for enhancing the magnetic charge of cholesterol. Our findings support that importance role of such nano-particles in hyperlipidemia which would be a potent cholesterol absorber and scavenger and might be a potential platform for treating such complication in pediatric nephrotic syndrome and other cholesterol-burden diseases.

Chapter four

Discussion

Discussion:

Examination the level of Selectin, VAP-1 and Omentine-1 in nephrotic syndrome group compared to control

The clinical manifestation of nephrotic syndrome is distinguished by substantial amounts of protein being excreted in the urine. Proteinuria is to blame for hypoalbuminemia, which in turn contributes to hyperlipidemia, edema, and a variety of other health issues. This problem may be traced back to its core cause, which is an increase in the permeability that occurs via the damaged basement membrane of the renal glomerulus. It is the result of an abnormality in the glomerular permeability, which may be primarily caused by an intrinsic renal sickness in the kidneys or secondarily induced by congenital infections, diabetes, systemic lupus erythematosus, neoplasia, or the use of certain medicines. In any case, it is the outcome of an abnormality in the glomerular permeability. (**Raina and Krishnappa.,2019)**

Adipose tissue is now known to be a hormonally active organ that releases a large number of bioactive proteins regulating not only body weight and energy homeostasis, but also insulin resistance, blood lipids, endothelial health, coagulation, fibrinolysis and inflammation, Fat tissue secretes a number of Adipokines **(G.F. Watts, *et. al* ., 2001**).

It is already known that nephrotic patients have endothelial dysfunction demonstrated by a reduction in FMD (flow-mediated dilatation) (**Yildiz, *et. al* ., 2013**). Moreover, these patients also have high levels of endothelial activation biomarkers **(M. Tkaczyk, *et. al* ., 2008**). In the first part of our study, the relation of adipocytokines and nephrotic syndrome in different state.

The pattern of expression of E-selectin and vascular adhesion protein-1 in renal disease received relatively little attention in past research.

In the current study, the level of E-selectin and VAP-1 were increased in patient group when compared with control group and theses results were agreed with **(Yildiz, *et. al* .,2013)** and **(Inamdar, *et. al* ., 2020**). while the Omentin-1 level was decreased in patient which also agreed with **(Mohammed, *et. al* ., 2021)**

A preliminary report for previous steadies suggests that E-selectin is expressed by glomerular endothelial cells in some patients with acute glomerulonephritis, lupus nephritis, and IgA nephropathy, acute kidney injury, nephrotic syndrome, membranous nephropathy Interestingly, glomerular levels of E-selectin appear to be highest in patients with high circulating levels of TNFa **(Scott B, *et. al* ., 2000)**

Adhesion molecules mediate the initial rolling of inflammatory cells along endothelial cells and platelets in response to a pathological process. However, the role of adhesion molecules in the pathophysiology of pediatric renal disease syndrome is not well known. Unlike other adhesion molecules, E-selectin is only synthesized by endothelial cells when activated by interleukin-1 or tumor necrosis factor. the **(Yildiz, *et. al* .,2013)** found that E-selectin levels were increased in patients with nephrotic syndrome this result agree with our finding. in Figure (3.9) a comparison of serum level of Selectine in different age groups was performed. The level of Selectine increasing in case compered to control within the age groups. The possible reason for the increased level of E-selectin may be the presence of hyperlipidemia which is reported to enhance the secretion of IL-6 and TNF-alpha, on the other hand, hypercholesterolemia has been reported to increase superoxide anion production in endothelial cells , in fact, we found that cholesterol and triglyceride levels were positively related to E-selectin levels in such patients. Hyperlipidemia appears to be associated with endothelial damage. **(Nishida, *et. al* .,1997)**

Other markers of formative status or cellular activation (P-selectin and E-selectin) were not significantly different across different degrees of renal impairment. This is first time study Serum Vascular Adhesion Protein-1(VAP-1) concentrations with nephrotic syndrome. the concentrations have been reported to increase with increasing the duration of the disease. In a cross-sectional study of 262 nondiabetic patients, serum sVAP-1 concentrations were positively associated with albumin excretions and with severity of chronic kidney disease (CKD). **(Pannecoeck, *et. al* .,2015)**

Omentine-1 is a novel adipokine identified in visceral adipose tissue also this is this a first time to study Serum Omentine-1 in nephrotic syndrome. Omentin-1 serum levels in CKD patients were significantly lower compared to healthy controls. An increase in blood components and malnutrition have been associated with a decrease in the level of Omentin in the blood. Omentin levels were lower in CKD patients in stage 2 and 3 than in control patients**. (Tekçe, , *et. al* .,2014)** this result agree with our study in the lowering concentration of omentine-1 in kidney disease. An increase in inflammation and malnutrition components was correlated with a decrease in the serum level of Omentine-1. **(Tripepi, *et. al* .,2011)**

Study the difference between the level of lipid profile & level of atherogenic indices in nephrotic syndrome

Pediatric renal diseases are associated with a significant rise in CVD risk. that means lipid disorders or an elevated atherogenic index (AC, AIP, CRI.I, CRI.II and C. index could predict major kidney function decline. Lipid disorders (such as dyslipidemia) have been identified as critical contributing factors for atherosclerosis and cardiovascular disease (CVD) **(Gu D, *et. al* .,2005).** Blood lipid ratios may be more effective than traditional blood lipid parameters in monitoring early cardiovascular risk in children with NS **(Zhang RX, *et. al* .,2021).**

Atherogenic index of plasma (AIP), calculated as the logarithmically transformed ratio of triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C) (log [TG/HDL-C]), is a novel marker of atherosclerosis and CVD. In addition, related studies have shown AIP to be a more accurate predictor of CVD than traditional lipid parameters **(Edwards MK, *et. al* .,2017)**. Huang et al., 2020 has been also reported that AIP was linked to the complication of lipid in nephrotic syndrome cases. A recent study showed that AIP is a predictor of subclinical renal damage, defined as an eGFR between 30 and 60 mL/min/1.73 m^2 **(Fei Huang, *et. al* .,2021).**

Also, Atherogenic coefficient (AC)which is the ratio of non-high-density lipoproteins cholesterol (non-HDL-C) to high-density lipoproteins cholesterol (HDL-C). It is a diagnostic alternative, which has been used in predicting the risk of developing *cardiovascular* events **(Olamoyegun MA, *et. al* .,2016).**

Furthermore, Castelli's risk indexes (I & II): both called cardiac risk indexes which are two lipid ratios, the CRI-I is the ratio of TC to HDL-C, while the CRI-II is the ratio of LDL-C to HDL-C, with notable positive associations with *CVD* risk **(Igharo O, *et. al* .,2020).** many reports assessed and confirmed their positive correlation with

CVD **(Tecer, *et. al* .,2019).** While, Cholesterol index (C-index): was reported as a simple index that predicts the probability of developing CAD with greater accuracy than the other indices **(Ulusoy R, *et. al* .,2013).**

In NS, atherogenic index of plasma AIP, atherogenic coefficient (AC), castelli risk index-1 (CRI-1), castelli risk index-2 (CRI-2), and Cholesterol index were all significantly higher than those in control group ($P<0.001$).

However, only a few studies have revealed the potential clinical usefulness of AIP such as that performed by **(Smajic et al., 2018)**.

This study was focused on the mean differences in the atherogenic indices for potential confounders. All indices were significantly associated with the development of nephrotic syndrome Generally, the mechanisms through which dyslipidemia can potentially accelerate renal disease progression remain unclear. One of the possible mechanisms is that an increase in reabsorption of phospholipids and cholesterol by tubular epithelial cells is associated with dyslipidemia.

This reabsorption could then stimulate tubulointerstitial inflammation, foam cell formation, and tissue injury **(Abrass CK., 2004)**. Moreover, increased levels of lipoproteins could increase the formation of proinflammatory cytokines, thus inducing glomerulosclerosis **(Keane WF et al., 1993).**

Nephrotic syndrome results in elevated serum triglyceride, VLDL, and IDL levels; increased triglyceride contents of apoB-containing lipoproteins; and prolonged postprandial lipemia **(Jul, et al., 2016).**

These abnormalities are due to impaired VLDL and chylomicron clearance **(Shearer GC, et al., 2016).** LPL, hepatic lipase, VLDL receptor, and proper shuttling of lipids and apoproteins between HDL and apoB-containing lipoproteins are essential steps in maturation and clearance of VLDL and chylomicrons and generation of normal LDL. In addition, changes in the structure of these lipoproteins limit their effective

binding to the key receptors, their ability to activate lipolytic enzymes, and their proper lipid and apoprotein exchange with HDL **(Jul, et al., 2016).**

On the other hand, HDL abnormalities were also reported to impaired the VLDL and chylomicron metabolism. nephrotic syndrome results in lecithin cholesteryl ester acyltransferase (LCAT) deficiency and upregulation of CETP, which lead to a decrease in cholesterol ester content and an increase in triglyceride content of HDL and impaired maturation of cholesterol-poor to cholesterol-rich HDL. The scarcity of cholesterol-rich HDL, in turn, limits contribution of apoE and apoC to the nascent VLDL and chylomicrons. Because clearance of the ApoB-containing lipoproteins depends on apoE and apoC for their binding to the endothelium and activation of LPL, HDL abnormalities contribute to the impaired VLDL metabolism in nephrotic syndrome **(Vaziri, 2016).**

Since it has been reported that the expression of genes encoding the main steps in phospholipid and triglyceride synthesis are upregulated in the liver of animals with nephrotic syndrome **(Zhou Y, *et al*., 2008).** It was confirmed that Nephrotic syndrome might be result in increased expression of the key enzymes involved in fatty acid biosynthesis including acetyl coenzyme A (CoA) carboxylase, fatty acid synthase, elongation of very long chain fatty acids 2 and 6, and downregulation of genes encoding proteins involved in fatty acid catabolism in the liver **(Vaziri ND, 2014).**

other have suggested that these abnormalities, in addition to impaired clearance of triglyceride-rich lipoproteins, increased production of fatty acids, triglycerides, and phospholipids may contribute to the hyperlipidemia in nephrotic syndrome **(Jul, et al., 2016).** The abnormalities of lipid metabolism in nephrotic syndrome described

previously can cause serious consequences: accumulation of atherogenic LDL, IDL, and chylomicron remnants, coupled with impaired HDL-mediated reverse cholesterol transport, contributes to the development and progression of atherosclerosis and cardiovascular disease; impaired delivery of lipid fuel to the skeletal muscles and adipose tissue, occasioned by lipoprotein lipase deficiency and dysfunction, results in the reductions of body mass and diminished exercise capacity **(Vaziri ND, 2014).** uptake of abnormal lipoproteins by glomerular mesangial cells promotes glomerulosclerosis and reabsorption of the filtered albumin and other lipid-containing proteins leads to accumulation of lipids and cytotoxicity in proximal tubular epithelial cells, events that can result in the loss of nephrons and development and progression of chronic kidney disease ; and an increase in plasma LP(a) in nephrotic patients increases the risk of the thromboembolic and cardiovascular complications **(Vaziri, 2016).**

Similarly, to the all of these complications in patients with NS such as the increased risk of atherosclerosis and thromboembolism are linked to dysregulated lipid metabolism and dyslipidemia **(Agrawal S, et al., 2018).** Which completely was reflected in increasing the levels of atherogenic indices.

Using the “alternative lipid window,” understanding the condition of dyslipidemia at a glance in the acute phase of NS is easy. Specially for patients belong to the case of hyperlipidemia, it implies that among those patients with hyper levels could be exposed to the risk of endothelial cell damage.

Using these criteria, the majority of NS patients were found exhibit a considerable risk of developing premature vascular disease **(Keisuke Sugimoto, et al., 2020).** In our point of view, theses indices provided novel clues on the atherogenic

mechanisms in such cases, which might reflect the inflammatory signals more effectively than lipid panel.

Study the structural features of chitosan/iron oxide nanoparticles CS-Fe_3O_4 NPs and Application CS- Fe_3O_4 NPs as a lipid lowering agent.

Chitosan nanoparticles are important materials that are widely used in many biological, engineering, and food industries Magnetic Fe_3O_4–chitosan nanoparticles were prepared by the covalent binding of chitosan (CTS) onto the surface of magnetic Fe_3O_4 nanoparticles. Interesting characteristics of chitosan include its polycationic nature, biodegradability, biocompatibility, bioactivity, and nontoxicity were the main motivation behind this work.

The formation of **Fe_3O_4** NPs and CS- **Fe_3O_4** nanocomposite was monitored by SEM images and UV-Visible spectroscopy. The spectral analysis was carried out in the range of 200-1000 nm and. The synthesized **Fe_3O_4** NPs exhibited a spectral absorbance shift at 268 nm. It was reported earlier that the spectral absorbance around 250 nm is a characteristic feature of **Fe_3O_4** NPs**.**

In the CS- **Fe_3O_4** nanocomposite FTIR spectrum, the highest FTIR peaks obtained at 3437 cm-1 were attributed to O-H and C-H stretch vibrations of phenols and alkynes. FTIR spectral bands at 1643, 1366, 1512, 1126, 906, and 839 cm-1 respectively correspond to N-H bend, N-O, C-H, O-H, and C=O stretching of amino (-NH2), nitro compounds, alkyl groups phenols (O-H stretch) and carboxylic (–COOH) groups***)* (Vasantharaj, et al., 2018).** The other short peaks observed from 500 to 800 cm^{-1} were assigned to the presence of a metal-oxygen (Fe-O) **(Rajiv, et al., 2017).** The Presence of other functional groups like amino, nitro, and alkyl groups may be derived from chitosan ($C_{56}H_{103}N_9O_{39}$) **(Bharathi, et al., 2019).**

While the synthesized chitosan iron oxide nanoparticles exhibit positively charged surfaces with zeta potentials, the surface charge was dramatically changed to positive values (+ 18.3 mV). This confirms the presence of -NH2 groups on the surface of CS- **Fe_3O_4** nanocomposite in a protonated form, and thus establishing the presence of chitosan on the prepared nanocomposite **(Bharathi, et al., 2019).** Similar findings were documented by Shi et al. who have reported positive zeta potential surface charge of CS- **Fe_3O_4** NPs **(Shi, et al., 2012).**

The prepared chitosan iron oxide nanoparticles were examined by XRD The **(CS-Fe_3O_4)** pattern, the characteristic diffraction peaks observed around 2θ = 32º, 36º, 41º, 50º, 54º, 57º, 62º, 64º, and 74º respectively corresponding to (104), (110), (113), (024), (116), (018), (214), (300), and (109) orientation planes and were indexed to a rhombohedral crystalline structure, Similar finding was reported by **(H. Muthukumar, et al., 2015).**

The 2θ peak obtained at nearly 23º in the given XRD data may be due to the crystallization of the chitosan phase in the prepared CS **Fe_3O_4** nanocomposite and the other extra peaks (*) may be due to the crystallization of the bioorganic phase in the prepared **Fe_3O_4** NPs and CS- **Fe_3O_4** nanocomposite. The average crystallite size of **Fe_3O_4** NPs was found to be 46 nm whereas the crystallite size of CS- **Fe_3O_4** nanocomposite was calculated to be 55 nm.

CS- **Fe_3O_4** NPs have shown a good effect as a lipid-lowering agent. The pool level of serum lipid profile in the presence of CS- **Fe_3O_4** NPs was decreased positively with increasing concentration of the CS- **Fe_3O_4** NPs after incubation at 37c^o as compared to the pre-test. It was found that **CS- Fe_3O_4 NPs** have the ability to lower lipid content indeed reducing the cholesterol accumulation in the pool samples by ~

85%, reducing the TG level in the pool samples by 84.5%, and reducing the LDL level in the pool samples by 81%.

These results confirmed that **CS- Fe_3O_4 NPs** might be used as cholesterol sinks for enhancing the magnetic charge of cholesterol. Our findings support the importance role of such nano-particles in hyperlipidemia which would be a potent cholesterol absorber and scavenger and might be a potential platform for treating such complications in pediatric nephrotic syndrome and other cholesterol-burden diseases.

Conclusions and Recommendations

Conclusions

- The proposed biomarkers and dyslipidemia were linked successfully in the diagnosis of endothelial dysfunction in children with nephrotic syndrome There was a highly association between nephrotic syndrome and E-selectin, Omentin, Vascular adhesion protien-1 as well as lipid penal.
- According to the ROC analysis, the serum concentration of E-selectin and VAP-1 level in the patient group were shown a good performance for prediction nephrotic syndrome patients
- An attempt has been made in this study to establish serum lipid pattern in the IRAQ/Kerbala children with nephrotic syndrome. it was confirmed that nephrotic experience hyperlipidemia which predisposes to cardiovascular disease. The atherogenic indices "alternative lipid window" are relatively easy to obtain using routine biochemical parameters and help understand the state of hyperlipidemia in patients with NS. Therefore, an advocate was made to use these indices among the hallmarks of the biochemical during investigation nephrotic syndrome patient for better management.
- Magnetic **Fe_3O_4** –chitosan nanoparticles were fabricated by the covalent binding of CTS on the **Fe_3O_4** nanoparticles. The analyses of TEM and XRD indicated that the **Fe_3O_4** –chitosan nanoparticles were monodispersed and regular spheres with a range diameter of (33-55) nm. These microspheres may apply to the magnetic-field-assisted lipid lowering processes.

Recommendations

- A study of other types of adipokines and their relationship to the clinical complications of pediatric nephrotic syndrome such as ghrelin, Obestatin which are another newly described adipocytokine that reported to be a ghrelin-associated peptide.
- Preparation of other nanocomposites with Nano-chitosan (such as ZnONPs) which might be directly related to lowering lipid levels and showing outstanding effectiveness.
- It might be a good idea to heightened the awareness of the associated exacerbated risks of cardiovascular complications, progressive kidney disease and thromboembolism in such cases and correlated the atherogenic indices as a risk factor for eGFR decline.

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Appendixes

University of Kerbala

College of Medicine

Department of chemistry & Biochemistry

جامعة كربلاء

جامعة كربلاء

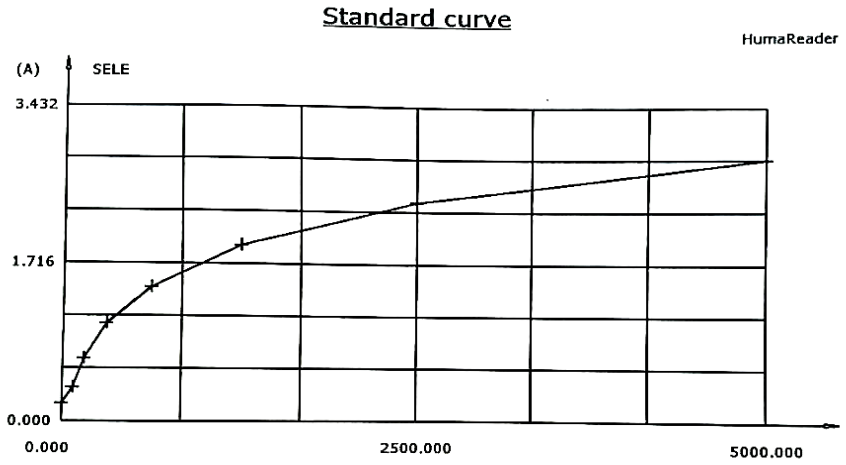
Case NO :

Research Questionnaire

Dear Participants, I am inviting you to participate in this research by completing the following survey.

Name of Participants:						
Mobile			Age:		Gender:	
Body mass index		Weight			length	
Level of education :			Residency :			
Marital state :			Occupation :			
Chronic disesease	Diabetic	hypertension	Hypercholesterolemia		Others	

Fig 1: questionnaire form

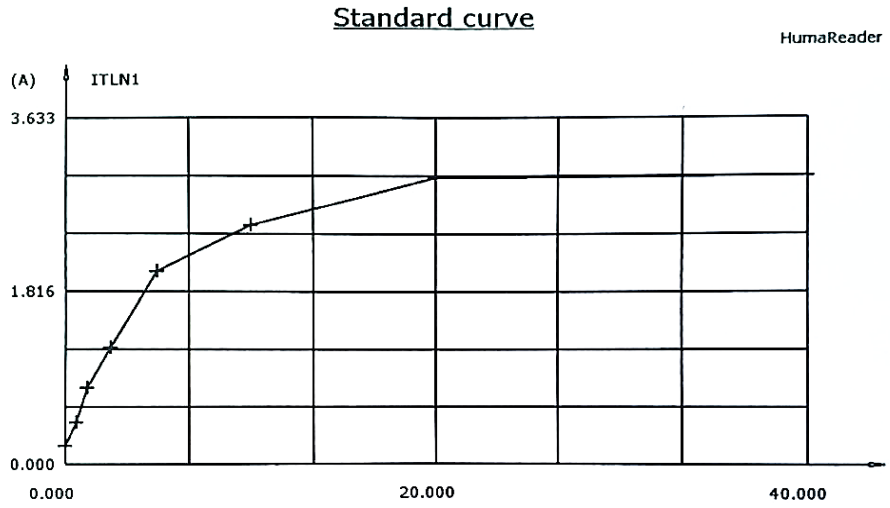


Program: SELE SELE Caculation: Curve Wavelength(nm): 450

Standard sample	#1	#2	#3	#4	#5	#6	#7	#8
Concentration(pg/ml)	0.000	78.130	156.250	312.500	625.000	1250.000	2500.000	5000.000
Abs(A)	0.186	0.363	0.671	1.050	1.456	1.912	2.385	2.860

Operator: Checker:
Print date: 04/22/2009

Fig 2: Standard carve of selectin



Program: ITLN1 ITLN1 Caculation: Curve Wavelength(nm): 450

Standard sample	#1	#2	#3	#4	#5	#6	#7	#8
Concentration(ug/ml)	0.000	0.630	1.250	2.500	5.000	10.000	20.000	40.000
Abs(A)	0.191	0.437	0.807	1.226	2.025	2.511	3.006	3.027

Operator: Checker:
Print date: 04/22/2009

Fig 3: standard carve of Omentine-1

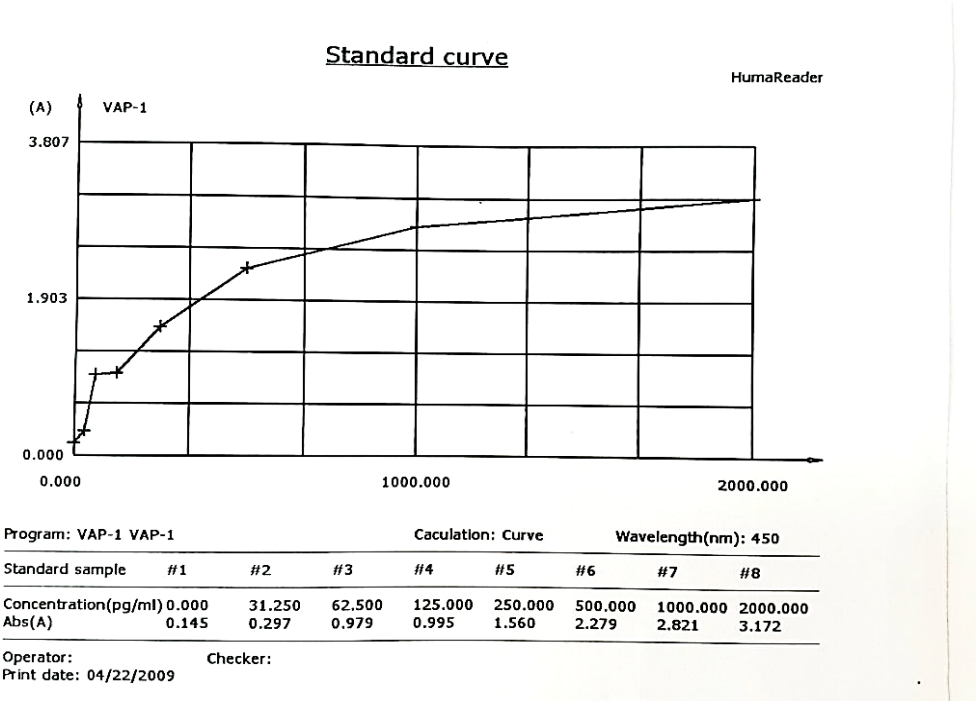


Fig 4: standard carve of Vap-1

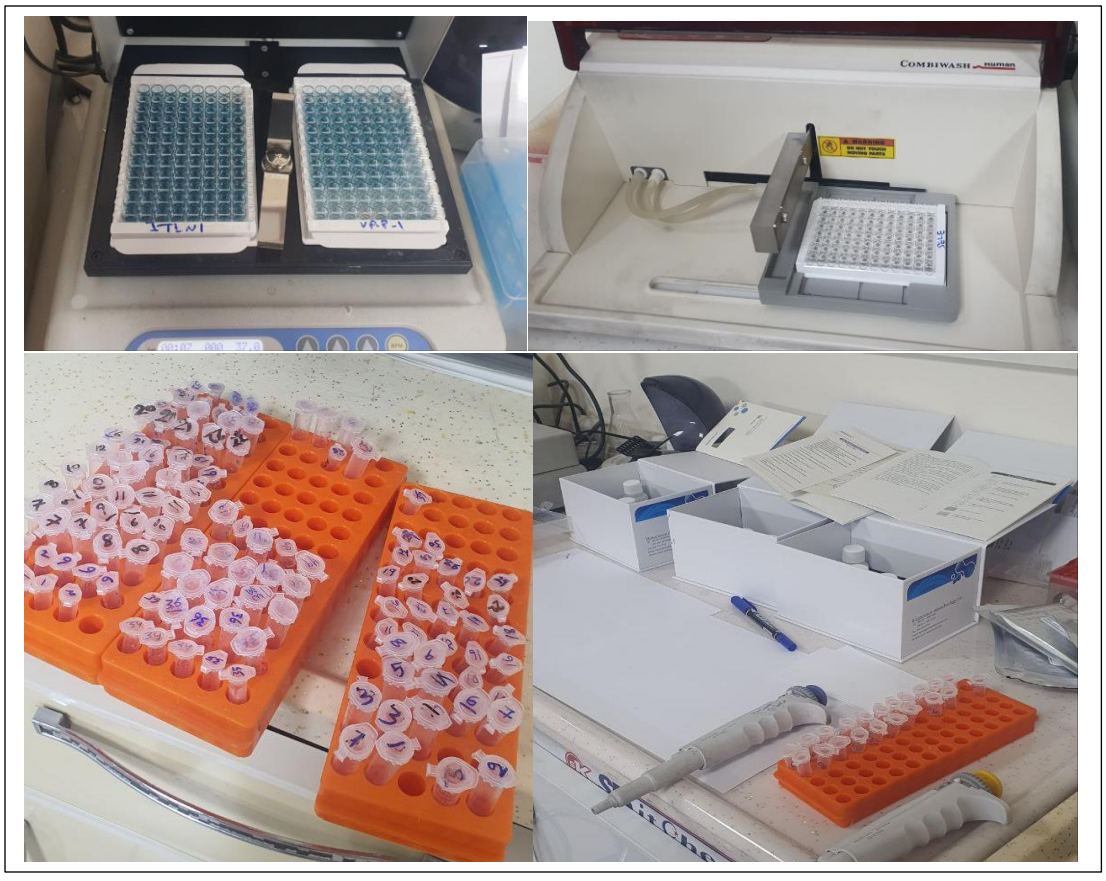


Fig 5: some picture for ELISA technique

Fig 6: some picture for Nano preparation

Fig 6: some picture for Nano application on pooling sample

الخلاصة:

المتلازمة الكلوية هي اضطراب في الكلى يتسبب في إفراز الجسم للكثير من البروتين في البول. الأسباب الأكثر شيوعًا هي العوامل الوراثية يمكن أن تشمل الأسباب الأخرى الأمراض التي تصيب أجزاء أخرى من الجسم والالتهابات وبعض الأدوية أيضا وتشمل الأعراض وجود الكثير من البروتين في الادرار وانخفاض مستويات الألبومين في الدم، وتورم الجسم (الوذمة).

تسبب المتلازمة الكلوية عند الأطفال العديد من المضاعفات في العديد من أعضاء الجسم وفي بعض الحالات قد تسبب الوفاة. هدفت هذه الدراسة إلى إيجاد المعلمات الكيميائية التي يمكن أن تتنبأ بخلل وظيفي في بطانة الأوعية الدموية لدى الأطفال المصابين بالمتلازمة الكلوية بسبب المستويات العالية من الدهون بالإضافة إلى العوامل الأخرى التي تضمنت تقدير مستوى:

- E-selectin
- Omentin-1
- Vascular adhesion protein-1 (VAP-1)

درس أيضًا التفضيلات التشخيصية لها كمعامل كيميائي حيوي في المتلازمة الكلوية باستخدام تحليل (ROC)علاوة على ذلك، قمنا بإعداد ودراسة السمات الهيكلية للجسيمات النانوية للكيتوزان / أكسيد الحديد (CS-Fe3O4 NPs) وفحص تطبيقات هذه الجسيمات النانوية كعامل لخفض الدهون في الأطفال المصابين بالمتلازمة الكلوية.

الية العمل: صممت هذه الدراسة كدراسة حالة وضبط، اجمالي 52 مشاركا مصابين بالمتلازمة الكلوية و48 طفل سليم تم جمع عيناتهم من مستشفى كربلاء التعليمي للأطفال، مديرية صحة كربلاء / العراق.

ومن ناحية أخرى في مختبرات كلية الطب / جامعة كربلاء، تم تحضير المركب النانوي من الشيتوزان وأكسيد الحديد، وتم قياس خصائص الجسيمات النانوية في وزارة العلوم والتكنولوجيا، وقسم البيئة وبحوث المياه باستخدام المجهر الالكتروني والتحليل الطيفي باستخدام الاشعة تحت الحمراء والتحليل بالأشعة السينية وغيرها من تقنيات التشخيص الاخرى.

أظهرت نتائج الدراسة زيادة في مستويات الكوليسترول في الدم، والدهون الثلاثية، والالبومين في الادرار، و E-selectin ، و VAP-1 مع وجود فروق ذات دلالة إحصائية.

وأظهرت أيضًا انخفاضًا في مستوى الألبومين في الدم و Omentin-1 بشكل ملحوظ في المرضى مقارنة بالمجموعة الضابطة (الاصحاء) . وتم تطبيق تجارب المركبات النانوية أكسيد الشيتوزان والحديد لخفض الكوليسترول مختبريا على عينات المرضى المصابين بالمتلازمة الكلوية ووجد أن CS-Fe3O4 NPs لديها القدرة على خفض مستوى الدهون بالفعل قللت من تراكم الكوليسترول في العينات بنسبة ~ 85٪ ، وخفض مستوى الدهون الثلاثية في العينات بنسبة 84.5٪ وخفض مستوى LDL في العينات بنسبة 81٪.

في الختام، تم ربط المؤشرات الحيوية المقترحة وخلل شحميات الدم بنجاح في تشخيص الخلل البطاني عند الأطفال المصابين بالمتلازمة الكلوية. تم تحضير الجسيمات النانوية المغناطيسية Fe3O4 – chitosan بنجاح عن طريق الارتباط التساهمي لـ CTS على جسيمات Fe3O4 النانوية. تم تطبيق هذه الكرات المجهرية على عمليات خفض الدهون بمساعدة المجال المغناطيسي.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء / كلية الطب

قسم الكيمياء والكيمياء الحياتية

دور بروتين التصاق الأوعية الدموية -1 والسيليكتين و الاومنتين -1 في الخلل الوظيفي البطاني ودراسة دور المواد النانوية كعامل لخفض الدهون للأطفال المصابين بالمتلازمة الكلوية

الرسالة مقدمة الى مجلس كلية الطب / جامعة كربلاء كجزء من متطلبات نيل شهادة الماجستير في الكيمياء السريرية

من قبل

احمد داود سلمان حسين الدهش

بكالوريوس علوم الكيمياء – كلية العلوم – جامعة كربلاء 2014

بأشراف

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رنا مجيد حميد

الأستاذ المساعد الدكتور

اثير حميد عودة الغانمي

2023 م

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